Food Intake, Body Weight Gain, and Body Composition of the Young Obese (ob/ob) Mouse[1]

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ABSTRACT Estimates of food intake and body weight gain were obtained in obese (ob/ob) mice from 7 to 56 days of age. Milk intake was estimated daily from 7 to 21 days of age; there were no differences between obese and lean mice. From 14 to 21 days of age, obese mice gained more body weight than lean mice. At 21 days of age, the carcasses of obese mice contained about twice as much fat as the carcasses of lean mice did, whereas the protein content was not different. Mice were weaned at 21 days of age, and individually fed a stock diet or a high-fat diet. During the first several days after weaning, obese males consumed less food than did the lean males. After approximately 28 to 35 days of age, obese mice consumed more food and gained more weight than lean mice. At 56 days of age, obese mice contained 4 to 5 times as much body fat as did lean mice, but contained significantly less body protein than lean mice. For the 5 week post-weaning period, obese mice converted 3 to 4 times more dietary energy to body energy than did lean mice, whereas obese mice consumed only 20 to 40% more energy. At the same time, obese mice converted only about 70% as much dietary protein to body protein as did lean mice. The high-fat diet markedly enhanced the apparent energy efficiency in obese mice. The present studies suggest that alterations in energy metabolism, as well as in protein metabolism, may play an important role in the development of obesity in these mice. Hyperphagia may be of secondary importance. J. Nutr. *107:* 1715–1723, 1977.

INDEXING KEY WORDS food intake · body weight gain · body composition · obesity · ob/ob mice

Genetically obese rodents have been utilized extensively as animal models in obesity studies (1). In most of those studies, adult animals have been used. However, recent evidence suggests that metabolic alterations may already be present in these animals at an early age. In the genetically obese (ob/ob) mouse, an increased body fat concentration (2, 3) and adipocyte cell size (4), a decreased oxygen consumption (5) and body temperature (6), as well as alterations in locomotor activity (7), have been reported to appear within the first 3 weeks of age. While hyperphagia has been closely associated with the development of obesity in older animals (1, 8), there is a paucity of data on food intake of the young obese mouse. The possible role of nutrition in the early development of the obese syndrome has not been elucidated.

The present study was designed to follow the food intake and growth pattern of obese and lean mice from 1 to 8 weeks of age. Before weaning at 3 weeks of age,

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TABLE 1
Diet composition

	Energy content[3]	Percentage of energy[4]		
		Fat	Protein	Carbohydrate
	kcal/g	%	%	%
Stock diet[1]	4.19	25	22	53
High-fat diet[2]	7.16	82	18	0

[1] Wayne Lab-Blox. Allied Mills Inc., Chicago, Illinois. [2] Percentage by weight: casein 31.2; tallow 47.0; corn oil 7.8; methione 0.5; mineral mix 6.3 (see ref. 10); vitamin mix 0.6 (see ref. 11); choline 0.3; cellulose 6.3. [3] Determined in a bomb calorimeter. [4] Computation based on 4 kcal per g protein and carbohydrate and 9 kcal per g fat. The carbohydrate content of the stock diet was computed by difference. It was assumed that the fiber in these diets was not utilized by the mouse. The stock diet contained 4.5% fiber.

milk intake of the pups was estimated daily. From 3 to 8 weeks of age, obese and lean mice were fed a high-carbohydrate stock diet or a high-fat semipurified diet. Food intake and body weight changes were monitored. Body composition was determined in 3 and 8 week old mice.

MATERIALS AND METHODS

Heterozygote breeding mice (C57BL/6J ob/+) [2] were housed in solid bottom cages with wood shavings for bedding, and fed ad libitum a stock diet.[3] Pregnant dams were removed from the breeding cages and placed in separate similar cages. The average litter contained 6 to 7 pups. Litters containing less than 4 pups or more than 9 pups were not utilized in the experiment. Pups were individually identified at 5 days of age. Lights in the temperature-controlled (22–24°) room were turned on at 0700 hours and off at 1900 hours each day. All mice had free access to water.

From 7 to 21 days of age, estimates of milk intake and growth rate were recorded daily. To obtain estimates of milk intake, pups were separated from their dam at 0900 hours daily and returned to the cage of their dam 6 hours later. Pups were allowed access to water, but not to food during this time. Pups were weighed at 0900 hours, 6 hours later, and again after 1 hour of suckling. The body weight gain of each pup during 1 hour of suckling was utilized as an estimate of the milk intake (9). The sum of the milk intake of all the pups in the litter was utilized as an estimate of milk production of the dam. Body weights measured daily at 0900 hours were utilized to determine the growth rate of the pups. Body weight differences between 0900 hours and 6 hours later were recorded as body weight loss during fasting. The obese (ob/ob) and lean (ob/+ or +/+) mice were identified retrospectively after the obesity became obvious by visual inspection.

At 21 days of age, obese mice were identified by their lower oxygen consumption (5) and by their relatively fat appearances. From 21 to 56 days of age, male and female obese mice and their lean littermates were individually housed in metal cages with wire-screen bottoms. Identification of the obese mice utilized in the feeding experiment was also confirmed retrospectively by visual inspection. They were fed a pelleted stock diet or a high-fat diet (table 1). Food consumption was determined daily.

Male and female obese mice and their lean littermates were killed at 21 and 56 days of age. Food residue in the stomach was removed from the carcass. The carcass was then softened in an autoclave at 100° for 15 minutes prior to homogenization. The homogenate was heated in a water bath at 50°, mixed, and sampled. A chloroform/methanol (2:3) mixture was used to extract fat from the carcass homogenate. Carcass fat was determined gravimetrically. The nitrogen content of the carcass was determined by a semi-microKjeldahl method (12). The protein content was computed by multiplying the nitrogen content by 6.25. A bomb calorimeter [4] was utilized to determine the energy content of mouse carcasses. The carcass homogenates were dried (13) in a vacuum oven at 50° for 24 hours prior to combustion in the calorimeter.

Statistical analysis of the results was conducted with a 2 or 3-factor model of crossed classification (14).

RESULTS

The milk production for 15 dams is shown in figure 1. The rate of milk pro-

[2] Jackson Laboratory, Bar Harbor, Maine.
[3] Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois.
[4] Parr Adiabetic Calorimeter with Oxygen Bomb, Parr Instrument Co., Moline, Illinois.

TABLE 2

Milk intake, body weight gain and body weight loss during fasting in mice

Age	Male		Female		MS_E[3]	ANOVA[4]
	Obese (15)[1]	Lean (30)	Obese (10)	Lean (30)		
days						
			Milk Intake (mg/hr)			
7–14	250[2]	240	220	220	8,869	NS
14–20	280	260	240	300	13,886	NS
			Daily Body Weight Gain (mg)			
7–14	360	320	370	310	320	P
14–20	190	110	190	140	447	P
			Body Weight Loss (mg per 6 hr fasting)			
7–14	50	70	50	70	767	P
14–20	100	130	110	150	9,838	NS

[1] Number of mice. [2] Mean; estimated to the nearest 10 mg. [3] MS_E = Mean Square Error. [4] Analysis of Variance. P designates a significant ($P < 0.05$) phenotype effect. NS indicates not significant.

duced for the 1 hour period increased from 7 to 15 days of lactation, and then declined rather rapidly, especially from 19 to 21 days of lactation.

From 7 to 20 days of age, young obese and lean mice consumed an equal amount of milk during the 1 hour suckling periods. Similar results were obtained when pups suckled for 90 minutes before weighing. However, obese mice gained more body weight than their lean siblings (table 2). The pups exhibited a normal growth pattern (15); they grew faster from 7 to 14 days of age than from 14 to 20 days of age. The growth curves of the mice during this period are shown in figure 2. At 21 days of age, the body weights of obese mice were heavier ($P < 0.05$) than their lean counterparts. The young mice lost body weight when they were isolated from their mother for 6 hours. Obese mice lost less weight than mice from 7 to 14 days of age, but not from 14 to 20 days of age. All mice lost less body weight from 7 to 14 days of age than from 14 to 20 days of age.

The ratio of the daily weight gain to weight loss during the 6 hour isolation period was calculated from 14 to 20 days of age; the values were 1.65 ± 0.12 and 0.84 ± 0.07 for the obese and lean mice, respectively. When values within the 99% confidence intervals were then used to predict the phenotype of an additional 7 litters, 6 of the 7 obese pups were correctly identified whereas 37 of the 39 lean mice were properly identified. Two lean mice were mistakenly identified as obese and one obese mouse was incorrectly identified as lean. When the values were calculated for the 2 week period (7 to 20 days of age), similar results were obtained. It may be possible to refine this technique into a useful tool for screening and identifying young obese and lean mice.

At 21 days of age, both male and female obese mice contained about twice the car-

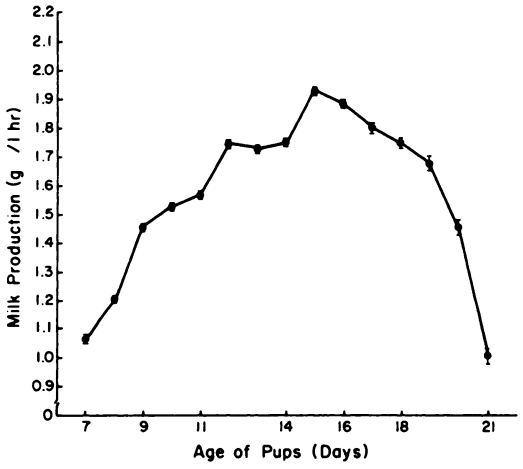

Fig. 1 Lactation curve of 15 dams. Five to eight pups suckled each dam. Each point is mean ± SEM of 15 dams.

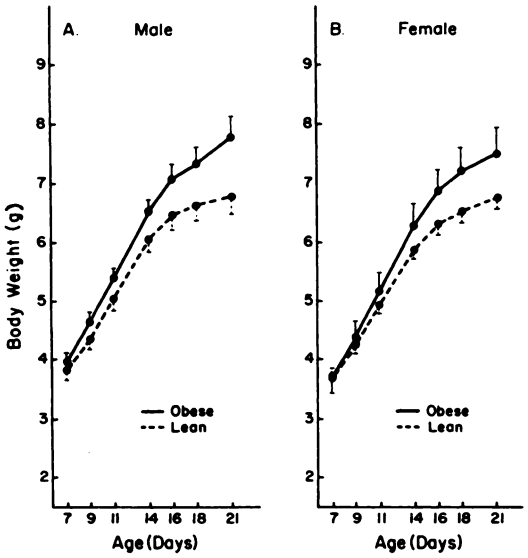

Fig. 2 Growth curves of mice before weaning. Each point is mean ± SEM. There are 15 obese males, 30 lean males, 10 obese females, and 30 lean females.

cass fat and total body energy observed in their lean littermates; the carcass protein content was not influenced by the phenotype (table 3). On a concentration basis, obese mice contained about 27% fat, and 16% protein; lean mice contained about 14% fat, and 18% protein.

During the first several days immediately after weaning, the mice showed some differences in adaptation to the diets (table 4). When the results from both diets were averaged from day 21 to day 23, obese males consumed less energy and gained less body weight than lean males, while obese females consumed less energy but gained as much weight as did their lean counterparts. From 23 to 25 days of age, obese mice generally consumed less energy and gained less body weight than lean mice. Male and female mice fed the stock diet consumed similar amounts of energy and gained similar amounts of weight, while males fed the high-fat diet consumed less energy and gained less weight than females. From 25 to 28 days of age, obese mice fed the stock diet still consumed less energy but generally started to gain more weight than lean mice. Obese mice fed the high-fat diet consumed more energy and gained more weight than their lean sibs. After 28 days of age, the effects of the diet and phenotype on body weight gain and food intake in mice became more pronounced (table 5); the obese mice gained more body weight than the lean mice. From 35 to 42 days of age, obese mice fed the stock diet started to consume more energy than lean mice, whereas obese mice fed the high-fat diet started to consume more energy than the lean mice at an earlier age (25–28 days of age).

Before 21 days of age, the sex of the mice did not influence the observations. From 28 to 56 days of age, the sex of mice had only a minimum effect on food intake, but body weight gain of the mice (except from 42–49 days of age) was influenced by their sex. Male and female obese mice gained body weight at similar rates, but lean males gained more weight than lean females.

Growth curves of the mice from 21 to 56 days of age are summarized in figure 3. Consumption of the high-fat diet increased the body weight of the obese mice, whereas lean mice fed the high-fat diet had body weights similar to those observed in lean

TABLE 3

Body weight, fat, protein and energy content of mice at 21 days of age

Body	Male		Female		MSE[3]	ANOVA[4]
	Obese	Lean	Obese	Lean		
Weight, g	9.20[1]	8.18	9.17	7.85	1.59	P
Fat, g	2.58	1.19	2.25	0.94	0.17	P
Protein,[2] g	1.43	1.48	1.47	1.42	0.04	NS
Energy, kcal	26.0	13.4	25.6	12.8	18.6	P

[1] Mean for eight mice per group. [2] Protein = nitrogen content × 6.25. [3] MS_E = Mean Square Error. [4] Analysis of Variance. P designates a significant ($P < 0.05$) phenotype effect. NS indicates not significant.

TABLE 4

Effect of diet on body weight gain and food intake of mice

Age	Diet[1]	Male		Female		MS_E[3]	ANOVA[4]
		Obese	Lean	Obese	Lean		
days							
		Body weight at 21 days of age (g)					
	S	9.0[2]	8.0	8.4	8.0	1.63	P
	HF	7.3	6.5	8.0	7.2		
		Body weight gain (g)					
21–23	S	0.1	1.0	0.4	0.8	0.29	P, P × S
	HF	−0.2	0.4	1.0	0.7		
23–25	S	0.9	1.4	1.0	1.3	0.20	P, S, D × S
	HF	0.4	0.9	1.7	1.7		
25–28	S	1.8	1.8	1.9	1.5	0.20	P, D
	HF	3.1	2.4	3.0	2.7		
		Food intake (kcal)					
21–23	S	10	18	12	19	40.4	P, D, S
	HF	<1	5	10	9		
23–25	S	14	22	16	22	48.4	P, S, D × S
	HF	9	14	25	21		
25–28	S	28	33	32	36	67.9	D, S, P × D
	HF	39	32	44	40		

[1] S = stock diet; HF = high-fat diet. [2] Mean for eight mice per group. [3] MS_E = Mean Square Error. [4] Analysis of Variance. P, D, and S designate significant ($P < 0.05$) phenotype, diet and sex effects, respectively.

mice fed the stock diet. Obese mice fed the high-fat diet weighed more than lean mice by 35 days of age, whereas obese mice fed the stock diet weighed more than lean mice after 42 to 49 days of age.

Energy, fat, and protein retention of the mice from 21 to 56 days of age were computed from the results of carcass analysis, and are shown in table 6. The results from both diets were averaged; at 56 days of age, obese males contained 52% fat and 12% protein; lean males contained 16% fat and 20% protein. For the females, obese mice contained 57% fat and 10% protein; lean mice contained 21% fat and 18% protein. Clearly, the obese mice retained more energy and exhibited a higher energy efficiency than lean mice. Obese mice retained 4 to 5 times as much energy and were about 3 times as efficient in converting dietary energy to body energy as their lean counterparts. Consumption of the high-fat diet enhanced the energy retention and energy efficiency to a greater extent in obese mice than in lean mice. As expected, fat retention was closely related to energy retention. Both obese and lean females retained more fat than did their male counterparts.

Protein intake of mice was calculated and is shown in table 5. Obese mice had a higher protein intake than lean mice. Mice consumed more protein when fed the stock diet than when fed the high-fat diet; the protein to energy ratio was also higher in the stock diet. Obese males retained less protein than did lean males; however, differences were not observed when obese and lean females were compared. Regardless of diet fed, obese mice were less efficient in converting dietary protein to body protein than lean mice. However, the high-fat diet caused a higher protein retention and protein efficiency than the stock diet.

DISCUSSION

As early as 7 to 14 days of age, small differences in body weight gain were evident in the obese mice. It has been suggested that increased milk intake may be a congenital factor in the development of obesity (7). However, no differences in milk intake were observed, suggesting that the obese mice might have a greater feed

TABLE 5

Effect of diet on weekly body weight gain and food intake of mice

Age	Diet[1]	Male		Female		MS_E[3]	ANOVA[4]
		Obese	Lean	Obese	Lean		
days							
				Weekly body weight gain (g)			
28–35	S	4.0[2]	5.2	4.9	3.6	2.4	P, D, S, P × D, P × S
	HF	10.1	7.4	9.2	5.5		
35–42	S	5.2	3.1	5.6	2.3	1.7	P, D, S, P × D, D × S
	HF	9.9	4.6	8.4	1.7		
42–49	S	5.0	1.7	4.6	0.4	1.5	P, D, P × D, P × S
	HF	6.2	2.4	7.4	1.1		
49–56	S	4.2	1.1	3.7	0.5	1.0	P, D, S, P × D
	HF	6.8	1.3	5.2	0.9		
				Weekly food intake (kcal)			
28–35	S	82	93	97	94	404	P, D, P × D
	HF	136	104	138	106		
35–42	S	118	110	131	102	376	P, D, P × D
	HF	166	130	164	118		
42–49	S	141	112	145	101	248	P, D, P × D, P × S
	HF	167	123	178	112		
49–56	S	150	108	145	100	227	P, D, P × D
	HF	174	118	179	117		
				Total food intake (kcal)			
21–56	S	541	496	577	474	4,574	P, D, P × D
	HF	694	528	737	522		
				Total protein intake (g)			
21–56	S	31.9	29.2	34.2	27.0	10.45	P, D, P × S
	HF	25.8	19.6	27.4	19.4		

[1] S = stock diet; HF = high-fat diet. [2] Mean for eight mice per group. [3] MS_E = Mean Square Error. [4] Analysis of Variance. P, D, and S designate significant ($P \leq 0.05$) phenotype, diet and sex effects, respectively.

efficiency than lean mice. It is also possible that the 1 hour estimate of milk intake did not reflect the total daily milk intake. The lactation curve obtained was similar to previous reports (9, 16), although the daily milk yield was somewhat lower than reported previously (9). Genetic background (17), as well as the number of pups suckled per litter (18), would influence the results. Losses in body weight of the pups due to urination were not considered in the estimation of milk intake. Milk intake data for the 21st day were not presented because solid food was found in the gut of pups at this age. Pups have been reported to eat solid food when they were 15 to 16 days old (19). However, intake of solid food in pups less than 21 days of age was not quantitated in the present study.

Obese pups from 7 to 14 days of age lost less weight than lean mice during 6 hours of fasting; an observation similar to that found in adult obese and lean mice (20). Adult obese mice excrete much less creatinine than lean mice, during the first 36 hours of fasting, suggesting that the obese mice may utilize less muscle mass than lean mice during the fast (21). Possibly obese mice rely to a greater extent on fat metabolism during a fast than do lean mice. However, the composition of the weight loss in the young pups during the fast is unknown.

At 21 days of age, obese mice contained about 130% more body fat, but were only 14% heavier than lean mice; an observation in agreement with a report by Chlouverakis et al. (3). Adipocyte cell size is increased in obese mice as early as 12 to 17 days of age (4). Similarly, the genetically obese rat (fa/fa) may have an increased body fat content as early as 14

days of age.[5] These observations suggest that significant differences in energy metabolism probably occur very early in the life of the obese mouse.

The time required for mice to adapt to a new diet immediately after weaning varied with the diet consumed and sex. Obese males just maintained their body weight immediately after weaning whereas both obese and lean females started to gain weight immediately after weaning, especially when the high-fat diet was fed. Mice were placed in wire bottomed cages in a room maintained at 22 to 24° when weaned. This may have stressed the obese males, which are known to adapt poorly to cold (22, 23), to a greater extent than the other mice. The critical temperature, which is defined as the temperature below which there is an increase in the metabolic rate, for young mice is 30 to 32° (24, 25).

In the present study, obese mice did consume more food than the lean mice during the 5 weeks feeding period. However, even when obese and lean mice were pair-fed from 24 days of age (26) or when the body weight of the adult obese mice was reduced to the level of the lean mice (27–30), obese mice still contained more fat than lean mice. These studies as well as those of others (31, 32) indicate that excess food intake can enhance the degree of the obesity, but may not be necessary for its development.

The obese mice, in agreement with earlier reports (reviewed in ref. 1), were much more efficient in retaining dietary energy than the lean mice when either one of the experimental diets was used. Comparisons between the stock diet and the high-fat diet have to be made with reservation because one was formulated with common feedstuff and the other with semipurified ingredients. It is possible that the observed differences due to diet might have been caused by factors in the diets other than the primary source of energy, i.e. carbohydrate versus fat. Nevertheless, the present study demonstrates that diet composition may influence energy efficiency to a greater extent in obese mice than in lean mice. The high-carbohydrate stock diet was selected because it was available in pelleted form, which greatly facilitated collection of food intake data in these young

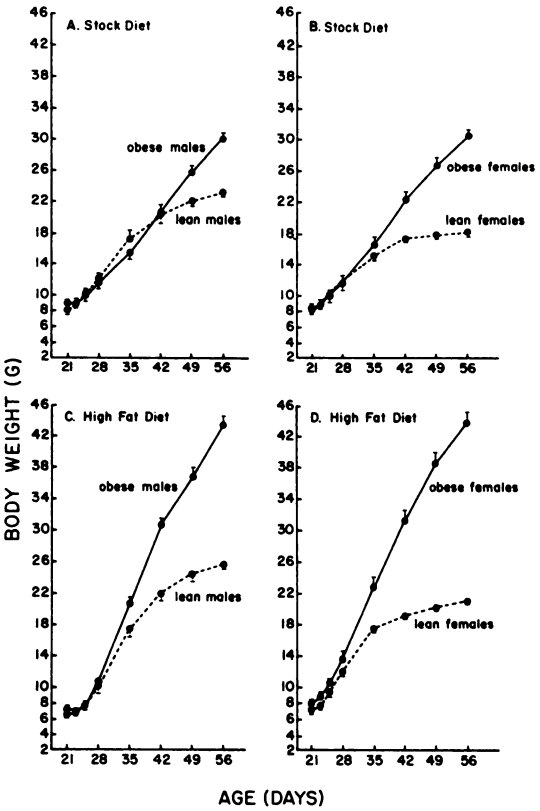

Fig. 3 Growth curves of mice after weaning. Each point is mean ± SEM of 8 mice per group.

mice. In other studies, a high-fat diet has also been shown to potentiate the development of obesity in rodents (34–37). The obese mouse has a lower oxygen consumption (38, 39), a lower body temperature (6), and lower locomotor activity (7, 40, 41) than lean mice. The involvement of these factors in the dietary-induced potentiation of obesity is not clear.

Obese animals also showed differences in their ability to retain dietary protein. While obese mice consumed more protein than lean mice, they retained less protein. Lean mice retained 35 to 45% their body energy as protein, while obese mice retained only 7 to 11%. These values are similar to those reported by others (29, 32). Direct estimates of muscle mass also indicate a difference in protein accumula-

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TABLE 6

Effect of diet on energy, fat and protein retention in mice from 21 to 56 days of age

Diet[1]	Male		Female		MS_E[3]	ANOVA[4]
	Obese	Lean	Obese	Lean		
			Energy retention (kcal)			
S	108[2]	35	118	28	188.4	P, D, P × D, P × S
HF	210	45	233	42		
			Energy efficient (%)[5]			
S	19.9	7.3	20.4	6.3	2.1	P, D, P × D, P × S
HF	30.4	8.4	31.7	8.0		
			Fat retention (g)			
S	11.9	2.5	13.5	2.6	3.2	P, D, S, P × D, P × S
HF	20.0	2.9	23.6	3.8		
			Protein retention (g)			
S	2.4	3.2	2.0	2.0	0.1	P, D, S, P × S
HF	3.0	3.4	2.5	2.2		
			Protein efficiency (%)[6]			
S	7.6	11.0	6.0	7.3	1.4	P, D, S, P × D, P × S, D × S
HF	11.5	17.5	9.2	11.6		

[1] S = stock diet; HF = high-fat diet. [2] Mean for eight mice per group. [3] MS_E = Mean Square Error. [4] Analysis of Variance. P, D, and S designate significant ($P \leq 0.05$) phenotype, diet and sex effects, respectively. [5] Energy retention in (kcal)/Energy intake in (kcal). [6] Protein retention in (g)/Protein intake in (g).

tion between obese and lean mice (2). The role of protein metabolism in the development of obesity in these mice remains to be elucidated.

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