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A double-blind trial of the effect of docosahexaenoic acid and vitamin and mineral supplementation on aggression, impulsivity, and stress

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Objectives Although a series of well-designed studies have reported that supplementation with vitamins/minerals and omega-3 fatty acids reduces the incidence of aggressive behavior, to date, the relative contribution and interaction between these nutrients has not been examined. The aim was therefore to consider the relative contribution of supplementation with multivitamins/minerals and/or docosahexaenoic acid (DHA) on laboratory-based measures of aggression, impulsivity, and stress.
Methods In a double-blind randomized trial, four groups of young adult men without a history of aggressive or impulsive behavior received a placebo ($n = 42$), multivitamins/minerals ($n = 43$), DHA ($n = 47$) or both ($n = 41$) for 3 months.
Results With the Picture-Frustration Task, DHA decreased the display of aggressive behavior. DHA also decreased impulsivity as measured using the GoStop Impulsivity Paradigm that examines the ability to inhibit already initiated behavior. Although a multivitamin and mineral supplement did not influence these measures, it did decrease perceived stress.
Conclusions The influence of supplementation on aggression and impulsivity can be conveniently studied in a sample without a history of antisocial behavior, using laboratory-based measures. No evidence was found of a synergistic interaction between vitamins/minerals and DHA. Copyright © 2013 John Wiley & Sons, Ltd.

KEY WORDS—aggression; DHA; fatty acids; impulsivity; minerals; vitamins

INTRODUCTION

There is a series of randomized, placebo-controlled double-blind studies that have reported, in those with a history of antisocial behavior, that supplementation with vitamins/minerals, *n*-3 fatty acids (*n*-3 FAs) or both reduces the incidence of aggressive behavior (Schoenthaler *et al*., 1997; Gesch *et al*., 2002; Zaalberg *et al*., 2010). To date, the topic has been largely examined under real-life condition, such as a prison, making the topic difficult and expensive to study. Up to now, measures of actual behavior have often proved to be sensitive to supplementation, although questionnaire measures have not. To facilitate work in this area, a major objective was to consider whether an influence of micronutrient and fatty acid supplementation could be demonstrated using laboratory-based measures of aggressive tendencies, in a sample without a history of antisocial behavior. If such measures respond to supplementation, then they will offer the means of conveniently examining the composition of supplements, allowing an optimal formulation to be established that can be examined subsequently in real-life situations.

Gesch *et al*. (2002) found that the disciplinary record of young male offenders improved by 26% following 13 vitamins/12 mineral and fatty acid supplementation [80 mg/day eicosapentaenoic acid (EPA) and 44 mg/day docosahexaenoic acid (DHA)]. The levels of fatty acid in this study were, however, much lower than those often used when examining the impact on behavior. Zaalberg *et al*. (2010), however, replicated the study using higher doses of fatty acids, 400 mg DHA and 400 mg/EPA, and found that aggression and rule-breaking decreased by 34%. However, a problem when studying fatty acids is in maintaining the blind, and although this was achieved in the Gesch *et al*. (2002) study, it was not by Zaalberg *et al*. (2010). Although a meta-analysis of eight studies found that the incidence of aggression was significantly less in those taking fatty acid supplements (Benton 2007), these may not be the only active ingredients. In imprisoned juveniles, Schoenthaler *et al*. (1997) reported that the incidence of violence was 28% less after consuming a 12 vitamin/11 mineral supplement (without fatty acids) rather than a placebo. Similarly, over a 4-month period,

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schoolchildren with a history of problem behavior, who took 12 vitamins/11 minerals, were disciplined significantly less frequently (Schoenthaler and Bier 2000). Thus, there is evidence that both vitamins/minerals and DHA, when given alone, reduce aggressive behavior. The possibility that there may be a synergistic interaction between vitamins/minerals and fatty acids has not been examined previously.

It may be important that a positive response to supplementation has tended to consider behavior in the real world rather than the laboratory. Both Gesch *et al*. (2002) and Zaalberg *et al*. (2010) used questionnaires of emotional control, anger, and aggression, but although supplementation changed actual behavior, questionnaire measures were not influenced. Research on this topic will be expensive, slow and difficult if it can only be carried out in institutions such as a prison. Therefore, a range of laboratory measures was examined with the aim of developing a paradigm that could be used in a normal population. Such a paradigm would greatly help to establish the active ingredients and appropriate doses before the topic is further studied in 'real-world' situations.

The Picture-Frustration Test (Rosenzweig, 1976), a semi-projective technique that has been used widely to monitor the aggressive responses, has been found to respond to fatty acid supplementation (Hamazaki *et al*., 1996, 1998, 2002). Rosenzweig and Adelman (1977) concluded that there was evidence of the tests validity on a range of dimensions, including actual behavior and changes in behavior after frustration had been induced. There are also reports that the response to this test reflects individual differences in physiology. Those with a tendency for blood glucose levels to fall to lower levels elicited more externally directed aggression (Benton *et al*., 1982; Donohoe and Benton, 1999).

In this area, an approach that has not been taken is to use laboratory-based measures of impulsivity that tap dimensions, not measured by traditional questionnaires, that have been reported to be associated with a range of antisocial and impulsive behaviors including attention deficit hyperactivity disorder, conduct disorder (CD), and aggressive behavior (Oosterlaan *et al*. 1998; Wodushek and Neumann, 2003; Bjork *et al*. 2000; Dougherty *et al*. 1999, 2003a; Mathias *et al*. 2002). As nutritional supplements have been found to influence measures of actual behavior, it seemed possible that this type of performance test might prove susceptible. It may be relevant that the relationships between questionnaire and behaviorally based measures of impulsivity have been reported to be nonsignificant (Krishnan-Sarin *et al*. 2007; Reynolds *et al*. 2004, 2008) or at the best limited (Lane *et al*., 2003).

Thus, the study aimed to see if, in those not chosen for their aggressive history, fatty acid and vitamin/mineral supplementation influenced laboratory-based measures of aggression and related behaviors.

METHODS

Participants

Two hundred and two male students were recruited, following a circular email, if they had not taken *n*-3 fatty acids or vitamin/mineral supplements during the previous 6 months and did not regularly consume seafood (defined as consuming seafood less than once a fortnight). They gave written informed consent and were paid £60 for taking part in the study that was approved by the local ethics committee. Their mean age was 20.9 years, and they were by self-report healthy, not taking medication for any ailment, and had no history of disorders associated with the liver or gastrointestinal tract. In the event, one hundred and seventy-three completed the trial, although the reasons for dropping out are unknown, as they failed to attend a second time when requested. As it was the examination period, other matters may have taken priority. It was also stated initially that blood samples would be taken to ensure adherence to the protocol, although in the event for logistic reasons, this did not occur. However, those who had not taken the supplements as they believed noncompliance would be discovered. Those who completed the trial were asked if they had experienced any side effects or whether they could guess the nature of their particular supplements.

Procedure

Baseline measures of aggression, impulsivity, cognition, and mood were assessed, although presently, only the aggression and impulsivity measures are reported. Randomly, and under a double-blind procedure, participants were allocated to one of four conditions: (i) vitamins/minerals and DHA, $n = 50$; (ii) DHA and placebo, $n = 51$; (iii) vitamins/minerals and placebo, $n = 51$; and (iv) placebo and placebo, $n = 50$. After consuming the supplements for 12 weeks, the test battery was again administered. The duration was chosen as in previous studies over this period, significant response was produced: for example, Zaalberg *et al*. (2010) gave supplements between 1 and 3 months. The randomization was computer generated, and until data collection had been finished, the blind was retained by an individual who never met the subjects.

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Supplements

Multivitamins/minerals. Each active tablet (Centrum Advance 50+ from Pfizer Inc, NY, USA) contained vitamins A (800 μg), B_1 (1.4 mg), B_2 (1.75 mg), B_6 (2 mg), B_{12} (2.5 μg), biotin (62.5 μg), folic acid (200 μg), niacin (20 mg), C (100 mg), D (5 μg), E (15 mg), K (30 μg), and pantothenic acid (7.5 mg). In addition, several minerals were administered: calcium (162 mg), phosphorus (125 mg), magnesium (100 mg), potassium (40 mg), chloride (36.3 mg). iron (5 mg), iodine (100 μg), copper (500 μg), manganese (2 mg), chromium (40 μg), molybdenum (50 μg), selenium (30 μg), zinc (5 mg) as well as lutein (500 μg). The doses tended to offer from 100% to 150% of the recommended daily intake, although with a lower percentage for some nutrients. The placebo was identical in color, size, and appearance and was made of the filler used in the active tablets. The particular formulation was chosen, as it offered a similar profile of micronutrients, in doses that were close to the supplements used in the Gesch *et al.* (2002) and Zaalberg *et al.* (2010) studies, although in addition, the presently used supplement contained vitamin K.

Docosahexaenoic acid. Each active capsule (Efalex from Efamol Ltd., Leatherhead, UK) weighed on average 506.5 mg and contained the following fatty acids: 14:0 myristic (0.1%, 0.5 mg), 16:0 palmitic (1%; 4.6 mg), 16:1 hexadecenoic (0.4%, 1.8 mg), 16:2 hexadecadienoic (0%, 0 mg), 17:0 heptadecanoic (0.2%; 1.0 mg), 16:3 hexadecatrienoic (0%, 0 mg), 18:0 stearic (3%, 13.1 mg), 18:1 oleic (5.4%, 23.4 mg), 18:0 *cis*-vaccenic (1%, 4.2 mg), 18:2 linoleic (0.8%, 3.4 mg), 18:3 γ-linolenic (0%, 0 mg), 18:3 α-linolenic (0.3%, 1.4 mg), 18:4 octadecatetraenoic (0.4%, 1.7 mg), 20:0 icosanoic (0.7%, 2.8 mg), 20:1 icosenoic (2.7%, 11.6 mg), 20:2 (*n*-9) icosadienoic (0.4%, 1.8 mg), 20:3 (*n*-9) icosatrienoic (0%, 0 mg), 20:3 (*n*-6) icosatrienoic (0.2%, 0.9 mg), 20:4 (*n*-6) arachidonic (2.2%, 9.3 mg), 20:3 (*n*-3) icosatrienoic (0.3%, 1.1 mg), 20:4 (*n*-3) icosatetraenoic (0.7%, 2.8 mg), 20:5 (*n*-3) icosapentaenoic (7.7%, 32.1 mg), 22:0 (*n*-3) docosanoic (0.4%, 1.7 mg), 22:1 (*n*-11) cetoleic (2.2%, 9.3 mg), 22:1 (*n*-9) erucic (0.5%, 2.1 mg), 22:4 (*n*-6) docosatetraenoic (0.5%, 2.0 mg), 22:5 (*n*-6) docosapentaenoic (4.1%, 16.9 mg), 22:5 (*n*-3) docosapentaenoic (2.9%, 11.9 mg), 24:0 tetracosanoic (0.4%, 1.5 mg), 22:6 (*n*-3) docosahexaenoic (54.9%, 224.2 mg), 24:1 tetracosenoic (1.7%, 6.8 mg), minor components (4.9%), alpha tocopheryl acetate (0.75 mg), and mixed tocopherols (1.5 mg).

Each placebo capsule weighed on average 496.7 mg and contained the following 14 fatty acids: 14:0 myristic (0.6%, 3.0 mg), 16:0 palmitic (28%; 130.7 mg), 16:1 hexadecenoic (0.2%, 0.7 mg), 17:0 heptadecanoic (0%; 0 mg), 18:0 stearic (3.7%, 16.7 mg), 18:1 oleic (41.2%, 187.5 mg), 18:1 (*n*-7) *cis*-vaccenic (1.2% 5.5 mg), 18:2 linoleic (21.2%, 96.5 mg), 18:3 α-linolenic (2.2%, 9.7 mg), 20:0 icosanoic (0.4%, 1.8 mg), 20:1 icosenoic (0.4%, 1.8 mg), 22:0 (*n*-3) docosanoic (0.3%, 1.1 mg), 22:1 docosenoic (0.1%, 0.5 mg), 24:0 tetracosanoic (0.1%, 0.5 mg), minor components (0.4%), alpha tocopheryl acetate (0.76 mg), and mixed tocopherols (1.5 mg).

Subjects were instructed to take three of these capsules each day for 12 weeks that provided 672 mg DHA/day. Thus, in total, all subjects consumed one micronutrient tablet and three fatty acid capsules a day. The final supplements were consumed the day before the final testing session, so it may be assumed that any response reflected chronic rather than acute consumption.

Picture-Frustration Task

The Picture-Frustration Test (Rosenzweig 1976) consists of cartoon pictures portraying two people in a frustrating situation. Each picture contained two speech bubbles. One was already filled with speech that was irritating, and the participant responded by writing in the second bubble the first thing that came to mind. The responses were placed into one of four categories by somebody blind to the supplements that had been consumed. (i) Extra-aggression: aggression directed towards another person; (ii) intra-aggression: self-directed guilt, fault or blame; (iii) inner-aggression: frustration or hostility that was not directed to anybody; and (iv) neutral: a nonaggressive response.

Buss–Perry Aggression Scale

The Buss–Perry Aggression Questionnaire (Buss and Perry 1992) assesses four aspects of aggressive behavior: physical aggression, verbal aggression, anger, and hostility. Participants rank statements about their temperament using a seven-point Likert scale ranging from 1 (extremely uncharacteristic of me) to 7 (extremely characteristic of me). Examples include "If I have to resort to violence to protect my rights, I will" (physical aggression); "I often get into arguments" (verbal aggression); "I flare up quickly but get over it quickly" (anger); and "I sometimes feel that I have gotten a raw deal out of life" (hostility). In addition, the scores were added to produce an overall score.

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Perceived Stress Scale

The Perceived Stress Scale (Cohen *et al*. 1983) assesses the extent to which stressful thoughts and feeling have been experienced during the last month. For example, "In the last month, how often have you been upset because of something that happened unexpectedly?" The participant responded on a scale ranging from 0 = never to 4 = very often. An overall score was calculated.

GoStop Impulsivity Paradigm

The GoStop Impulsivity Paradigm (Doughtery *et al*. 2003) measures the ability to inhibit an already initiated response. A five-digit number was presented on a computer screen for 500 ms followed by a 500-ms blackout. A second number then appeared for 500 ms followed by a 500-ms blackout. If the numbers were identical, the mouse button had to be pressed before the second number disappeared. However, the response was to be withheld if a "stop" signal appeared; that is, the second number was identical but changed from black to red. The stop signals were presented 50, 150, 250 or 350 ms after stimulus onset. Two blocks of trials were considered where in each block, there were 40 stop trials, 40 no-stop trials, and 80 novel trials where the two numbers were different, and no response was required. There were 10 stop trials for each of the four periods of delay.

TIME

The TIME paradigm investigates the perception of time as impulsive individuals perceive time to pass more slowly (Barratt and Patton 1983). A button started the test and was clicked again when a minute was estimated to have passed (Dougherty *et al*. 2003c). The mean of five trials was reported.

Single Key Impulsivity Paradigm (SKIP)

The SKIP assesses the tolerance of delayed reward (Dougherty *et al*. 2003d). The longer a subject waits, the higher the reward; that is, more points are earned. A mouse click began the task, and a second click resulted in a reward. Two counters displayed the most recent and cumulative reward over a 20-min session. Subjects were able to infer that responses at a faster rate earned smaller rewards. The output measures were the total number of responses that reflected the ability to wait for later and larger rewards, the longest delay between responses and the average delay between responses for the entire session.

Statistical analysis

The questionnaire measures and impulsivity measures were analyzed using a three-way analysis of variance: DHA/placebo × Vitamins and minerals/placebo × Before/after supplementation. In this analysis of variance the first two were between and the third within-subject factors. In addition, with the impulsivity measures, a secondary analysis examined the possibility that the response to supplementation might differ in those who at baseline displayed either high or low levels of impulsivity. That is, supplementation might selectively influence those who at baseline were more impulsive. With each measure, arbitrarily, a 50/50 median split was created to the extent that the distribution allowed this to occur. With the GoStop task, a median split could potentially be made with each of the four delays so it was decided to create the two groups using the 350-ms condition, as this is the most sensitive measure (Dougherty *et al*. 2003b). Thus, two groups were created: those with initially lower or higher levels of impulsivity. These data were analyzed using a four-way analysis of variance: DHA/placebo × Vitamins and minerals/placebo × Lower/higher baseline impulsivity as between-subjects factors and Before/after supplementation as a within-subject factor.

RESULTS

Importantly, the scores of those who subsequently consumed the four combinations of capsules did not differ significantly prior to supplementation on any measure of stress, aggression or impulsivity. The average weight of the four groups similarly did not differ significantly. Very few side effects were mentioned more than once, and those stated were of a minor nature. The average number of side effects was similar in the four groups: placebo 1.98 (0.02), vitamins/mineral 1.98 (0.02), DHA 1.19 (0.04), and both 1.95 (0.03). The rate of drop out was similar in all four groups; 42 of those taking both placebos finished the trial, 43 of those taking vitamins/minerals, 47 of those taking DHA and 41 of those taking both.

When asked at the end of the trial whether they believed they had been taking vitamins/minerals as opposed to a placebo, examination of these data using chi-square found that the pattern of response did not significantly differ depending on the combination of substances consumed. A total of 26.6% of those taking the placebo falsely guessed that they had been taking the active tablets, a figure that compared with 25% of those who actually were taking the active ingredients and who correctly guessed that they were. These data

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strongly suggested that the blind with vitamins and minerals was successful. In contrast, of those taking the DHA, 60% correctly guessed that they had been consuming the active capsule. In total, 53% of the sample was able to correctly guess that they had taken either the DHA or placebo, leaving 47% who were either uncertain or incorrect. Of those taking the placebo, 26.6% falsely believed that they had been taking DHA. When asked whether they experienced anything that lead them to believe they were taking active substances, rather than a placebo, a minority made any relevant response. There were few comments such as "sure I am taking omega-3 due to a fish tasting burp" or the "the fishy taste led me to believe I was taking omega-3." However, those taking a placebo on occasions believed they had consumed the active ingredients, making comments such as "mental alertness improved and have obtained higher exam grades than usual" or "more alert in the mornings and more energetic throughout the day". Conversely, some taking the active tablets reported negative side effects, for example, "sometimes lost concentration for long periods of time" or that he "didn't feel any different and therefore guessed that they were placebo."

Figure 1 reports that with the Picture-Frustration Test, there was a significant DHA × Before/after supplement interaction, $F(1, 169) = 4.01$, $p < 0.05$, with the extra-aggression scale; DHA consumption reduced aggressive responses. Similarly, there was a trend for intra-aggression to be lower after DHA, $F(1, 169) = 3.21$, $p < 0.07$. The effect size as indicated by partial eta-squared was 0.28 with extra-aggression and 0.30 with intra-aggression. The vitamin/mineral supplement failed to significantly influence these measures, although there was again a trend for scores to be lower after consumption when extra-aggression was examined, $F(1, 169) = 3.26$, $p < 0.07$; effect size 0.24. The interaction between these supplements was nonsignificant, $F(1, 169) = 1.25$, ns. With the measures of inner-aggression and neutral responses, on no occasion was a supplement influential.

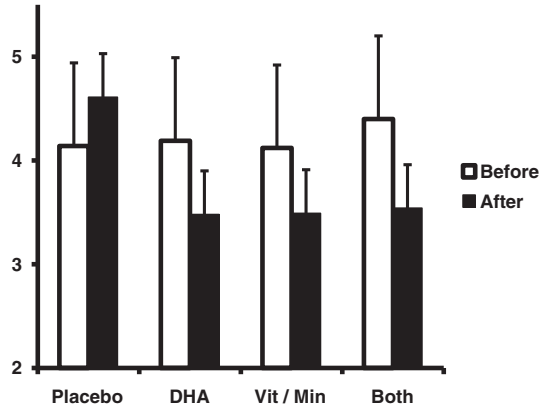

Figure 1. The influence of supplementation on extra-aggression. The data are mean instances of extra-aggression on the Picture-Frustration Test ± standard error of the mean before and after supplementation. Higher scores reflect the expression of more aggressive responses. DHA, docosahexaenoic acid

The possibility, that some individuals guessing that they had consumed DHA influenced the findings, was examined by considering the change in extra-aggression during the study using a two-way analysis of variance: DHA/placebo consumed × Whether at the end of the study you guessed you were taking DHA, placebo or did not know. The effect of which capsule you rightly or wrongly believed you had consumed did not significantly predict changes in extra-aggression, $F(2, 163) = 1.45$, ns. Similarly, the interaction between the type of capsule taken and the guess as to which had been consumed was nonsignificant, $F(2, 163) = 0.48$, ns. Thus, there was no reason to believe that the belief that you were taking DHA, rather than taking DHA as such, accounted for this finding.

When the Buss–Perry questionnaire was examined, the total score was not influenced by supplementation. The Vitamins/minerals × Before/after supplement was nonsignificant, $F(1, 167) = 0.80$, ns. The mean scores of those taking the placebo was 92.8 (2.66) before supplementation and 90.7 (2.87) afterwards. The comparable figures for those taking vitamins/minerals were 97.7 (2.73) and 94.8 (2.96). The influence of DHA was also nonsignificant, $F(1, 167) = 0.27$, ns. The mean score of those taking the placebo was 94.8 (2.71) before supplementation and 93.0 (2.94) afterwards, with the means for those taking DHA being 95.7 (2.67) and 92.5 (2.89). The interaction between supplements was again nonsignificant, $F(1, 167) = 1.70$, ns. With the three subscales, similarly on no occasion were scores influenced by supplementation. After supplementation, there was a correlation of 0.30 between the total score from the Buss–Perry questionnaire and the extra-aggression measure from the Picture-Frustration Test indicating that these tests have only 9% of variance in common.

The consideration of the Perceived Stress Scale resulted in a significant interaction between the consumption of DHA and vitamins/minerals, $F(1, 168) = 6.26$, $p < 0.02$. Post-hoc analysis revealed a significant difference between the placebo/placebo group and the vitamin and mineral/placebo group ($p < 0.007$; effect size 0.33). Stress scores increased with the placebo, as the study was carried out during the examination period, although they decreased when vitamins/minerals were consumed (Figure 2).

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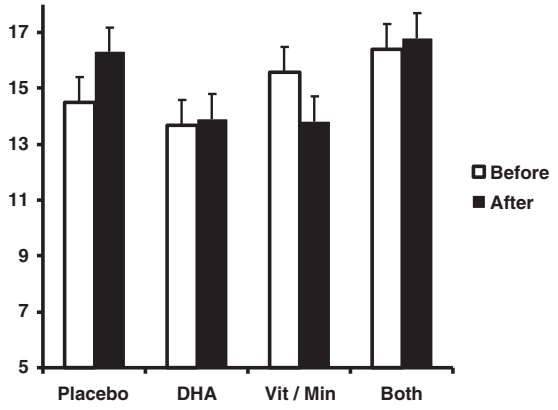

Figure 2. The influence of supplementation on perceived stress. The data are mean total scores from the Perceived Stress Scale over the study ± standard error of the mean. A positive score was associated with more stress, and a negative score with less stress. There was a significant difference between the placebo and vitamin/mineral groups ($p = 0.007$). DHA, docosahexaenoic acid

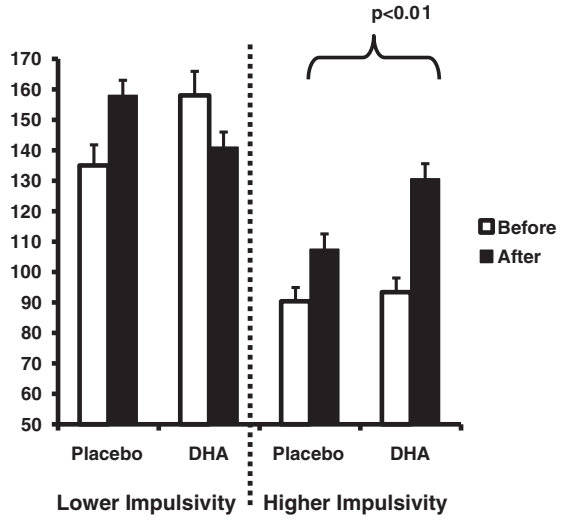

Figure 3. The influence of docosahexaenoic acid (DHA) on the inhibition of responding in the GoStop task in those with higher and lower baseline impulsivity. The data are the percentages of trials where a response was not made when they were to be inhibited, reported as a mean ± standard error of the mean. A higher score indicates lower impulsivity. The data distinguish those who at baseline were more or less able to inhibit responses. DHA selectively and significantly influenced the responses of those who initially displayed greater impulsivity. In this group at baseline, the performance was similar, but after taking DHA as opposed the placebo, significantly more responses were inhibited ($p < 0.01$)

When the various measures of the three impulsivity tests were analyzed, there was only on one occasion a significant finding, an interaction between DHA and vitamin/mineral consumption with the GoStop task, $F(1, 164) = 4.01$, $p < 0.05$. Post-hoc tests failed to find significant differences between any two groups. However, it seemed possible that the general failure to find an influence of supplementation may have reflected the use of a sample where the baseline level of impulsivity was low, making a beneficial response difficult to demonstrate. To consider this possibility, the baseline scores were divided into halves: those who had initially higher and lower levels of impulsivity. Supplementation was predicted to selectively benefit those with initially greater impulsivity.

Using this approach with the GoStop task taking DHA produced significant results. The percentage of occasions that inhibition was demonstrated markedly declined with increasing delay: it occurred with 83% of trials with 50 ms, 65% with 150 ms, 42% with 250 ms, and only 23% with 350 ms delays. Given that with the 50-ms delay many subjects responded at or very close to a maximum rate, these data were excluded from the analysis, as improvement was not possible. Therefore, the measure reported is the total percentage of inhibited responses on the stop trials, when the responses to each of the other three delays were added. When the percentage of inhibited responses was examined, there was a three-way interaction: DHA/placebo Initially higher/lower impulsivity × Before/after supplementation, $F(1, 164) = 7.46$, $p < 0.007$. Figure 3 reports that the performance of those who were more able to inhibit responses at baseline did not differ depending on whether they subsequently consumed DHA or placebo. In contrast, those who were more impulsive at baseline, that is, they were less able to inhibit their responses, produced a DHA/placebo Before/after supplement interaction, $F(1,94) = 4.39$, $p < 0.04$. Initially, responses did not differ depending on the supplement they subsequently consumed. However, after supplementation, the percentage of responses that were inhibited was significantly greater if they had consumed DHA rather than a placebo ($p < 0.01$). The Vitamin and mineral/placebo × Before/after supplement interaction failed to achieve statistical significance, $F(1, 94) = 0.63$, ns. The Vitamin/mineral DHA × Before/after supplementation was also non-significant, $F(1, 94) = 1.18$, ns.

The possibility that the partial breaking of the blind may have influenced the findings was considered in those who at baseline were more impulsive. A two-way analysis of variance DHA/placebo × Whether you guessed that you were taking DHA, the placebo or did not know was considered. Neither the nature of what you thought you were consuming [$F(2,78) = 0.12$. ns] nor the interaction with whether DHA or the placebo was actually being taken [$F(1,78) = 0.10$, ns] achieved statistical significance.

The results with the TIME and SKIP paradigms are presented in Table 1. Neither the higher nor lower impulsivity groups were affected by supplementation

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Table 1. Changes in impulsivity depending on supplementation in those with greater or lesser impulsivity at baseline

	Placebo		Vitamins/minerals		Placebo		DHA		
	Before	After	Before	After	Before	After	Before	After	
TIME estimation	63.87	61.05	63.4	61.62	63.71	61.35	63.56	61.32	DHA × Group x Time (1, 164) = 0.83, ns
(Less impulsive)	(0.53)	(0.48)	(0.53)	(0.47)	(0.52)	(0.47)	(0.54)	(0.48)	Vit × Group × Time (1, 164) = 0.89, ns
TIME estimation	57.17	60.01	57.48	59.86	57.31	59.13	57.35	60.73	Four-way interaction (1, 164) = 0.0, ns
(More impulsive)	(0.79)	(0.71)	(0.86)	(0.76)	(0.86)	(0.77)	(0.79)	(0.71)	
TIME view time	11.97	9.06	10.92	8.51	11.94	9.49	10.94	9.48	DHA × Group × Time (1, 164) = 0.00, ns
(Less impulsive)	(0.52)	(0.66)	(0.47)	(0.59)	(0.48)	(0.61)	(0.51)	(0.61)	Vit × Group × Time (1, 164) = 0.20, ns
View time	4.98	5.66	5.16	5.73	4.87	5.70	5.27	5.69	Four-way interaction (1, 164) = 0.63, ns
(More impulsive)	(0.44)	(0.56)	(0.53)	(0.76)	(0.51)	(0.65)	(0.46)	(0.58)	
SKIP total responses	(48.15)	(47.70)	11.26	65.95	11.14	60.61	11.79	52.62	DHA × Group × Time (1, 164) = 0.01, ns
(Less impulsive)	(48.15)	(47.70)	(49.73)	(49.27)	(48.15)	(47.70)	(49.73)	(49.27)	Vit × Group × Time (1, 164) = 0.01, ns
SKIP total responses	392.58	247.40	300.52	165.83	390.82	250.39	302.28	162.83	Four-way interaction (1, 164) = 0.11, ns
(More impulsive)	(49.81)	(49.35)	(49.78)	(49.32)	(51.21)	(50.74)	(48.34)	(47.89)	
SKIP IRT	7.34	89.04	7.97	153.43	6.67	78.33	8.63	164.14	DHA × Group × Time (1, 163) = 0.10, ns
(Less impulsive)	(29.40)	(43.92)	(27.15)	(40.55)	(28.26)	(42.22)	(28.33)	(42.32)	Vit × Group × Time (1, 163) = 1.03, ns
SKIP IRT mean	181.98	206.26	220.01	208.25	225.98	205.91	176.01	208.61	Four-way interaction (1, 163) = 0.02, ns
(More impulsive)	(27.09)	(40.48)	(30.54)	(45.63)	(29.22)	(43.65)	(28.52)	(42.60)	
SKIP longest delay	593.16	544.61	619.47	731.88	631.10	650.36	581.53	626.12	DHA × Group × Time (1, 164) = 1.39, ns
(Less impulsive)	(30.63)	(47.25)	(37.05)	(57.14)	(32.69)	(50.43)	(35.24)	(54.35)	Vit × Group × Time (1, 164) = 4.56, $p < 0.03$
SKIP longest delay	113.54	432.05	105.83	346.07	96.20	297.21	123.16	480.91	Four-way interaction (1, 164) = 1.12, ns
(More impulsive)	(36.56)	(56.39)	(31.20)	(48.13)	(35.94)	(55.43)	(31.92)	(49.23)	

DHA, docosahexaenoic acid; SKIP, Single Key Impulsivity; ns, not significant; IRT, Inter-response time.
The first two columns report the main effect of the influence of vitamin/mineral supplementation and the third and fourth columns the main effect for DHA. Data for those who at baseline displayed greater or lesser impulsivity are reported separately. The *F* values for both the main effects and the four-way interaction are reported in the right-hand column.

on the TIME paradigm measure. The only SKIP measure that was significantly affected by supplementation was the longest delay. There was a significant Vitamins and minerals/placebo × Initial higher/lower impulsivity interaction [$F(1, 164) = 4.56$, $p < 0.03$], although examination of the means failed to find significant differences between groups, and they were not further examined, as it appeared likely that it was a chance finding.

DISCUSSION

Although supplementation did not significantly affect any of the Buss–Perry scales, this is a self-report measure, and in this area, self-report measures poorly predict real-life aggressive behavior (Gesch *et al*. 2002, Zaalberg *et al*. 2010). However, the taking of DHA resulted in a significantly lower incidence of extra-aggression in the Picture-Frustration Test, and there was also a trend for intra-aggression to decrease (Figure 1). Although a paper-and-pencil test, the Picture-Frustration Test asks for samples of behavior and thus is different in nature from the Buss–Perry test that measures an enduring 'trait' that can be expected to be less susceptible to daily changes. The finding that DHA decreased a measure of aggressive behavior (Figure 1) is consistent with other findings using the same test (Hamazaki *et al*., 1996, 1998, 2002). There is also epidemiological evidence of an association between lower hostility and greater DHA and total fish consumption (Iribarren *et al*. 2004), perhaps reflecting the importance of the *n*-6 : *n*-3 ratio as *n*-6 levels have been found to be higher in violent individuals (Virkkunen *et al*. 1987). As it has been proposed that *n*-3 FA prevents aggression only in the presence of a stressor (Hamazaki *et al*. 2002; Hallahan *et al*. 2007), we can speculate that it may be relevant in the present study that subjects were tested at the time of stressful end-of-year examinations.

The creation of a placebo is a consistent problem for any study of DHA, as the smell, or fishy flavored eructation, can suggest that you are taking the active capsule. For example, at the beginning of the Zaalberg *et al*. (2010) study, 49% guessed they were taking the active variant, and by the end, the figure had risen to 75%. These figures compare with the present study where at the end, 60% guessed correctly. There are, however, several reasons to suggest that the extent that the blind for DHA was partially broken did not impact on the present findings. There is no reason to suspect widespread and consistent expectations about the association between DHA and aggression or an unfamiliar measure of impulsivity. The responses of those who did or did not correctly believe they took a particular capsule did not differ statistically. Finally, the Gesch *et al*. (2002)

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study, where the blind appeared to be entirely maintained, reported similar findings to Zaalberg *et al*. (2010) where the blind was partially broken.

The use of DHA offers only one possible approach, as EPA has also been suggested to reduce antisocial and aggressive behaviors (Zannarini and Frankenburg, 2003). The relative amounts of *n*-3 and *n*-6 FA need to be considered as both Gesch *et al*. (2002) and Zaalberg *et al*. (2010) included a small source of *n*-6 FA in their supplements. The dose of DHA in addition needs to be examined as Gesch *et al*. (2002) gave 44 mg, Zaalberg *et al*. (2010) 400 mg and the present study 672 mg/day.

One explanation for the previous positive findings with vitamin/minerals supplementation (Schoenthaler *et al*. 1997; Schoenthaler and Bier 2000) is that they reflected the use of participants who at baseline were deficient in micronutrients. A previous study of a similar sample to the one presently reported used biochemical methods to establish vitamin status (Benton *et al*. 1997). Ascorbic acid, cyanocobalamin, alpha-tocopherol, and folic acid retinol levels tended to be adequate, although with riboflavin and pyridoxine the status of a substantial minority was either borderline or deficient. The thiamin and biotin status of a minority was also marginal. Therefore it is possible that the vitamin status of the present sample was not universally good, although in the absence of an assessment of dietary status, such matters can only be the subject of speculation. Clearly, future studies would benefit from the reporting of biochemical measures of micronutrient status. Although the present study failed to find a significant effect of micronutrients, with extra-aggression, it approached significance ($p = 0.07$). It is thus possible that the present study was under-powered in this respect.

The possibility of an inadequate intake of micronutrients was, however, supported by the finding that perceived stress (Figure 2) declined in those taking vitamin and mineral supplementation, whereas it increased when taking a placebo. The finding that multivitamin and minerals supplementation decreased perceived stress appears to be a robust phenomenon, as it replicates several previous studies. After 28 days of supplementation, significant reductions in anxiety and perceived stress were reported (Carroll *et al*. 2000). Similarly, a B-vitamin complex/mineral supplement decreased ratings of stress (Kennedy *et al*., 2010), and when measured in a work situation, a vitamin B complex supplement was found to decrease reports of 'personal strain' (Stough *et al*. 2011). In healthy older men, a multivitamin/mineral supplement reduced scores on a stress scale (Harris *et al*. 2011). A recent meta-analysis of supplementation with multivitamins/minerals reported that they significantly reduced perceived stress (standardized mean difference = 0.345, 95% CI: −0.467, −0.224, $p = 0.001$; Long & Benton, 2013).

A major question posed was whether there was evidence of a synergistic interaction between DHA and vitamins/mineral supplementation. There was, however, no evidence of either an additive or synergistic interaction with either questionnaire measures of aggression or measures of impulsive behavior. The stress measure did, however, produce an interaction between the different types of supplement (Figure 2), although if anything a combination of DHA and vitamins/minerals had less ability to reduce stress than the latter supplement by itself. The origin of this interaction is uncertain, and clearly, there is much to be established about the interaction between food supplements. One approach would be to consider pharmacokinetics and examine the influence of the interaction between both types of supplement on absorption, distribution, metabolism, and excretion.

The possibility that performance measures of impulsivity might prove particularly sensitive to dietary manipulations was considered. Yet impulsivity is a multifaceted construct with a range of different measures that consider largely independent dimensions (Dougherty *et al*. 2005): the ability to inhibit a response once it has been initiated, the ability to tolerate a delay to have a greater reward and the ability to judge the speed at which time is passing. Given that the present sample had no history of impulsive behavior, it is perhaps not surprising that initial analyses found no impulsivity measure to be significantly affected. However, in those initially more impulsive, GoStop performance benefited from DHA (Figure 3). It is relevant that the GoStop test has previously been associated with clinical disorders with elements of impulsivity including CD and oppositional defiant disorder (Bjork *et al*., 2000). Adolescents with disruptive behavior disorder have been found to perform the GoStop and SKIP tasks poorly, although the GoStop measure was the more sensitive (Dougherty *et al*. 2003a). Response inhibition, as measured with the GoStop task, was found to be impaired in children with attention deficit hyperactivity disorder and CD (Oosterlaan *et al*. 1998). Women with a history of childhood aggression performed the GoStop task less well (Mathias *et al*. 2002).

A laboratory-based paradigm that allows a consideration of the composition of supplements and the doses of nutrients will allow this area to more rapidly progress. The present study found the Picture-Frustration Test (Figure 1) and the Go Stop measure, at least in those selected for having higher initial levels of impulsivity (Figure 3), responded to supplementation. As

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such, these measures could be used to systematically explore the characteristics of supplements, although there will still be a need to consider whether similar responses occur under real-life conditions.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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