Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 163–171

Journal of Nutritional Biochemistry

The effect of fish oil on physical aggression in schoolchildren — a randomized, double-blind, placebo-controlled trial

Miho Itomura[a], Kei Hamazaki[a], Shigeki Sawazaki[b], Makoto Kobayashi[c], Katsutoshi Terasawa[d], Shiro Watanabe[a], Tomohito Hamazaki[a,*]

[a]*Division of Clinical Application, Department of Clinical Sciences, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan 9300194*

[b]*First Department of Internal Medicine, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan 9300194*

[c]*Faculty of Pedagogy, Toyama University, Toyama, Japan 9308555*

[d]*University Hospital, Toyama Medical and Pharmaceutical University, Toyama, Japan 9300194*

Received 28 December 2003; received in revised 10 August 2004; accepted 15 October 2004

Abstract

Objectives: The aim of the study was to investigate whether fish oil supplementation affected Japanese schoolchildren's behavior, with changes in aggression over time as the primary endpoint.

Design and subjects: A placebo-controlled double-blind study with 166 schoolchildren 9–12 years of age was performed. The subjects of the fish oil group ($n=83$) took fish oil-fortified foods (bread, sausage and spaghetti). These foods were provided in amounts such that each subject in the fish oil group had an intake of 3600 mg of docosahexaenoic acid + 840 mg of eicosapentaenoic acid (EPA)/week for 3 months. The rest (the controls, $n=83$) took control supplements. At the start and end of the study, psychological tests were performed to assess their aggression.

Results: Physical aggression assessed by Hostility-Aggression Questionnaire for Children in girls increased significantly (median: 13 to 15, $n=42$) in the control group and did not change (13 to 13, $n=43$) in the fish oil group with a significant intergroup difference ($P=.008$) with baseline as covariate. The changes in physical aggression scores over time and those of the ratio of EPA/arachidonic acid in RBC (ΔEPA/AA) were significantly correlated in girls who agreed to blood collection ($r=-.53$, $P=.01$, $n=23$). On the contrary, there were no significant changes in physical aggression in boys. Aggression against others (extraggression) assessed by Picture Frustration Study did not change in the control group (median: 5 to 5) but increased significantly in the fish oil group (4 to 5) with a significant intergroup difference ($P=.02$) with baseline as covariate. These changes in extraggression might be explained partly by significantly lower baseline values of extraggression in the fish oil group ($P=.02$) than in the control group. There were no significant correlations between Δextraggression and ΔEPA/AA in blood-sampled children ($n=49$). Impulsivity of girls assessed by parents/guardians using the diagnostic criteria for attention deficit/hyperactivity disorder of *DSM-IV* was reduced in the fish oil group (1 to 0) with a significant ($P=.008$) intergroup difference from the control group (1 to 1). There were no significant correlations between Δimpulsivity and ΔEPA/AA in blood-sampled girls. In males, impulsivity reduced in both groups without any intergroup differences.

Conclusion: There is a possibility that changes in fatty acid nutrition might affect physical aggression especially in girls.

Keywords: DHA; EPA; Fatty acid composition; Hostility; Aggression; Impulsivity; Schoolchildren

1. Introduction

Docosahexaenoic acid (DHA) of n-3 series is an important component of cell membranes of the retina and brains. Stevens et al. [1,2] showed that a number of behavior problems in boys such as temper tantrums and sleep problems were reported more often in subjects with lower total n-3 fatty acid concentrations in the plasma phospholipid fraction. Stordy [3] reported that dark adaptation improved in five dyslexic patients after supplementation with DHA-rich fish oil for 1 month. However, with regard to the symptoms related to attention deficit/hyperactivity disorder (AD/HD), a 4-month period of DHA supplementation (345 mg/day), which increased plasma phospholipid DHA concentrations 2.6-fold compared with

* Corresponding author. Tel.: +81 76 434 7615; fax: +81 76 434 5057.
E-mail address: hamazaki@ms.toyama-mpu.ac.jp (T. Hamazaki).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2004.10.009

the placebo group, did not improve any objective or subjective measure of AD/HD symptoms in a randomized, double-blind, placebo-controlled trial [4].

We have been investigating the effects of DHA-rich fish oil on behavior. In our first randomized, placebo-controlled, double-blind study to observe the effect of DHA on aggression [5], young students took either 1.5–1.8 g of DHA or placebo oil for 3 months. At the start and the end of the study, their aggression against others (extraggression) was assessed by a psychological test, Picture Frustration Study (PF Study) [6]. In the DHA group, extraggression did not change between the start and the end of the study, whereas it significantly increased in the control group because of the presence of stressor (final exams) at the end of the study. In another placebo-controlled, double-blind study, 40 Thai subjects aged 50–60 years were recruited from a university and nearby villages. They took 1.5 g DHA/day or placebo oil for 2 months. Extraggression was assessed by a PF Study revised for Thai people at the start and the end of the study. Just prior to that PF Study at the end of the study, subjects were asked to watch a stressful videotape as a stressor component. In the group of university employees, extraggression did not change over time with placebo, whereas extraggression significantly decreased in the DHA group. The DHA administration favorably controlled extraggression in at least elderly white-collar workers [7].

As shown above, DHA-rich fish oil affects human behavior. However, such data were not available for normal schoolchildren. We, therefore, asked Japanese schoolchildren who were 9–12 years old to participate in the present study to investigate whether fish oil supplementation affected their behavior, especially aggression.

2. Materials and methods

2.1. Subjects

Two hundred thirty schoolchildren whose parents/guardians responded to our advertisements recruiting study volunteers for the present study were invited to meeting places of four geographical regions: Inuyama, Fukuyama, Kanazawa and Seki cities. A total of 50 children were not able to visit us for an interview and psychological testing. The rest, 180 schoolchildren, were in the fourth to sixth grades of elementary schools. One of them was excluded because she was found to be allergic to flour products. Then, the available children were 88 boys and 91 girls 9–12 years of age. They were stratified according to areas, gender, age and body mass index and randomly divided into either a fish oil group (89 subjects) or a control group (90 subjects) in a double-blind manner (Fig. 1). The ethics committee of Toyama Medical and Pharmaceutical University approved the present study. Written informed consent was obtained from each subject's parent/guardian after full verbal and written explanation in front of both subjects and their parents/guardians. We did not obtain written informed consent from children because we asked parents/guardians to give informed consent only if their children agreed.

2.2. Study design

All subjects were asked to maintain their physical activity level and consume their habitual diets during the study period of 3 months. They were also asked to eat bread rolls, steamed bread, sausages and spaghetti containing either DHA-rich fish oil or control oil. Two rolls of bread (300 mg DHA each), two pieces of steamed bread (600 mg DHA each), and three sausages (600 mg DHA each) per week were taken. Sometimes a serving of spaghetti, which contained 300 mg DHA, was eaten as a Saturday or Sunday brunch. These foods were provided in amounts such that each subject in the fish oil group had an intake of 3600 mg of DHA [and 840 mg of eicosapentaenoic acid (EPA)] per week. The fatty acid contents of provided foods are shown in Table 1. Control foods contained 50% soybean and 50% rapeseed oil instead of fish oil. Subjects in the control group took about 4200 mg more linoleic acid per week from the test foods than those in the fish oil group (Table 1).

Subjects were asked to come to their regional meeting places on the first day and the last of the study. Psychological tests, physical tests, other questionnaires than psychological tests (see below) and blood sampling, if their parents/guardians agreed, were performed and compared. In order to test the blind of their foods, parents/guardians were asked at the end of the study to choose one answer among the following three: fish oil-fortified food, "hard to tell" or control food.

2.3. Psychological tests

The Hostility-Aggression Questionnaire for Children (HAQ-C) was performed. This questionnaire was a Japanese version [8] of the Buss-Perry Aggression Questionnaire [9]. This questionnaire contains 27 multiple-choice questions [four choices were provided: not at all (point = 1), somewhat (2), moderately so (3) and very much (4)], and each question belongs to one of the following four categories: physical aggression, verbal aggression, anger and hostility. Here are example questions for each category: physical aggression ("If somebody hits me, I hit back."), verbal aggression ("I can't help getting into arguments when people disagree with me."), anger ("Some of my friends think I'm a hothead.") and hostility ("At times I feel I have gotten a raw deal out of life."). The reliability and validity of the questionnaire were well documented [10].

Another psychological test (PF Study) was also performed. This test was originally developed by Rosenzweig and Rosenzweig [6]. We used a Japanese version [11]. In this psychological test, subjects were asked to look at 24 pictures illustrating a frustrating situation and to write their first reactions (feelings) with a couple of sentences. Their reactions were regarded as an aggression, and aggression against others was diagnosed as extraggression. Thirteen

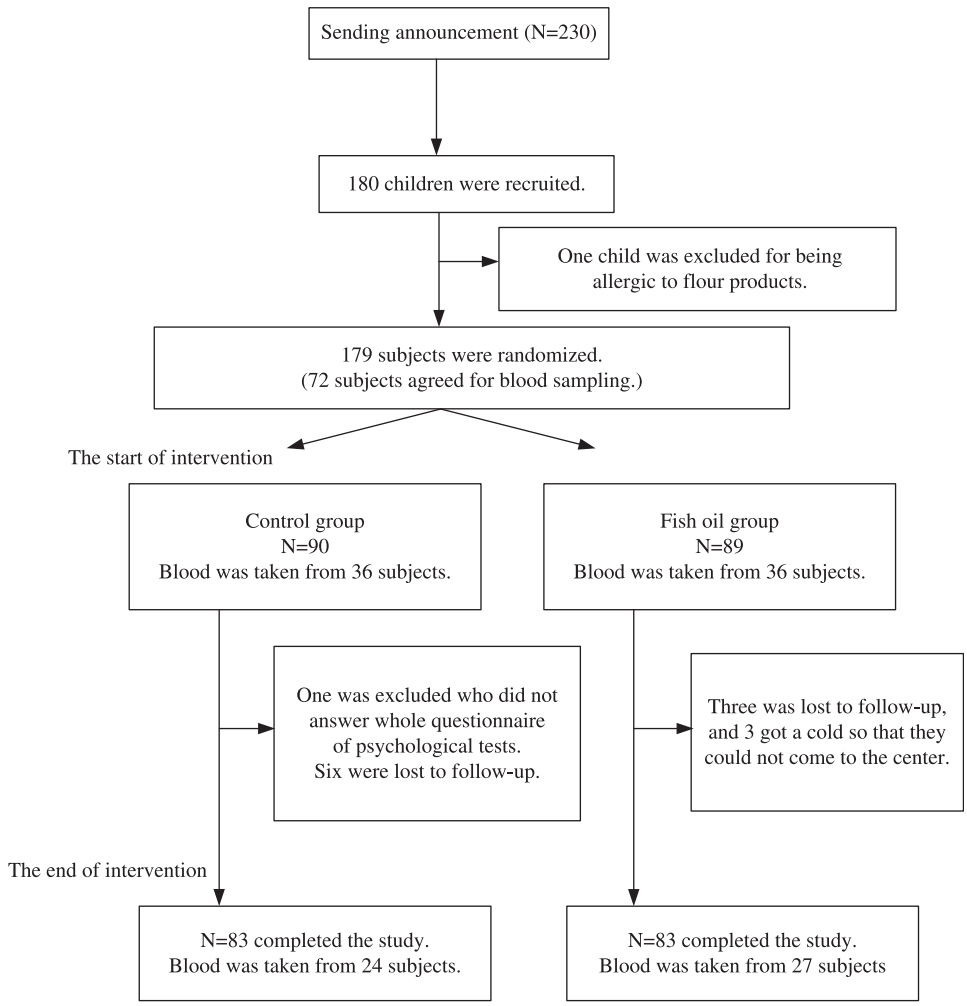

Fig. 1. Trial profile.

pictures were already out of date, and those pictures were replaced with modern pictures created by one of the present authors (SS). PF Study was assessed in a blind manner by one (MI) of the present authors who had enough experience in PF Study assessment. The reliability and validity of this test was also documented [11].

Table 1
Fatty acid contents of supplements per piece

	Total weight (g)	Total oil (g)	DHA (mg)	EPA (mg)	Linoleic acid (mg)	Linoleic acid of control foods (mg)
Bread roll	45	5.6	300	70	520	860
Steamed bread	50	10	600	140	800	1480
Sausage	50	4	600	140	270	990
Spaghetti	100	5	300	70	800	1140
Total/week (estimates)		43	3600	840	3600	7800

All subjects were asked to take 3600 mg DHA per week for 3 months from the foods listed above on the assumption that even the foods provided to the control group had DHA as shown above, since the present study was a double-blind study. The fatty acid data were calculated from the fatty acid composition of the ingredients of each food. The control foods contained essentially neither EPA nor DHA except that a control sausage had 40 mg of EPA and 80 mg of DHA.

Attention deficit, hyperactivity and impulsivity were assessed using the diagnostic criteria for AD/HD disorder of *DSM-IV*. Here are example questions for each category: attention deficit ("often fails to give close attention to details or makes careless mistakes in schoolwork, work or other activities"), hyperactivity ("often fidgets with hands or feet or squirms in seat") and impulsivity ("often blurts out answers before questions have been completed"). Rating was performed by parents/guardians of the subjects, assigning point 0 for no, 1 for "hard to tell" and 2 for yes.

2.4. Lipids analysis of red blood cells and plasma fatty acid composition

RBCs were washed three times with saline and were kept at −80°C until fatty acid analysis. The total lipids of RBC (100 μl) were extracted according to Bligh and Dyer [12]. The total phospholipid fraction separated by thin-

Table 2
Baseline characteristics of the randomized subjects

	Group	
	Control ($n=83$)	Fish oil ($n=83$)
No. of subjects		
Male/Female	41/42	40/43
Age	10.4±0.9	10.3±0.9
Body mass index	17.7±2.5	17.6±2.7

There were no significant differences between the two groups.

layer chromatography was transmethylated and analyzed with a Shimadzu gas-liquid chromatograph GC-14A, equipped with a DB225 capillary column (J&W Scientific Folsom, CA).

2.5. Food analysis and other questionnaires

Food intake was estimated with Eiyokun ver 3.0 (Kenpakusha) using a food frequency questionnaire completed at the start and the end of the study. Each subject's family counted unconsumed test foods every week and reported the consumption rate at the end of the study. Subjects' parents/guardians were also asked to guess their randomization status at the end of the study.

2.6. Statistical analysis

The primary endpoint of the present study was changes in aggression measured with HAQ-C and PF Study. Paired blood samples (at both the start and end of the study) were available only from 51 subjects from two areas (Fukuyama and Kanazawa). Two pairs of them, one in the fish oil group and the other in the control group, were deleted from the statistical calculation of fatty acid composition because the percentage of arachidonic acid (AA) and the sum of the polyunsaturated fatty acids in those two subjects were extremely low at the start of the study (Grubbs-Smirnov test for outlier). The data of those two subjects other than fatty acids were included for calculation. As for baseline characteristics of the randomized subjects and fatty acid composition, data are expressed as means±S.D., and the parametric methods were used. Intergroup comparison was done with ANCOVA using baseline as covariate. The unpaired t test was used for the comparison of baseline and food analysis between the two groups. The paired t test was used for detection of intragroup changes. As for the scores of HAQ-C, PF Study and *DSM-IV* questionnaires, many of the data were not normally distributed. Consequently, data are expressed as medians (25th percentile–75th percentile), and intergroup comparisons of baseline data were treated nonparametrically (Mann–Whitney U test). However, because changes in those parameters over time were nearly normally distributed, two-way ANOVA was applied to those changes with the treatment groups and gender as factors. These procedures enabled us to calculate the effect of gender. If ANOVA showed that gender was a significant factor, both male and female data were also shown separately. Wilcoxon matched-pairs sign-rank test was used for intragroup comparison. Correlations between changes of scores of psychological tests over time and those of fatty acid composition were analyzed by a least-square method. Subjects' guess about their randomization was analyzed by a chi-square test. Statview (Japanese version 5; SAS Institute, CA) was used for statistical analysis. $P<.05$ was considered as significant.

3. Results

3.1. Analyzed subjects and fatty acid composition

One hundred sixty-six subjects (81 boys and 85 girls) completed the study. Their baseline characteristics are shown in Table 2. Table 3 shows the changes over time in the fatty acid composition of the total phospholipid fraction of RBC of 49 subjects. Eicosapentaenoic acid was significantly increased in the fish oil group. Although

Table 3
The fatty acid composition (area%) of RBC phospholipids

Fatty acid	Control ($n=23$)			Fish oil ($n=26$)				Intergroup comparison
	Baseline	End		Baseline		End		
16 : 0	21.8±1.3	21.8±2.2		21.1±1.1	f	22.6±2.0	d	.03
18 : 0	15.3±0.8	15.6±0.6		15.6±1.3		15.8±1.2		NS
18 : 1 n-9	12.5±0.7	12.8±0.8	b	12.2±0.7		13.0±1.8	b	NS
18 : 2 n-6	8.7±1.1	9.5±1.0	c	9.1±0.9		9.0±1.2		.006
20 : 4 n-6 (AA)	11.9±1.0	12.6±1.3	a	12.0±1.4		11.8±1.1		.08
20 : 5 n-3 (EPA)	1.0±0.3	1.2±0.4	c	0.9±0.2	e	1.4±0.3	d	.0009
22 : 0	1.5±0.3	1.3±0.2	b	1.5±0.2		1.3±0.2	b	NS
22 : 5 n-3	1.7±0.2	1.8±0.3	a	1.7±0.2		1.6±0.2	b	.004
22 : 6 n-3 (DHA)	6.4±0.9	6.6±0.9		6.1±0.9		7.1±0.8	d	.06
24 : 0	3.6±0.8	3.1±0.6	c	3.7±0.6		3.2±0.6	c	NS
EPA/AA	0.086±0.03	0.098±0.026	b	0.074±0.02	e	0.117±0.033	d	.0001
n-6/n-3	2.7±0.4	2.7±0.4		2.9±0.4		2.5±0.4	d	.0004

Subjects in the DHA group took 3600 mg DHA/week for 3 months. AA, arachidonic acid.
Intragroup comparison: a, $P<.1$; b, $P<.05$; c, $P<.01$; d, $P<.001$. Intergroup comparison at baseline: e, $P<.1$; f, $P<.05$. NS, not significant.

Table 4
Scores of HAQ-C

HAQ-C	Control (n=41/42)		Intragroup comparison	Fish oil (n=40/43)		Intragroup comparison	Gender-adjusted intergroup comparison	Effect of gender
	Baseline	End		Baseline	End			
Verbal aggression								
Total	13 (11–15)	12 (11–15)		13 (11–14)	13 (11–14)		NS	NS
Physical aggression								
Total	14 (12–16.8)	15 (13–18)		14 (12–16)	13 (12–16.8)		.04	.0009
Male	15 (13–17)	14 (13–18)		15 (12.7–18.3)	14.5 (12–17)		NS	
Female	13 (10.3–15)	15 (13–18)	.0004	13 (11–15.5)	13 (11–16)		.008	
Anger								
Total	11 (9–14)	12 (10–14)		11 (9–13)	11 (9.25–13.9)		NS	NS
Hostility								
Total	12 (10–14.8)	13 (11–15)	.02	13 (10–15)	13 (12–16)		NS	NS

Values are shown as median (25th percentile–75th percentile). n=males/females. NS, not significant.

DHA increment was significant within the fish oil group, the difference was only marginally significant between the two groups (P=.06). The biggest changes in Table 3 were EPA/arachidonic acid (EPA/AA) in the fish oil group. Therefore, in the subsequent correlation analyses, we used changes of EPA/AA ratios in the total phospholipid fraction of RBC (ΔEPA/AA). Linoleic acid was significantly increased in the control group with a significant intergroup difference. There might be a possibility that we obtained blood samples only from subjects with high compliance. However, this did not seem the case because compliance of test foods was very similar for both the blood-sampled children and others (both were more than 90%).

The daily intake of DHA from habitual food was essentially the same among the four areas, and the averaged daily intake of DHA from habitual food was 410±280 mg in the control group and 440±320 mg in the fish oil group (not significantly different). The intakes of total energy and macronutrients were not significantly different between the two groups (data not shown).

3.2. Psychological tests

Because the effect of gender on physical aggression assessed by HAQ-C was significant (P=.0009, Table 4), it is shown separately. It increased significantly in the female control group (P=.0004) and did not change in the female fish oil group with a significant intergroup difference (P=.008, Fig. 2). However, there were no significant changes over time in boys (Table 4). The changes in physical aggression scores over time (Δphysical aggression) and ΔEPA/AA were significantly correlated in the girls whose blood samples were collected (Fig. 3).

Extraggression scores by PF Study increased significantly in the fish oil group [4 (3–5) to 5 (3.5–6), P=.005] and did not change in the control group [5 (3.1–6) to 5 (3–6.5)] with a significant intergroup difference with baseline as covariate (P=.02 with no significant gender effect). However, baseline extraggression in the fish oil group was significantly lower than in the control group (P=.02). ΔExtraggression was not significantly correlated with

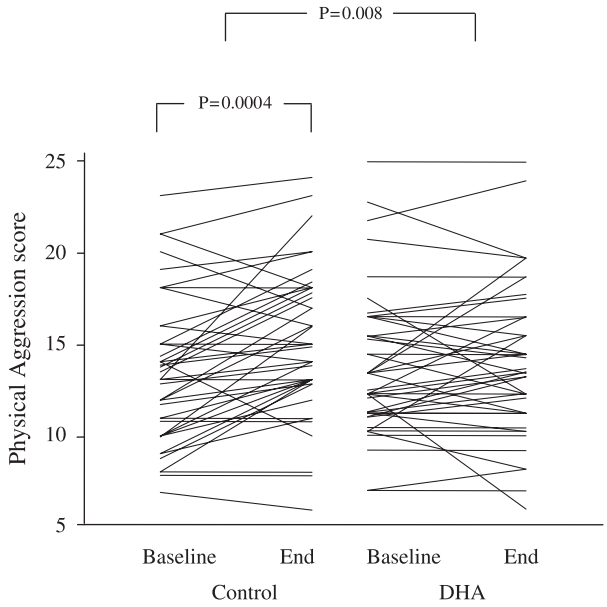

Fig. 2. Scores of physical aggression assessed with HAQ-C in girls. Schoolchildren (85 girls) took either fish oil-fortified or control foods for 3 months.

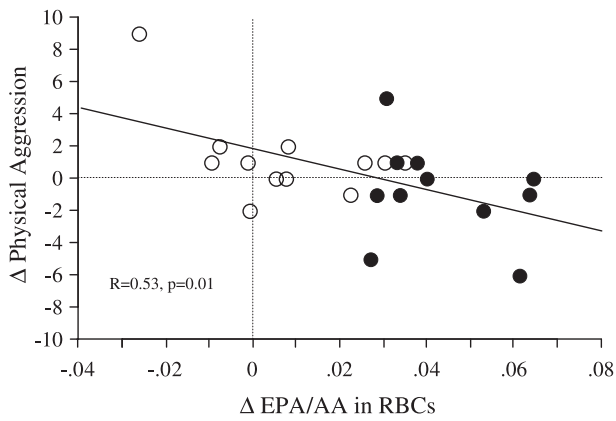

Fig. 3. Simple regression between ΔEPA/AA and Δphysical aggression in 23 girls. Eicosapentaenoic acid levels were measured in the total phospholipid fraction of RBC. ○, the control group; •, the fish oil group.

Table 5
Scores of *DSM-IV* questionnaire assessed by parents/guardians

	Control (n=41/42)		Intragroup comparison	Fish oil (n=40/43)		Intragroup comparison	Gender-adjusted intergroup comparison	Effect of gender with baseline as covariate
	Baseline	End		Baseline	End			
Inattention								
Total	8 (5–9)	6 (3–9)	.001	8 (5–11)	7 (4–10)	0.003	NS	NS
Hyperactivity								
Total	2 (1–3)	1 (0–2)	.0001	2 (1–4)	1 (0–3)	0.001	NS	NS
Impulsivity								
Total	1 (0–2)	1 (0–2)		1 (0–2)	0 (0–2)	0.002	NS	.04
Male	1 (0–3)	1 (0–2)	.02	1 (0–2)	1 (0–2)	0.04	NS	
Female	1 (0–2)	1 (0–2)		1 (0–2)	0 (0–1)	0.02	0.008	

Values are shown as median (25th percentile–75th percentile). n=males/females. NS, not significant.

ΔEPA/AA in blood-sampled children (P=.9, n=49).

Table 5 shows the results of diagnostic questionnaires of *DSM-IV* for AD/HD assessed by parents/guardians. There were no significant intergroup differences. However, in girls, impulsivity was reduced in the fish oil group, and this reduction was significant compared with the control group (Fig. 4). ΔImpulsivity was not correlated with ΔEPA/AA in blood-sampled girls (P=.99, n=26). ΔLinoleic acid was not correlated significantly with changes in physical aggression, extraggression or impulsivity (data not shown).

3.3. Quality of the blind and compliance of test foods

Because fish oil-containing rolls smelled like fish if heated, we asked all subjects and their parents/guardians not to toast them. However, some did so anyway. In addition, during cooking, the fish oil-containing spaghetti sometimes smelled like fish. Consequently, quite a number of families were convinced that the food which they had been provided contained fish oil. At the end of the study we asked parents/guardians to guess their foods in two areas, Inuyama and Seki. In the fish oil group, the numbers of their guesses as fish oil, "hard to tell" and control were 30, 2, 20, respectively; and in the control group those numbers were 15, 3, 36, respectively. The ratio of correct answers was significantly higher than that of incorrect answers (P=.008).

Consumption rates of test foods were over 90% and not different between the fish oil and control group.

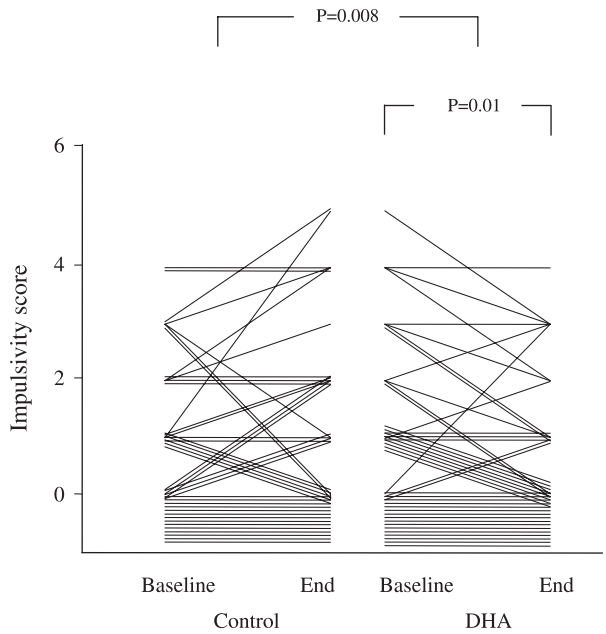

Fig. 4. Scores of impulsivity assessed by parents/guardians using *DSM-IV* questionnaire in girls.

4. Discussion

The present study had 166 normal schoolchildren as subjects and was the biggest intervention trial of n-3 fatty acid administration as far as normal schoolchildren were concerned. There has been reported one intervention trial with n-3 fatty acid using 64 normal Thai schoolchildren as subjects [13]. In that Thai study, the days absent from school was less in a group of subjects who took fish oil-containing foods than those of a control group. However, there was no significant difference in scores of psychological measures between the two groups. Their psychological measures to assess behavior were different from ours. Consequently, it is difficult to compare our results with theirs.

In the present study we chose bread, sausage and spaghetti as a vehicle of fish oil, considering that compliance with these foods were better than capsules and that children and their parents/guardians would easily agree with those foods. As shown in Results, compliance with those foods was excellent, but quality of the blind was not high. This was because provided foods smelled like fish when heated. In another intervention trial in which we used fermented soybean milk as an EPA/DHA vehicle, subjects could not detect any fish odor at all, and the blind was perfect [14]. A kind of drink, which is not supposed to be cooked, therefore, is a better vehicle in terms of blinding than what can be heated.

Docosahexaenoic acid concentrations in RBC significantly increased over time in the fish oil group (Table 3),

whereas the intergroup difference in the DHA concentrations with baseline as covariate only tended to be different ($P=.06$). The foods used in the present study contained nonfish oil, which might dilute the effect of DHA supplementation. It is possible that the high metabolic rate in rapidly growing children might shift the metabolism of DHA to combustion instead of its incorporation into tissues.

Extraggression was increased in the fish oil group compared with the control group. These changes were different from what could be expected from our previous experiments with students [5] and with elderly white-collar Thai subjects [7], in which the direction of changes in extraggression was downward in the DHA groups compared with the control groups. One of the major differences between the present study and those two studies [5,7] was the absence of a stressor component in the present study. When planning the present study, we thought a possibility to load schoolchildren with some stressor at the end of the study just before the second PF Study in order to increase their extraggression and hostility, but frustrating schoolchildren to such an extent that they might change their behavior even temporarily might not be ethically acceptable. Therefore, we gave up the idea of frustrating them. If there was no stressor, DHA supplementation did not decrease extraggression but might even increase it with marginal significance [15]. A similar phenomenon might have happened in the present study. Another point to discuss about extraggression is that there was a significant difference in this measure between the fish oil and control groups at baseline. Significant changes over time in extraggression between the two groups might be explained at least partly by the rule of regression to the mean. The finding that there was no correlation between Δextraggression and ΔEPA/AA suggested that this might be a chance finding. Moreover, PF Study took about 20 min to finish. Subjects of the present study might be tired of putting sentences in the second PF Study performed at the end of the present study. Actually, simple answers like "yes" and "no" increased about 10% in the second PF Study. Those simple answers made it difficult to differentiate individual scores, resulting in similar scores in both fish oil and control groups in favor of the tendency of regression to the mean. PF Study, if planned to be performed twice, may not be a good measure for schoolchildren since they may be accustomed to and tired of it.

As shown in Table 4, physical aggression was significantly increased in the female control group, whereas it was stable in the female fish oil group. It is unclear from the present study why changes in physical aggression over time were significantly different between the two female groups. However, the activity of serotonergic neurons in the prefrontal cortex may be involved [16]. It is also unclear why physical aggression was significantly increased in the control female group. n-6 Fatty acids were significantly increased in the control group. This might have something to do with the changes in the female control group. However, the findings that there were no correlations between Δlinoleic acid and Δphysical aggression were against this hypothesis. Although the harmful effects of linoleic acid on general health have already been reported [17], there seem no investigations about the effects of linoleic on behavior yet.

In the female control group, physical aggression increased by two points (13 to 15, Fig. 2 and Table 4). These changes meant either one-point increases in two questions related to physical aggression of HAQ-C or a two-point increase in one such question (see Psychological Tests section). These differences can be considered as changes within normal limits [8]. However, we do not think that it is negligible because the effect size (the mean difference between before and after treatment divided by 1 S.D.) in the control group was 0.42, which is near "medium" suggestive of clinical significance [18]. Different from adults, children aged 9–12 years may not be good at verbal aggression yet. In this sense, physical aggression is an easier method to express their anger, hostility or aggression for them. Consequently, controlling physical aggression by fish oil is rather practical and important than in the case of verbal aggression or extraggression.

Our research group recently performed a double-blind test of 2 months to investigate the effects of administration of DHA-rich foods on behaviors of AD/HD children aged 6–12 years and found that visual short-term memory and errors of commission of continuous performance significantly improved (probably because of learning effects) in the control group compared with the changes over time in the DHA group [19]. The authors of that study suggested that DHA might depress the brain noradrenergic system taking into account that attention of AD/HD children was not improved. The careful reevaluation of that study revealed that aggression (including physical aggression), the sum of the scores rated separately by parents and teachers, was significantly reduced in the DHA group compared with the control group [20]. Norepinephrine is related with aggression [21]. Therefore, DHA or EPA/AA ratios in tissues might control aggression through depressing the noradrenergic system in the brain. This mechanism might be important in the case of girls in the present study.

Gesch et al. [22] performed a double-blind, placebo-controlled, randomized trial with 231 young adult prisoners for an average study period of 142 days. One group of prisoners given nutritional supplements containing vitamins, minerals and essential fatty acids were compared with control prisoners taking placebos with regard to antisocial behavior. Compared with placebos, those receiving active supplements committed an average of 26.3% fewer offenses. Although active supplements contained EPA and DHA, the daily intake from the supplements was only 80 mg EPA and 44 mg DHA, much smaller than the dosage of the present study. Consequently, it is difficult to compare that study [22] and ours. Buydens-Branchey et al. [23] performed an

observational study with 24 patients addicted to cocaine and found that plasma levels of DHA in aggressive patients ($n=6$) were only two thirds of those nonaggressive patients. Again, the comparison with our study is difficult since the number of aggressive cocaine patients was so small.

In the present study, fish oil supplement beneficially influenced impulsivity of girls compared with control foods. Impulsivity was not the primary endpoint of the present study, and the number of statistical calculations was large in the present study; moreover, there were no correlations between changes in impulsivity and fatty acid composition. These points suggest a possibility that this finding might be merely a chance finding. However, our finding about impulsivity was similar to the finding of Hibbeln et al. [24] that there was a significant inverse single regression between impulsivity and plasma EPA concentrations in 50 suicide attempt patients. Besides, the P value of intergroup difference in impulsivity was less than .01 in girls. Consequently, this finding should not simply be ignored. Further investigations are necessary.

We treated male and female subjects equally in the present study; however, the reactions to fish oil administration in male and female children were apparently different. We did not ask how many of girls had already menstruated in the present study, and were not able to discuss the effect of menstrual cycles in depth. It was recently reported that scores of hostility and anxiety in young healthy female adults in Japan positively correlated with the serum levels of long-chain saturated fatty acids, but not in males [25]. We have also found similar correlation in female junior high school students, but again not in males (unpublished data). Sexual distinction about relations between fatty acids and behavior, therefore, is not a rare finding, although the reason(s) is not clear.

In conclusion, we suggest that physical aggression might be affected by fatty acid nutrition especially in girls. Further studies on fish oil in schoolchildren were warranted.

Acknowledgments

This study was partly supported by grants from Japan Fisheries Association and Foundation for Total Health Promotion. The authors are very grateful to Tokunori Matsui (Noble Co.), Shuuji Nakajima (Nippon Suisan Kaisha), Hiroko Hamatani, Shizuko Takebe, Mingming Huan, Hiroto Nishizawa, Nina Hamazaki and Yuuhiro Yamazaki for their technical assistance.

References

[1] Stevens LJ, Zentall SS, Deck JL, Abate ML, Watkins BA, Lipp SR, et al. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 1995;62:761–8.

[2] Stevens LJ, Zentall SS, Abate ML, Kuczek T, Burgess JR. Omega-3 fatty acids in boys with behavior, learning, and health problems. Physiol Behav 1996;59:915–20.

[3] Stordy BJ. Dark adaptation, motor skills, docosahexaenoic acid, and dyslexia. Am J Clin Nutr 2002;71(Suppl):323S–6S.

[4] Voigt RG, Llorente AM, Jensen CL, Fraley JK, Berretta MC, Heird WC. A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. J Pediatr 2001;139:189–96.

[5] Hamazaki T, Sawazaki S, Itomura M, Asaoka E, Nagao Y, Nishimura N, et al. The effect of docosahexaenoic acid on aggression in young adults. A placebo-controlled double-blind study. J Clin Invest 1996;97:1129–33.

[6] Rosenzweig S, Rosenzweig L. Guide to research on the Rosenzweig Picture-Frustration (P-F) Study, 1934–1974. J Pers Assess 1976;40: 599–606.

[7] Hamazaki T, Thienprasert A, Kheovichai K, Samuhaseneetoo S, Nagasawa T, Watanabe S. The Effect of docosahexaenoic acid on aggression in elderly Thai subjects — a placebo-controlled double-blind study. Nutr Neurosci 2002;6:37–41.

[8] Yamasaki K, Sakai A, Soga S, Ohdo O, Shimai T, Ohtake K. Reconstruction of subscales in the Hostility-Aggression Questionnaire for Children (HAQ-C) and considerations of aggressiveness construct. Naruto Kyouikudaigaku Kenkyukiyou 2001;16:1–10 [in Japanese].

[9] Buss AH, Perry M. The aggression questionnaire. J Pers Soc Psychol 1992;63:452–9.

[10] Sakai A, Yamasaki K, Soga S, Oashi O, Shimai S, Otake K. Development of the aggression questionnaire for elementary school-aged children. Jpn Sch Health 2000;42:423–33.

[11] Hayashi K, Sumita K, Ichitani Y, Nakata Y, Hata K, Tsuda H, et al. PF-Study Kaisetsu. Kyoto: Sankyobo; 1987 [in Japanese].

[12] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem 1959;37:911–7.

[13] Thienprasert A, Kheovichai K, Sukanand S, Hamazaki T. Attendance and school performance in Thai children: a randomized double-blind placebo-controlled trial of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). In: Cunnane SC, editor. 5th Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) 2002 Montreal; 7–11 May, p. 46 [Abstract].

[14] Hamazaki K, Itomura M, Huan M, Nishizawa H, Watanabe S, Hamazaki T, et al. N-3 long chain fatty acids decrease serum levels of triglycerides and remnant-like particle-cholesterol in humans. Lipids 2003;38:353–8.

[15] Hamazaki T, Sawazaki S, Nagao Y, Kuwamori T, Yazawa K, Mizushima Y, et al. Docosahexaenoic acid does not affect aggression of normal volunteers under nonstressful conditions. A randomized, placebo-controlled, double-blind study. Lipids 1998;33:663–7.

[16] Hibbeln JR, Umhau JC, George DT, Salem Jr N. Do plasma polyunsaturates predict hostility and depression? World Rev Nutr Diet 1997;82:175–86.

[17] Hamazaki T, Okuyama H. The Japan society for lipid nutrition recommends to reduce the intake of linoleic acid. A review and critique of the scientific evidence. World Rev Nutr Diet 2003;92: 109–32.

[18] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, NJ: Lawrence Earlbaum Associates; p. 25.

[19] Hirayama S, Hamazaki T, Terasawa K. The effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder — a placebo-controlled double-blind study. Eur J Clin Nutr 2004;58:467–73.

[20] Hamazaki T, Hirayama S. Letter to the Editor. Re: The effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder — a placebo-controlled double-blind study. Eur J Clin Nutr 2004;58:838.

[21] Haller J, Makara GB, Kruk MR. Catecholaminergic involvement in the control of aggression: hormones, the peripheral sympathetic, and central noradrenergic systems. Neurosci Biobehav Rev 1998;22: 85–97.

[22] Gesch CB, Hammond SM, Hampson SE, Eves A, Crowder MJ. Influence of supplementary vitamins, minerals and essential fatty

acids on the antisocial behaviour of young adult prisoners. Br J Psychiatry 2002;181:22–8.

[23] Buydens-Branchey L, Branchey M, McMakin DL, Hibbeln JR. Polyunsaturated fatty acid status and aggression in cocaine addicts. Drug Alcohol Depend 2003;71:319–23.

[24] Hibbeln JR, Enstrom G, Majchrzak S, Salem Jr N, Traskman-Benz L. Suicide attempters and PUFAs: lower plasma eicosapentaenoic acid alone predicts greater psychopathology. In: Hamazaki T, editor. 4th Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) 2000; Tsukuba, 49 June, p. 191 [Abstract].

[25] Yanagisawa A, Shimojima Y, Yamagami M, Tsujii T, Kannda H, Tajino K. Serum levels of long-chain saturated fatty acids positively correlate with anxiety and hostility scores in healthy young Japanese women. In: Cunnane SC, editor. 5th Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) 2002; Montreal, 7–11 May, p. 145 [Abstract].