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BENEFICIAL EFFECTS OF DAILY DIETARY OMEGA-3 POLYUNSATURATED FATTY ACID SUPPLEMENTATION ON AGE-RELATED COGNITIVE DECLINE IN ELDERLY JAPANESE WITH VERY MILD DEMENTIA: A 2-YEAR RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Abstract: *Background:* Studies of the effects of dietary omega-3 polyunsaturated fatty acid intake on cognitive functions in elderly Japanese are lacking. In this 2-year randomized, double-blind, placebo-controlled trial, we primarily aimed to examine the effects of daily dietary docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) supplementation on cognitive functions in healthy elderly Japanese. *Methods:* Independently living community dwellers aged 57 years or over (n = 111, 72.4 ± 7.7 years) were randomized to active and placebo groups. The active group consumed fish sausages containing 1720 mg DHA and 407 mg EPA daily for 24 months; the placebo group consumed fish sausages containing olive oil daily for the first 12 months and fish sausages containing DHA and EPA daily for the next 12 months. Cognitive functions were assessed by using the Revised Hasegawa's Dementia Scale (HDS-R), Mini-Mental State Examination (MMSE), and Frontal Assessment Battery (FAB) and blood biochemistry was analyzed at the baseline and 6, 12, 18, and 24 months thereafter. *Results:* The mean baseline HDS-R, MMSE, and FAB scores were not significantly different between the placebo and the active groups. On average, the plasma and erythrocyte plasma membrane DHA and EPA levels significantly increased in the active group at 6 and 12 months, but were not significantly different between the groups at 24 months. Further, at 6 and 12 months, the mean total HDS-R, MMSE, and FAB scores were not significantly different between the groups, but the mean changes in FAB-subitem "Conflicting instructions" scores from the baseline to month 6 and MMSE-subitem "Language: copying" scores from the baseline to month 12 were significantly greater in the active group. Depending on the responses to the MMSE, the subjects were also grouped as responders and nonresponders. The mean changes in the total MMSE scores and MMSE-subitem "Attention and Calculation" scores from the baseline to month 12 were significantly greater in the responders of the active group. *Conclusion:* Long-term daily dietary DHA and EPA supplementation seems to have beneficial effects against age-related cognitive decline in otherwise healthy elderly Japanese with very mild dementia.

Key words: Interventional study, elderly, cognitive decline, omega-3 fatty acids, DHA, dementia.

Introduction

Alzheimer's disease (AD) is a neurodegenerative condition commonly affecting the elderly. It is characterized by short-term memory loss and cognitive impairment. The current therapeutic drugs have small but significant effects on cognition, behavior, and activities of daily living, but they do not affect the underlying disease process. Therefore, interest in the role of diet for the prevention and treatment of AD is increasing.

Numerous epidemiological studies have shown an inverse association between AD risk and dietary omega-3 polyunsaturated fatty acid (n-3 PUFA) intake. A population-based prospective study in Rotterdam showed that consumption of fish, an important source of n-3 PUFAs, is inversely related to the risk of dementia, particularly AD (1). In a prospective human study on the progression of AD, the total intake of n-3 PUFAs, particularly docosahexaenoic acid (DHA) but not eicosapentaenoic acid (EPA), was associated with decreased risk of AD (2). Moreover, patients with blood DHA concentrations in the highest quartile demonstrated lower risk of dementia than those with blood DHA

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concentrations in the three lowest quartiles, during a mean follow-up period of 9 years (3).

Loss of the brain DHA content in patients with AD is accompanied by loss of memory and learning (4, 5). Cognitive ability decreases in animals fed n-3 PUFA-depleted diets (6), but learning ability is restored when they are fed diets supplemented with DHA or EPA (7, 8). Furthermore, DHA supplementation improves cognitive functions in the AD rat model produced by intraventricular infusion of amyloid β peptide solution (9) and the APPsw (Tg2576) transgenic mouse model of AD (10). Taken together, these results suggest that dietary supplementation of n-3 PUFAs alters the risk of cognitive impairment with aging and/or the risk of AD over time.

Although a few studies of the effects of dietary DHA intake on cognitive functions in elderly people in Europe and the United States have been reported (11, 12), similar Japanese studies are lacking. In this 2-year randomized, double-blind, placebo-controled trial, we primarily aimed to examine the effects of daily dietary DHA and EPA supplementation on cognitive functions in apparently healthy elderly Japanese.

Methods

Participants

Healthy elderly community dwellers (≥57 years old) living independently in the county surrounding Kawamoto mountain, a remote area in Japan, were the subjects of the present investigation. The volunteers underwent medical examinations with anthropometric, blood biochemical, and cognitive function tests and answered two self-administered questionnaires: a general questionnaire and a brief dietary history questionnaire (BDHQ) (13).

The exclusion criteria were as follows:

(1) Evidence of delirium, confusion, or other disturbances of consciousness

(2) Evidence of a neurological disorder that could produce cognitive deterioration, including AD, Parkinson's disease, stroke, or brain tumor

(3) History of infective or inflammatory brain disease including those of viral, fungal, or syphilitic etiology

(4) Considerable head injury immediately preceding cognitive deterioration

(5) Current psychiatric diagnosis according to the DSM-IV criteria of depression, mania, or any other major psychiatric disorder

(6) Current diagnosis or history of alcoholism or drug dependence

(7) Evidence of a medical disorder that could produce cognitive deterioration, including renal, respiratory, cardiac, or hepatic disease, diabetes mellitus, and endocrine, metabolic, or hematological disturbances unless well controlled for more than 2 years

(8) Use of a psychotropic drug or any supplement that may significantly affect cognitive functioning during the trial

(9) History of hypersensitivity or allergy to fish or fish oil

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The protocol was approved by the Ethics Committee of Jinjukai Kato Hospital (approval number 2008-002), and all volunteers gave written informed consent before participation.

Anthropometrics and self-administered questionnaires

Body weight, height and blood pressure were measured by hospital nurses. The general questionnaire posed lifestyle questions, including those related to educational and medical histories. The participants were requested to answer the BDHQ by hospital nurses.

Blood sampling

Blood samples were obtained in the morning or afternoon, after ascertaining whether the participants had eaten breakfast or lunch. The samples were separated into two sets of tubes: tubes containing a serum isolator for blood biochemical analysis and tubes containing ethylenediaminetetraacetic acid (EDTA) for fatty acid analysis. The samples were separated into serum, plasma, and erythrocyte (RBC) aliquots and stored at −80oC within 8 h of collection.

Cognitive evaluations

The Revised Hasegawa'a Dementia Scale (HDS-R) (14), Mini-Mental State Examination (MMSE) (15), and Frontal Assessment Battery (FAB) (16) were used to evaluate cognitive functions. HDS-R, consisting of nine simple questions with a maximum score of 30, MMSE, consisting of 11 questions; FAB, consisting of six subitems are widely used for examining cognitive ability including dementia, onceptualization, mental flexibility, motor programming, sensitivity to interference, inhibitory control, and environmental autonomy. Subjects were excluded if they had an HDS-R score of less than 20.

Supplementation procedure

The subjects were randomized to active and placebo groups. All study staff and subjects were blinded to the food products. Subjects in both the groups were instructed to consume two fish sausages daily within 1 h, with or without food, and avoid altering their normal diet

during the study. Each fish sausage for the active group contained 860 mg DHA and 204 mg EPA ("Resara", Maruha Nichiro Foods, Inc., Tokyo, Japan). The placebo group received fish sausages containing olive oil, as well as 48 mg DHA and 12 mg EPA (17), for the first 12 months and the DHA- and EPA-enriched fish sausages for the next 12 months. All sausages were indistinguishable with regard to color, taste, and flavor. The number of fish sausages consumed (0, 1, or 2 pieces) was recorded by the subjects daily. Consumption compliance was assessed monthly to encourage protocol adherence. Efficacy was assessed at the baseline and 6, 12, 18, and 24 months thereafter.

Blood biochemical analysis

The DHA and EPA levels in plasma and the RBC plasma membrane (RBC-PM) were determined by direct transmethylation (18), as described previously (19). In brief, 100 μL of RBCs was suspended by vigorous mixing in 1.2 mL of 4 mM EDTA solution containing 0.005% BHT (2,6-di-t-butyl-4-methylphenol) and centrifuged at 10,000g for 15 min at 4°C. After the supernatant was discarded, the resultant pellet was resuspended by vigorous mixing in 1 mL of Dulbecco's PBS(-) containing 0.005% BHT and centrifuged at 10,000 rpm for 15 min at 4°C. This procedure was repeated once. The final pellet was homogenized in 250 μL of Dulbecco's PBS(-) containing 0.5% saponin and 0.005% BHT by using an ultrasonic homogenizer (Bioruptor®; Cosmobio Co., Tokyo, Japan) and subjected to the measurement of fatty acids and proteins. The fatty acid concentrations in plasma and the RBC-PM suspension were measured by capillary gas chromatography of the corresponding methyl esters prepared by transesterification with acetyl chloride. Chromatography was performed by using an Agilent 6850A gas chromatograph (Agilent Technologies, Santa Clara, CA) with a flame ionization detector and an automatic sampler utilizing a 25-m fused silica column with an internal diameter of 0.25 mm (DB-WAX P/N 122-7032; J&W Scientific, Folsom, CA). The identities of the peaks were established by comparing with those of reference compounds and, in part, by gas chromatography–mass spectrometry.

Plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, and glucose levels were determined with an automatic analyzer (BiOLiS 24; Tokyo Boeki Medical System Ltd., Tokyo, Japan). HbA1c was measured with a kit from TFB, Inc. (Tokyo, Japan). We used the Invader® assay to screen for APOE mutations as previously described (20). In brief, 3 μL of a primary probe–Invader® oligonucleotide mixture, containing 3.5 μmol/L Invader® oligonucleotide and 10 mmol/L β-(N-morpholino)propanesulfonic acid) (MOPS), was combined with 5 μL of 22.5 mM magnesium chloride per reaction. Eight microliters of the primary probe, Invader® oligonucleotide, and magnesium chloride mixture was added to each well of a 96-well plate. Seven microliters of 5 fmol/L synthetic target oligonucleotides, 10 μg/mL yeast tRNA (no target blank), and 15 ng/μL genomic DNA were added, and the mixture was denatured by incubation at 95°C for 10 min. After mineral oil (Sigma-Aldrich, St. Louis, MO) was overlayed in all the wells, the plate was incubated at 63°C for 4 h in a DNA thermal cycler (PTC-200; MJ Research, Watertown, MA) and maintained at 4°C until fluorescence was measured. A fluorescence microtiter plate reader (Cytoflour 4000; Applied Biosystems) with excitation at 485 nm/20 nm (wavelength/bandwidth) and emission at 530 nm/25 nm for FAM and excitation at 560 nm/20 nm and emission at 620 nm/40 nm for RED was used. The genotyping was analyzed by calculating the ratio of the net counts with the wild primary probe to the net counts with the mutant primary probe. The probes used in this study were designed and synthesized by Third Wave Technologies, Inc. (Madison, WI).

Statistical analysis

Results are expressed as means ± SD. Student's t-test for dependent or independent samples was used to evaluate differences in the demographic and baseline variables. In the case of significant differences, the changes in the active group were compared with those in the placebo group by using the Mann–Whitney U–test. All analyses were performed with SPSS ver 13.0 (IBM-SPSS Japan, Inc., Tokyo, Japan). All hypothetical tests were two sided; $P < 0.05$ was considered significant.

Results

Demographic and clinical characteristics at the baseline

One hundred eleven subjects (42 men and 69 women) completed the baseline assessments and were randomized to either group (Figure 1). One hundred two subjects completed the study.

The baseline characteristics of these groups are shown in Table 1. The groups were well matched and did not show significant differences in age, gender, body mass index, educational history, and blood pressure. Their mean total HDS-R, MMSE, and FAB scores were 27.6 ± 3.3, 27.8 ± 3.3, and 15.0 ± 2.3, respectively, suggestive of very mild cognitive impairment. These scores were not significantly different between the groups. The frequencies of the APOE ε2, ε3, and ε4 alleles were also not significantly different between the groups (Table 1).

Except for the individuals who withdrew from the study, the subjects showed high adherence to the study

Table 1
Baseline characteristics of study subjects

	Placebo (n = 54)	Active (n= 57)	P values
Gender (male/female)	21/33	21/36	0.845
Age (y)	72.9 ± 7.8	72.0 ± 7.6	0.537
BMI (kg/m2)	23.0 ± 3.4	23.0 ± 2.5	0.919
Education			
Score	1.8±0.7	1.6±0.6	0.337
Gender (male/female)	19/29	21/31	0.433
Less than high school (%)	35.4	44.2	
High school graduate (%)	58.3	51.9	
Technical school (%)	2.1	1.9	
College/postgraduate college (%)	4.2	1.9	
Blood pressure (mm Hg)			
Systolic	153.7 ± 21.9	149.1 ± 20.3	0.256
Diastolic	85.5±10.3	83.3±13.7	0.338
Cognitive function			
HDS-R score	27.5±2.6	27.7±3.9	0.816
MMSE score	27.7±2.6	27.8±3.9	0.849
FAB score	14.7±2.1	15.2±2.5	0.296
APOE4 No. (%)	(male/female)		1.000
0	41 (80.4) 14/27	45 (81.8) 13/32	0.642
1	10 (19.6) 5/5	8 (14.6) 4/4	
2	0 (0)	2 (3.6) 1/1	
Plasma biochemical parameters			
Total cholesterol (mg/dL)	208.1±37.5	214.0±36.6	0.410
LDL-cholesterol (mg/dL)	116.8±28.8	118.9±26.4	0.687
HDL-cholesterol (mg/dL)	69.6±16.0	74.4±19.4	0.164
Triglyceride (mg/dL)	119.9±75.6	106.5±65.3	0.319
Glucose (mg/dL)	115.8±30.2	114.3±28.1	0.784
HbA1c (%)	5.2±0.7	5.2±0.6	0.840
Fatty acid composition of erythrocyte plasma membrane			
Palmitic acid (%)	27.6±1.8	27.7±1.0	0.590
Stearic acid (%)	16.5±0.7	16.4±0.7	0.366
Oleic acid (%)	12.7±1.0	12.3±0.8	0.029
Linoleic acid (%)	7.9±1.0	8.0±1.2	0.632
Linolenic acid (%)	0.11±0.05	0.13±0.04	0.004
Arachidonic acid (%)	11.6±1.3	11.2±1.1	0.154
Eicosapentaenoic acid (%)	2.7±1.3	2.8±0.8	0.623
Docosapentaenoic acid (%)	2.7±0.4	2.7±0.5	0.862
Docosahexaenoic acd (%)	9.0±0.9	9.1±1.1	0.672
n-6/n-3	1.9±0.4	1.9±0.4	0.405

Values are mean + SD. BMI, body mass index; HDS-R, Revised Hasegawa's Dementia Scale; MMSE, Mini-mental State Examination; FAB, Frontal Assessment Battery; APOE4, allele producing the ε4 type of apolipoprotein E; LDL, low density lipoprotein; HDL, high density lipoprotein; n-6, n-6 polyunsaturated fatty acid; n-3, n-3 polyunsaturated fatty acid. Statistical significance for gender and APOE genotype frequency was analyzed by using the chi-square test. Statistical significance for educational history was analyzed by using the following scores: 1, less than high school; 2, high school graduate; 3, technical school; 4, college/postgraduate college.

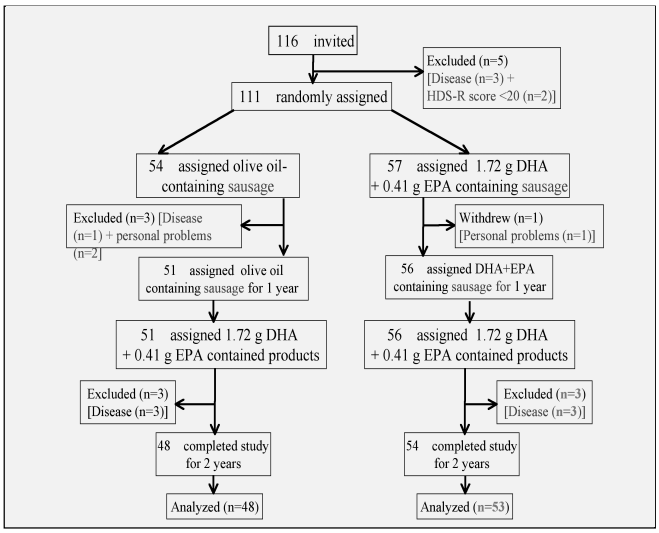
protocol for 2 years (86 ± 3.1 %), without a significant difference between the groups. Consumption compliance in the active group was evident by increases in the mean DHA and EPA levels in plasma and the RBC-PM. Notably, DHA/ EPA supplementation did not produce any side effects such as allergic response, excessive sweating, urination, palpitation and gastrointestinal irritation as well.

Plasma and RBC-PM fatty acid levels

"In the active group, the plasma DHA and EPA levels increased, on average, by 1.35 (from 5.33 ± 1.13 to 7.20 ± 1.42 mol%, P <0.0001) and 1.19-fold (from 4.10 ± 1.71 to 4.88 ± 1.36 mol%, P = 0.001), respectively, in plasma and by 1.14-fold (from 9.00 ± 1.00 to 10.23 ± 0.86 mol%, P < 0.0001) and 1.16-fold (from 2.76 ± 0.69 to 3.20 ± 0.63 mol%, P < 0.0001), respectively, in the RBC-PM during the first 6 months. On the other hand, in the placebo group, the DHA and EPA levels decreased on average, by 0.96-fold (from 5.36 ± 1.29 to 5.17 ± 0.99 mol%, P = 0.1859) and 0.79-fold (from 3.96 ± 2.01 to 3.14 ± 1.37 mol%, P = 0.0003), respectively, in plasma and by 0.94-fold (from 9.08 ± 1.02 to 8.60 ± 0.73 mol%, P = 0.0001) and 0.82-fold (from 2.90 ± 1.32 to 2.40 ± 0.89 mol%, P = 0.0001), respectively, in the RBC-PM. These mean values were sustained from 6 to 12 months in both the groups. However, at 24 months (as

they were fed fish oil-containing sausages for the last 12 months), the placebo group showed similar DHA and EPA levels in plasma and the RBC-PM to those in the active group. At 6 and 12 months, the mean RBC-PM arachidonic acid (AA) level was significantly lower in the active group than in the placebo group (P = 0.0019 at 6 months, P = 0.0007 at 12 months).

Figure 1
Flow diagram of participants throughout the study



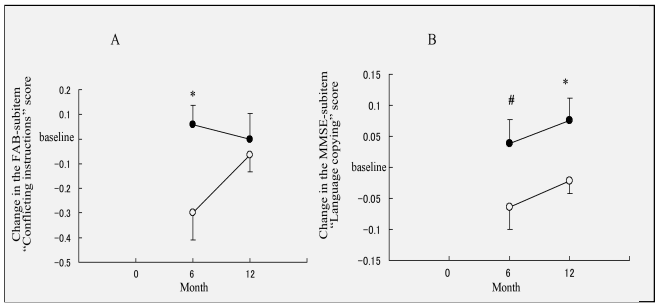
Cognitive function scores

At the baseline, the placebo and active groups showed no significant differences in their mean total HDS-R (27.5±2.6 vs. 27.7±3.9), MMSE (27.7±2.6 vs. 27.8±3.9), and the FAB (14.7±2.1 vs.15.2±2.5) scores (Table 1). Similarly, at 6 and 12 months, these scores were not significantly different between the groups (data not shown), and the mean total HDS-R, MMSE, and FAB scores at 6 and 12 months were not significantly different from those at the baseline.

When the scores of each subitem of the HDS-R, MMSE, and FAB were analyzed, the placebo and active groups showed significant differences in the mean changes in the FAB-subitem "Conflicting instructions" scores from the baseline to month 6 (16) (Figure 2A) and the MMSE-subitem "Language: copying" scores from the baseline to month 12 (Figure 2B).

Further, positive correlations were observed between the mean changes in the FAB-subitem "Conflicting instructions" scores from the baseline to month 6 and the mean changes in the DHA (r = 0.263, P = 0.009) and EPA levels (r = 0.207, P = 0.039) and DHA:AA ratio (r = 0.299, P = 0.003) in the RBC-PM from the baseline to month 6. Contrarily, an inverse correlation was noted between the FAB-subitem scores and the n-6:n-3 PUFA ratio in the RBC-PM (r = −0.266, P = 0.008).

Figure 2
Time course of the mean changes in the FAB-subitem "Conflicting instructions" (A) and MMSE-subitem "Language: copying" (B) scores from the baseline to month 12 in the placebo (open circles) and active (closed circles) groups. *P < 0.05 and # 0.05 < P < 0.1 versus the placebo group. At the baseline (month 0), the mean FAB-subitem "Conflicting instructions" score was 2.88 ± 0.33 in the placebo group and 2.77 ± 0.54 in the active group. Further, the mean MMSE-subitem "Language: copying" score at month 0 was 1.00 ± 0.00 in the placebo group and 0.92 ± 0.27 in the active group



Responder analysis

As all subjects consumed DHA- and EPA-enriched fish sausages for at least 1 year, both the groups were subdivided according to the mean changes in the total MMSE (ΔMMSE) scores over 12 months as follows: responders, total MMSE score change > 0; nonresponders, total MMSE score change < 0. The percentage of responders in the placebo and active groups was 60.4% and 35.8%, respectively.

The baseline data of the nonresponders and responders are shown in Table 2. At the baseline, these groups did not show significant differences in any parameter except the mean serum LDL-cholesterol level, which was significantly higher in the responders than in the nonresponders.

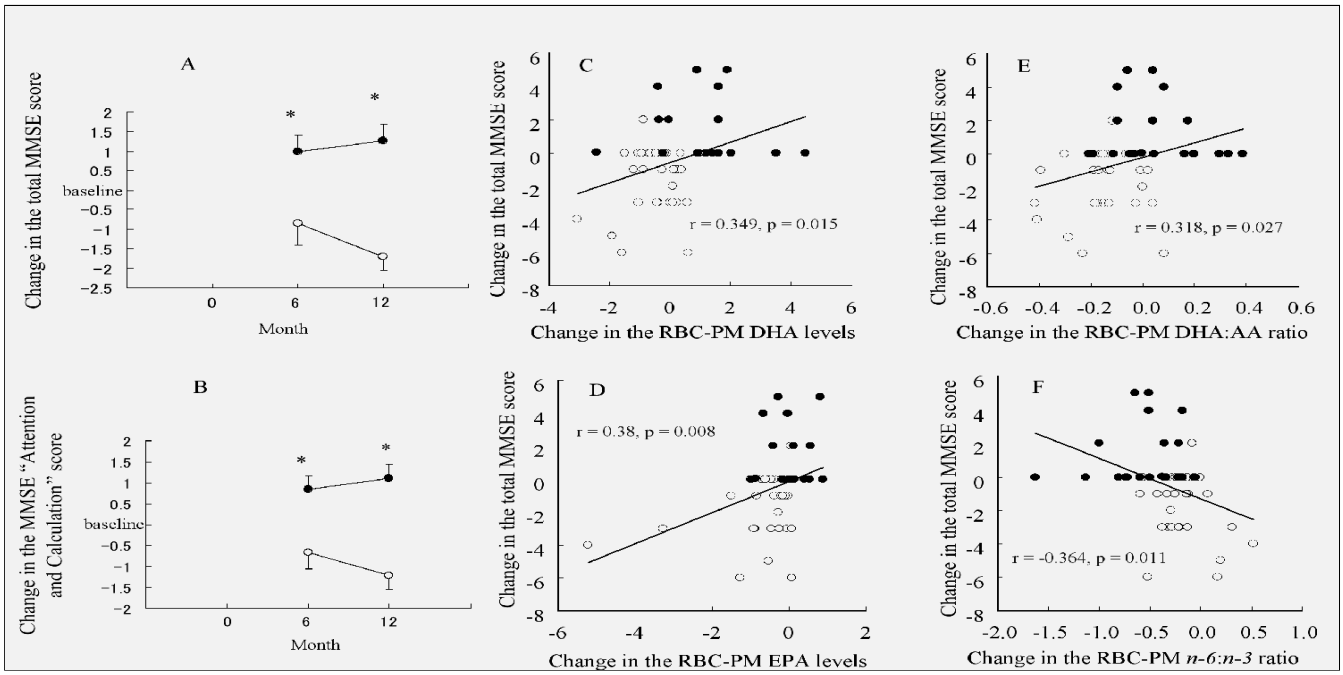
The responders of active group showed significantly increased mean DHA levels from the baseline to month 6 (9.1± 1.1 vs. 9.3± 1.0 mol%, P < 0.0001) and month 12 (9.1± 1.1 vs. 9.2± 1.0 mol%, P = 0.0038). These responders also had a decreased n-6:n-3 PUFA ratio in the RBC-PM at both 6 (P = 0.0026), and 12 (P = 0.0207) months, when compared with those of the placebo group.

The mean total MMSE score was significantly higher in the responders of the active group than those of the placebo group at 12 months (28.8 ± 2.0 vs. 27.0 ± 2.6, P = 0.0129). Further, in the active group, the mean total MMSE score was significantly higher at 6 (28.6 ± 2.3, P = 0.0237) and 12 months (28.8 ± 2.0; P = 0.009) than at the base line (27.6 ± 2.9). The placebo and active groups did not show significant differences in the mean total HDS-R and FAB scores at the baseline and 6 and 12 months.

The mean changes in the total MMSE scores from the

Figure 3

Time course of the mean changes in the total MMSE (A) and MMSE-subitem "Attention and Calculation" (B) scores from the baseline to month 12 in the placebo (open circles) and active (closed circles) groups. The relationships between the mean changes in the total MMSE scores and the mean changes in the DHA level (C), EPA level (D), DHA:AA ratio (E), and n-6:n-3 PUFA ratio (F) in the RBC-PM from the baseline to month 12 are also shown. At the baseline (month 0), the mean total MMSE score was 28.72 ± 2.37 in the placebo group and 27.58 ± 2.85 in the active group. Further, the MMSE-subitem "Attention and Calculation" score at month 0 was 4.45 ± 1.18 in the placebo group and 3.26 ± 1.94 in the active group. *P < 0.05 versus the placebo group



baseline to months 6 and 12 was significantly different between the placebo and the active groups (Figure 3A), but the mean changes in the total HDS-R and FAB scores were not significantly different. In the case of the HDS-R, MMSE and FAB subitems, the placebo and active groups showed significant differences in the mean changes in the MMSE-subitem "Attention and Calculation" scores from the baseline to months 6 and 12 (Figure 3B).

Positive correlations were observed between the mean changes in the total MMSE scores from the baseline to month 12 and the mean changes in the DHA (r = 0.349, P = 0.015; Figure 3C) and EPA levels (r = 0.380, P = 0.008; Figure 3D) levels and DHA:AA ratio (r = 0.318, P = 0.027; Figure 3E) in the RBC-PM from the baseline to month 12. However, an inverse correlation was noted between the mean changes in the total MMSE scores and the mean changes in the RBC-PM n-6:n-3 PUFA ratio (r = −0.364, P = 0.011; Figure 3F).

Discussion

The present study demonstrated that daily dietary supplementation with 1720 mg DHA and 407 mg EPA might protect against age-related cognitive decline in otherwise healthy elderly Japanese with very mild cognitive impairment. Specifically, the daily consumption of n-3 PUFA-enriched food increased the FAB-subitem "Conflicting instructions" score at 6 months and the MMSE-subitem "Language: copying" score at 12 months along with increases in the DHA and EPA level in the RBC-PM.

To the best of our knowledge, this is the first long-term, randomized, double-blind, placebo-controlled trial of the effects of dietary DHA and EPA supplementation on cognitive functions in healthy elderly Japanese. We used DHA- and EPA-enriched fish sausages rather than fish oil-containing soft capsules, because a case-control study (21), a prospective study (22), and the Rotterdam study (23) showed no association between supplemental intake of antioxidants and the risk of AD. The participants had customarily eaten DHA- and EPA-enriched fish sausages as snacks when they were young, which could explain their excellent compliance during this study. Such n-3 PUFA-enriched fish sausages are authorized as a special health food by the Japanese Ministry of Health, Labor and Welfare. In a previous study, this food product lowered the serum triglyceride levels in volunteers with initially slightly high levels (219 ± 13 mg/dL) (17), but it had no effect in volunteers with normal serum triglyceride levels (24). Similarly, long-

Table 2
Baseline characteristics of nonresponders and responders

	Nonresponders (n = 53)	Responders (n = 48)	P values
Gender (male/female)	19/34	19/29	0.837
Age(y)	72.5±7.9	70.7±6.6	0.234
BMI (kg/m2)	23.2±2.8	23.2±2.9	0.959
Education			
Score	1.6±0.6	1.7±0.7	0.404
Gender (male/female)	18/31	18/25	0.606
Less than high school (%)	44.9	34.9	
High school graduate (%)	51.0	58.1	
Technical school (%)	2.0	2.3	
College/postgraduate college (%)	2.0	4.7	
Blood pressure (mm Hg)			
Systolic	150.0±21.2	150.1±19.6	0.979
Diastolic	83.0±11.9	85.4±11.8	0.299
Cognitive function			
HDS-R	27.9±2.1	28.2±2.3	0.522
MMSE	28.2±2.1	28.3±2.6	0.861
FAB	15.1±1.9	15.4±1.8	0.449
Serum biochemical parameters			
Total cholesterol (mg/dL)	206.6±31.5	219.6±40.4	0.073
LDL-cholesterol (mg/dL)	112.5±25.2	127.0±28.3	0.008
HDL-cholesterol (mg/dL)	72.8±17.0	71.9±19.1	0.803
Triglyceride (mg/dL)	120.2±79.7	105.7±45.9	0.275
Glucose (mg/dL)	114.7±28.8	113.2±25.4	0.787
HbA1c (%)	5.1±0.6	5.3±0.7	0.289
Fatty acid composition of erythrocyte plasma membrane			
Palmitic acid (%)	27.7±1.0	27.3±1.8	0.149
Stearic acid (%)	16.5±0.7	16.6±0.7	0.305
Oleic acid (%)	12.7±0.9	12.4±1.1	0.095
Linoleic acid (%)	7.9±1.1	8.0±1.2	0.750
Linolenic acid (%)	0.1±0.0	0.1±0.1	0.217
Arachidonic acid (%)	11.5±1.1	11.2±1.2	0.141
Eicosapentaenoic acid (%)	2.6±0.7	3.0±1.3	0.050
Docosapentaenoic acid (%)	2.7±0.3	2.8±0.6	0.085
Docosahexaenoic acd (%)	9.0±0.9	9.1±1.1	0.619
n-6/n-3	1.9±0.3	1.9±0.4	0.465

Values are mean + SD. BMI, body mass index; HDS-R, Revised Hasegawa's Dementia Scale; MMSE, Mini-Mental State Examination; FAB, Frontal Assessment Battery; LDL, low density lipoprotein; HDL, high density lipoprotein; n-6, n-6 polyunsaturated fatty acid; n-3, n-3 polyunsaturated fatty acid. Statistical significance for gender was analyzed by using the chi-square test. Statistical significance for educational history was analyzed by using the following scores: 1, less than high school; 2, high school graduate; 3, technical school; 4, college/postgraduate college.

term n-3 PUFA-enriched food consumption did not affect the serum triglyceride levels in our subjects, because their baseline levels were not high (Table 2).

Epidemiological studies have shown that intake of fish oil is associated with reduced risk of neurological and psychiatric disorders, especially AD. Studies of participants in the population-based prospective Rotterdam study (25) have shown that fish consumption is inversely related to the risk of dementia, particularly AD. In a prospective human study on the progression of AD (2), the total intake of n-3 PUFAs, particularly DHA but not EPA, was associated with decreased risk of AD. Moreover, cross-sectional analyses have linked low plasma levels of DHA with the development of dementia and AD. In this study, regression analysis of the relationship between RBC-PM fatty acid levels and cognitive functions showed significant positive correlations between the n-3 PUFA levels and the cognitive function scores and significant negative correlations between the n-6:n-3 PUFA ratio and the cognitive function scores. In a Japanese epidemiological study, because female patients with AD consumed significantly lower amounts of fish and vegetables than control, their n-3 PUFA intake was significantly lower and their n-6:n-3 PUFA ratio was significantly higher (26). Many diseases in the elderly, such as atherosclerosis, thrombosis, cardiovascular disease, and cancer, are related to an increase in the n-6:n-3 PUFA ratio (27). These facts suggest that dementia is an age-related disease and decreased fish consumption by the elderly may be related to its onset.

We previously investigated the relationships among cognitive functions, nutrition, and fatty acid levels in plasma and the RBC-PM in 53 community dwellers (aged

≥ 65 years) in Shimane, Japan, over a 4-year follow-up period. The RBC-PM DHA levels were significantly higher in those who showed improvement or no change in their HDS-R scores than in those who showed a worse score (28). In the present study, among the 26 subitems (11 of the MMSE, nine of the HDS-R and six of the FAB), why the scores of only two subitems, namely "Language copying" of the MMSE and "Conflicting instructions" of the FAB, significantly decreased in the placebo group (at 6 and 12 months, when compared to those in the active group), remains to be clarified. The finding may be related to the fact that DHA-EPA supplementation has effects at the vital brain functional level, which is responsible for these two cognitive abilities. Our results are partially consistent with those of the Otsuka study (29), in which the total MMSE score of the patients with AD also decreased from the baseline by -1.5 and -1.9. These results suggest that even in elderly Japanese, who consume more fish than elderly people in the West, higher consumption of DHA may protect against age-related cognitive decline observed in very mild dementia.

Despite the promising findings of epidemiological studies, no effect of fish oil or DHA on AD has been observed in clinical studies (30-32). In a well-designed, randomized, double-blind, placebo-controlled trial of n-3 PUFA supplementation, no significant differences in the primary outcomes, namely the MMSE and AD Assessment Scale cognitive component (ADAS-cog) scores, were observed between the treated and the placebo groups. However, a subgroup analysis of mildly affected individuals (MMSE score > 27) demonstrated significant reduction in MMSE-assessed decline compared with a placebo subgroup (30). In this study, the mean total HDS-R, MMSE, and FAB scores of the participants suggested very mild cognitive impairment. The placebo group showed a faster decline, however, the mean changes in the MMSE- and FAB-subitem scores (Figure 3A and 3B) were significantly different from those in the active group. Cockburn and Kneel (31) also reported changes in MMSE scores of about 10.09 over 4 years in the elderly. Our findings are consistent with those reported by Yurko-Mauro et al. (12), who showed that 24-week supplementation with 900 mg/day DHA significantly improves episodic memory and learning functions in healthy adults with age-related cognitive decline. Therefore, we speculate that the beneficial effects of dietary n-3 PUFA supplementation may depend on the cognitive status at the start of treatment, with those in the earliest stages of the disease benefiting the most.

The lipid composition of the human brain may partially reflect dietary intake (34). In recent studies, DHA has been found to play a role in cognitive development, learning ability, neural plasticity, and synaptogenesis, all of which are involved in synaptic transmission and normal brain functioning (26). The fish sausages used in this study contained not only DHA but also EPA; therefore, the subjects in the active group received almost 400 mg/day of EPA. Long-term administration of EPA increases DHA levels in the RBC-PM, plasma, and corticohippocampal tissue and has beneficial effects on memory formation and protection in normal and AD model rats along with a corresponding decrease in oxidative stress and increase in the expression of synaptic plasticity-related protein (8). These results suggest that EPA protects against age-related cognitive decline after its conversion to DHA. However, the conversion rate from EPA to DHA through the desaturation or chain elongation system is limited in humans and has essentially no impact on the plasma DHA level (35, 36), suggesting that the protective effect of dietary DHA and EPA against age-related cognitive decline may be less than that of dietary DHA alone. On the other hand, higher levels of EPA in the RBC-PM are associated with better cognitive outcomes (37). In addition, the potential neuroprotective effects of n-3 PUFAs against amyloidogenesis, oxidative stress, and inflammation in AD have been described (38). The effects of dietary n-3 PUFA supplementation in humans often differ according to the source, such as pure DHA or fish oil products, including a combination of both DHA and EPA (39). Such disparate data suggest that EPA increases blood flow and nutrient supply as well as removal of toxic metabolites and proteins from the brain, which might otherwise augment age- and/or AD-related degeneration. Finally, the results suggest that DHA- and EPA-enriched food products are more effective than food products with DHA or EPA alone in preventing the effects of neuronal diseases such as dementia.

Conclusion

In this 2-year, randomized, double-blind, placebo-controlled trail, we observed beneficial effects of daily dietary DHA and EPA supplementation on cognitive functions in otherwise healthy elderly Japanese with very mild cognitive impairment. This suggests that dietary n-3 PUFA-enriched foods are effective only when consumed before the onset of dementia or when symptoms are mild.

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