Title

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Journal

Journal of Functional Foods, Volume 74

Published

November 2020

URL

https://doi.org/10.1016/j.jff.2020.104195

Beneficial effects of docosahexaenoic acid-enriched milk beverage intake on cognitive function in healthy elderly Japanese: A 12-month randomized, double-blind, placebo-controlled trial

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Abstract

Recent approaches to the management of dementia have focused mainly on continual preventive treatment. In this study, we investigated whether intake of 297 mg docosahexaenoic acid (DHA) and 137 mg eicosapentaenoic acid (EPA) per day, which is a smaller amount than in previous reports, would maintain higher cognitive function in healthy elderly people. Eighty-seven participants were enrolled in this 12-month randomized, double-blind, placebo-controlled trial. Cognitive function test, blood biochemical analysis and anthropometry were conducted at baseline, 6 and 12 months of DHA beverage intake. Intake of DHA-enriched milk beverage for 6 and 12 months significantly increased erythrocyte plasma membrane DHA and EPA levels. Furthermore, intake of DHA-enriched milk beverage for 12 months protected against age-related cognitive decline. The preventive mechanism of this small amount of DHA is not known. Thus, ~~futher~~further investigation is needed to reveal the mechanism underlying its role in cognitive function.Trial registration: UMIN Clinical Trial Registry, UMIN000031699.

Keywords: Docosahexaenoic acid; Short-term memory; Recall; Red blood cell plasma membrane; Clinical trial

1 Introduction

Aging and extended lifespan worldwide have led to increased risk of various aging-associated diseases. In particular, the population with dementia is rapidly growing around the world (Alzheimer's Disease International, 2015). Diagnosis of dementia may be categorized into early, middle, and late stages according to progression. Cognitive function is notably impaired in patients in the late stage, which decreases their quality of life as well as that of their caregivers.

Various hypotheses about the mechanism of dementia have been proposed, but the detailed molecular mechanism of dementia onset remains unknown and no fundamental remedy has yet been established. Therefore, recent approaches to the management of dementia have focused mainly on continual preventive treatment rather than medical treatment. Many studies have reported on the relationship between dementia prevention and consumption of particular foods, especially those high in docosahexaenoic acid (DHA) (Weiser, Butt, & Mohajeri, 2016, Hashimoto, Hossain et al., 2017).

DHA, an ω-3 poly unsaturated fatty acid, is suggested to play an important role in nerve cell membrane structure and function in the brain. DHA is known to be essential for the phospholipid layers of nerves. In fact, about 60% of the

brain is composed of lipids, 22% of which is DHA (Sinclair, 1975). DHA is important in brain and eye development from early childhood (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2014). Even in the elderly, DHA consumption is positively correlated with higher brain function (Dacks, Shineman, & Fillit, 2013; Noel et al., 2006; Solfrizzi et al., 2009; Yurko-Mauro, Alexander, & Van Elswyk, 2015). Particularly notable is that DHA is substantially decreased in the brain and serum of patients with Alzheimer's disease (AD), who comprise the majority of all dementia patients (Söderberg, Edlund, Kristensson, & Dallner, 1991).

In general, the absorbability of DHA is lower than that of other fatty acids (Chen et al., 1987). Ikeda et al. (1995) found that triglycerides consisting of DHA and eicosapentaenoic acid (EPA) are more slowly hydrolyzed by pancreatic lipase and have lower absorbability than triglycerides consisting of oleic acid. In another study, lipase secretion was halved in a group of elderly people aged 65 years or older compared with a group aged less than 65 years, even in those with normal lipase secretion (Ishibashi, 1999). In other words, DHA absorption was further decreased in the elderly group. DHA is often ingested as an encapsulated dietary supplement because it is susceptible to oxidative degradation, but its absorbability is notably higher when consumed as a constituent of food, such as fish (e.g., smoked salmon) (Visioli, Risé, Barassi, Marangoni, & Galli, 2003).

Cholecystokinin, a hormone involved in bile acid secretion, is important for the digestion and absorption of lipids. Cholecystokinin secretion is mainly stimulated by food constituents such as lipids and proteins (Liddle, Goldfine, Rosen, Taplitz, & Williams, 1985). Sakaguchi, Haraguchi, Oda, and Ueda (2013) noted that the DHA absorption rate is markedly higher when DHA is ingested after a meal compared with before. These reports suggest that meal timing and hormone secretion are associated with DHA absorption. In other words, efficient DHA digestion and absorption could be induced by the intake of food containing DHA, which may affect higher cognitive function, even if the amount of DHA intake is lower than previously reported (480 mg per day; Bo et al., 2017).

In this study, we investigated intake of a dairy milk beverage containing a small amount of DHA compared with amounts reported in previous studies investigating brain function. We examined whether intake of this beverage would maintain higher cognitive function in healthy elderly people.

2 Materials and methods

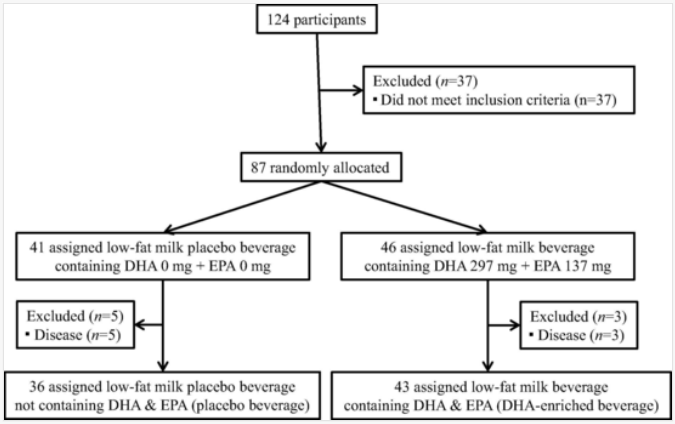
2.1 Participants

This was a 12-month randomized, double-blind, placebo-controlled, parallel-armed study. The participants were volunteers who underwent a medical examination that included anthropometry, blood biochemical assessment, and cognitive function and mental health tests. Participants were excluded if there was evidence of a medical disorder (including cognitive impairment, renal, respiratory, cardiac, or hepatic disease, diabetes mellitus, and endocrine, metabolic, or hematological disturbances) or use of any psychotropic drug/supplement that might significantly influence the outcomes of the study, or if they had hypersensitivity or allergy to low-fat milk beverages.

After screening, 87 participants were included in the investigation. The study was carried out in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The protocol was approved by the Ethics Committee of Shimane University (study number: 2899) and all participants provided written informed consent before participation.

The participants were randomly divided into two groups: a placebo group (n = 41), who ingested 200 mL of a low-fat milk beverage containing soybean oil; and a DHA group (n = 46), who ingested 200 mL of the same low-fat milk beverage containing 297 mg DHA (C22:6 [n − 3]) and 137 mg EPA (C20:5 [n − 3]) (Fig. 1). The group allocation was performed by stratified random assignment according to sex and age, as described previously (Sumiyoshi et al., 2019; Hashimoto, Matsuzaki et al., 2020; Hashimoto, Tanabe et al., 2020). The placebo and DHA beverages were consumed once daily for 12 months and were served in identical single-serving paper packages. The compositions of the placebo beverage and the DHA-enriched beverage are shown in Table 1. Both the participants and the researchers were blinded to group allocation. Both of the low-fat milk beverages were produced by Omu Milk Products Co., Ltd. (Fukuoka, Japan). The DHA-rich oil (PRORARE™) and soybean oil were provided by Fuji Oil Co., Ltd. (Osaka, Japan). We used soybean oil for the placebo low-fat milk beverage because soybean oil was one of the components of the DHA-rich oil (PRORARE™).

Fig. 1



Flow diagram of participants through the study. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Table 1

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Composition of placebo and DHA-enriched beverages.

Per 200 mL	Placebo beverage	DHA-enriched beverage
Energy (kcal)	100.2	100.2
Protein (g)	7.1	7.1
Carbohydrate (g)	10.4	10.4
Fat (g)	3.4	3.4
Ash (g)	1.5	1.5
DHA (mg)	0	297
EPA (mg)	0	137

Ingredient composition was measured by the following methods: air oven method to determine percent moisture content; combustion method (nitrogen conversion factor = 6.38) to measure protein content; Reese-Gottlieb method to measure amount of lipids; direct incineration method to determine amount of ash content; subtraction [100-{moisture (g) + protein (g) + lipid (g) + ash (g)}] to measure carbohydrates; gas chromatography to measure the amount of DHA and EPA. Energy was calculated assuming 4 kcal/g protein, 4 kcal/g carbohydrate and 9 kcal/g fat. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

2.2 Anthropometry, body composition, and assessment of dietary intake

Body weight, height, waist circumference, and systolic and diastolic blood pressure were measured by hospital nurses. Body composition was determined by bio-electrical impedance analysis (WB-150, Tanita Corporation, Tokyo, Japan). To investigate the effects of intake of the placebo and DHA milk beverages on health status and lifestyle during interventional trials, a general questionnaire was administered that posed lifestyle questions including those related to medical history. Dietary intake was estimated using a brief self-administered dietary history questionnaire for Japanese adults.

Dietary intake before and after the intervention was assessed using a validated, self-administered, brief diet history questionnaire (BDHQ) (Kobayashi et al., 2011). The BDHQ is a 4-page questionnaire with fixed portion sizes that inquires about the consumption frequency of selected food items to estimate the dietary intake of 58 food and beverage items in the preceding month. The food and beverage items listed on the BDHQ were selected from among foods commonly consumed in Japan, mainly from a food list used in the National Health and Nutrition Survey of Japan. Based on the provided responses to the BDHQ, an ad hoc computer algorithm estimated the amounts of 98 nutritional factors consumed during the previous month.

2.3 Blood sampling

Blood samples were collected at baseline before milk beverage intake and at 6 and 12 months of intervention in the morning or afternoon, after ascertaining that the participants had not consumed 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. After the clinical parameters were measured, the

samples were separated into serum, plasma, and erythrocyte (red blood cell [RBC]) aliquots, and after measuring the clinical parameters, the remaining serum and RBCs were stored at −80 °C within 8 h of collection until analysis.

2.4 Blood biochemical analysis and apolipoprotein E4 measurement

DHA and EPA levels in plasma and the RBC plasma membrane (RBC-PM) were determined by direct transmethylation (Hashimoto, Matsuzaki et al., 2020; Hashimoto, Tanabe et al., 2020), as described previously (Hashimoto et al., 1999). Briefly, 100 μL of RBCs was suspended by vigorous mixing in 1.2 mL of 4 mM EDTA solution containing 0.005% 2,6-di-t-butyl-4-methylphenol (BHT) and centrifuged at 10,000*g* for 15 min at 4 °C. After the supernatant was discarded, the resultant pellet was re-suspended by vigorous mixing in 1 mL Dulbecco's phosphate-buffered saline (PBS) (−) containing 0.005% BHT and centrifuged at 10,000*g* for 15 min at 4 °C. This procedure was repeated once. The final pellet was homogenized in 250 μL Dulbecco's PBS (−) containing 0.5% saponin and 0.005% BHT by using an ultrasonic homogenizer (Bioruptor®; Cosmobio Co., Tokyo, Japan) and subjected to measurement of fatty acid and protein concentration. 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 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 comparison with those of reference compounds and partially by gas chromatography–mass spectrometry.

Plasma total cholesterol, low- and high-density lipoprotein cholesterol, triglyceride, and glucose levels were determined using an automatic analyzer (BiOLiS 24; Tokyo Boeki Medical System Ltd., Tokyo, Japan). HbA1c was measured using a kit from TFB, Inc. (Tokyo, Japan).

Apolipoprotein E (APOE) gene mutations were measured as previously described (Hashimoto, Kato et al., 2017). APOE single nucleotide polymorphism genotyping was carried out by real-time polymerase chain reaction (7900HT; Applied Biosystems, Foster City, CA) using the TaqMan single nucleotide polymorphism genotyping assay for rs429358 and rs7412 with identification numbers C___3084793_20 and C____904973_10, respectively (Applied Biosystems).

2.5 Cognitive function evaluation test

Cognitive function was assessed using the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975), Hasegawa's Dementia Scale-Revised (HDS-R) (Imai & Hasegawa, 1994), and the Japanese version of the Montreal Cognitive Assessment (MoCA-J) (Fujiwara et al., 2010).

2.6 Statistical analysis

Results are expressed as means ± standard deviation (SD). Independent uniformly distributed data were compared using Student's *t*-test and independent non-uniformly distributed data were compared using Welch's *t*-test. Participant characteristics, cognitive test scores, and erythrocyte plasma membrane fatty acid levels at baseline, 6 months, and 12 months were analyzed by two-way (group × time) repeated measures analysis of variance (ANOVA) followed by Tukey's test. BDHQ values at baseline and 12 months were also analyzed by two-way repeated measures ANOVA. All analyses were performed using SPSS ver 13.0 (IBM, Armonk, NY), and p-values < 0.05 were considered significant.

3 Results

3.1 Demographic and clinical characteristics of the participants and assessment of dietary intake

Of the initial 124 participants, 87 healthy elderly participants met the eligibility criteria (Fig. 1). These 87 participants were randomly assigned into one of two groups, which were confirmed to have no significant differences in age, sex, cognitive function, blood parameters, and frequency of APOE ε2, APOE ε3, and APOE ε4 alleles (Table S1).

The 12-month trial was completed by 36 placebo group participants and 43 DHA group participants (Fig. 1). Except for those who withdrew from the study, the participants showed high adherence to the study protocol for 12 months (98.4% ± 0.03% for placebo group and 97.1% ± 0.06% for DHA group). Data obtained from the general questionnaire on lifestyle habits and medical history showed no differences between baseline and the end of the trial (12 months) (data not shown). No side effects that disturbed the daily lives of the participants were noted in either group.

There were no significant differences between the two groups in dietary intake information based on responses to the BDHQ (Table 2). Serum biochemical parameters did not differ between the two groups at 12 months (Table 3), indicating that DHA-enriched milk beverage intake did not affect hepatic function, renal function, or blood lipid metabolism.

Table 2

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Dietary intake assessed using the BDHQ before and after intervention in the placebo and DHA groups.

	Baseline		12 months		12 months − baseline	
	Placebo	DHA	Placebo	DHA	Placebo	DHA
Energy (kcal)	1961 ± 601	1846 ± 441	1948 ± 556	1837 ± 453	−13 ± 569	−9 ± 463
Protein (g)	75.8 ± 28.5	74.7 ± 21.6	78.2 ± 28.7	77.6 ± 18.5	2.4 ± 34.9	2.8 ± 23.1
Fat (g)	53.5 ± 21.0	51.3 ± 14.6	52.9 ± 19.1	53.0 ± 15.0	−0.7 ± 22.4	1.7 ± 15.7
Carbohydrate (g)	269.3 ± 78.8	243.5 ± 69.3	257.1 ± 73.8	237.3 ± 66.5	−12.2 ± 78.1	−6.0 ± 70.3
Total dietary fiber (g)	12.9 ± 5.1	13.3 ± 4.4	12.9 ± 5.1	12.9 ± 4.4	0.0 ± 5.4	−0.4 ± 3.6
Saturated fat (g) Monounsaturated fat (g)	14.3 ± 5.9	13.3 ± 4.3	14.0 ± 5.2	13.5 ± 4.0	−0.3 ± 6.4	0.2 ± 4.8
	18.9 ± 7.8	18.2 ± 5.7	18.5 ± 7.0	18.7 ± 5.8	−0.4 ± 8.1	0.5 ± 5.7
Polyunsaturated fat (g)	12.8 ± 4.8	12.6 ± 3.4	13.1 ± 4.9	13.4 ± 4.3	0.3 ± 5.5	0.8 ± 3.9
n-6 Polyunsaturated fat (g)	9.9 ± 3.5	9.7 ± 2.7	10.2 ± 3.7	10.4 ± 3.4	0.3 ± 3.9	0.7 ± 3.0
n-3 Polyunsaturated fat (g)	2.8 ± 1.4	2.8 ± 0.9	2.8 ± 1.4	3.0 ± 1.1	0.0 ± 1.7	0.2 ± 1.1
α-Linolenic acid (C18:3, n-3) (mg)	1506 ± 579	1490 ± 491	1562 ± 601	1619 ± 623	56 ± 659	126 ± 514
EPA (C20:5, n-3) (mg)	393 ± 280	399 ± 215	390 ± 297	411 ± 207	−2 ± 388	12 ± 245
DHA (C22:6, n-3) (mg)	659 ± 442	664 ± 442	645 ± 447	687 ± 320	−14 ± 599	22 ± 382

Values are means (per day) ± SD. There were no significant differences between the groups. Placebo group, n = 36; DHA group, n = 43. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Table 3

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Participant characteristics at baseline, 6 months, and 12 months in the placebo and DHA groups.

	Baseline		6 months		12 months	
	Placebo	DHA	Placebo	DHA	Placebo	DHA
Participants (men/women)	36 (16/20)	43 (22/21)				
Age (years)	70.1 ± 5.6	71.1 ± 5.9				
Height (cm)	156.0 ± 9.1	157.6 ± 8.6				
BMI (kg/m^2)	24.4 ± 3.2	22.6 ± 3.4	24.3 ± 3.4	22.3 ± 3.3	24.4 ± 3.3	22.7 ± 3.5
Blood pressure (mmHg)						
Systolic	149.8 ± 20.3	154.1 ± 18.5	137.9 ± 16.3	231.3 ± 18.0	147.0 ± 16.1	140.8 ± 17.5
Diastolic	85.3 ± 9.4	81.5 ± 10.3	75.8 ± 9.9	70.7 ± 10.1	86.1 ± 9.9	79.7 ± 11.7
Serum biochemical parameters						
GOT (U/L)	26.7 ± 0.7	26.7 ± 9.3	26.6 ± 13.4	27.5 ± 13.9	27.5 ± 12.9	27.5 ± 11.5
GPT (U/L)	22.4 ± 8.5	22.1 ± 10.2	24.9 ± 16.2	23.8 ± 13.3	27.6 ± 18.7	23.6 ± 13.1
γ-GTP (IU/L)	43.7 ± 50.3	45.5 ± 50.9	40.9 ± 47.0	41.6 ± 44.0	47.7 ± 68.5	37.9 ± 34.9
Albumin (g/dL)	4.3 ± 0.3	4.4 ± 0.3	4.4 ± 0.2	4.3 ± 0.3	4.4 ± 0.3	4.4 ± 0.3
Total cholesterol (mg/dL)	210.8 ± 45.0	206.9 ± 32.7	198.8 ± 27.4	191.6 ± 27.8	213.0 ± 36.9	206.5 ± 33.9

HDL cholesterol (mg/dL)	63.6 ± 15.8	67.0 ± 21.0	60.7 ± 14.5	63.2 ± 19.6	65.9 ± 16.8	67.3 ± 21.4
LDL cholesterol (mg/dL)	126.3 ± 40.1	122.5 ± 29.8	118.8 ± 21.9	109.2 ± 28.0	126.1 ± 31.5	119.3 ± 28.6
Triglyceride (mg/dL)	142.8 ± 75.9	125.5 ± 63.4	136.1 ± 79.0	134.8 ± 80.4	139.6 ± 77.0	136.6 ± 67.8
Glucose (mg/dL)	114.6 ± 20.8	110.6 ± 20.7	114.9 ± 18.1	120.9 ± 30.6	119.1 ± 24.1	117.3 ± 23.4
HbA1c (NGSP) (%)	5.9 ± 0.3	6.0 ± 0.6	5.8 ± 0.3	5.7 ± 0.4	5.9 ± 0.4	6.0 ± 0.7
Blood urea nitrogen (mg/dL)	17.5 ± 6.1	16.8 ± 4.8	18.9 ± 5.9	18.8 ± 4.6	18.4 ± 5.8	18.1 ± 5.5
Creatinine (mg/dL)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2

Values are means ± SD. There were no significant differences between groups. Placebo group, n = 36; DHA group, n = 43. BMI, body mass index; DHA, docosahexaenoic acid; GOT, glutamate–oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; γ-GTP, gamma-glutamyl transpeptidase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NGSP, National Glycohemoglobin Standardization Program; SD, standard deviation.

These results suggest the potential safety of consuming the DHA-enriched milk beverage for 12 months.

3.2 Cognitive function

Mean total MMSE, HDS-R, and MoCA-J scores of all the participants were 27.9 ± 3.3/27.1 ± 4.0, 27.3 ± 3.8/26.1 ± 5.7, and 23.6 ± 4.5/22.9 ± 5.4 (placebo group [n = 41)/DHA group [n = 46]), respectively, which were suggestive of age-related cognitive decline (Table S1). There were no differences between the two groups in the baseline scores according to the total cognitive function evaluation (Table 4). Additionally, no significant differences were found for any values compared with baseline in either group (Table 4).

Table 4

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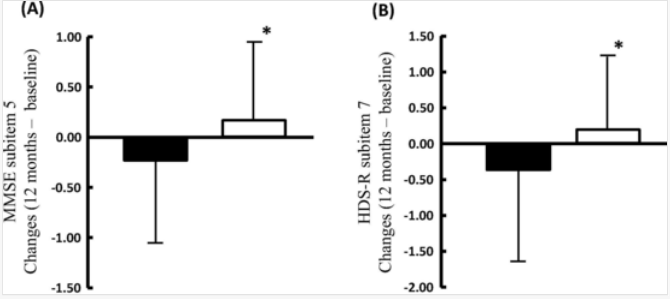
Cognitive test scores of participants who completed the study.

	Baseline		6 months		12 months		Change (12 months – baseline)	
	Placebo	DHA	Placebo	DHA	Placebo	DHA	Placebo	DHA
MMSE	28.0 ± 3.3	27.4 ± 3.5	27.6 ± 3.3	27.3 ± 3.9	27.7 ± 3.7	27.4 ± 4.5	−0.3 ± 1.8	−0.1 ± 2.3
HDS-R	27.2 ± 3.9	26.6 ± 5.3	26.6 ± 5.2	26.5 ± 5.4	26.6 ± 5.4	26.5 ± 5.8	−0.6 ± 2.7	−0.2 ± 1.8
MoCA	23.7 ± 4.6	23.5 ± 4.9	24.5 ± 5.2	23.6 ± 5.7	24.6 ± 5.3	24.1 ± 5.5	0.8 ± 2.3	0.5 ± 2.3

Values are means ± SD. There were no significant differences between groups. Placebo group, n = 36; DHA group, n = 43. DHA, docosahexaenoic acid; HDS-R, Hasegawa's Dementia Scale-Revised; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; SD, standard deviation.

However, analysis of the MMSE and HDS-R subitems, which were categorized as short-term memory, revealed a significantly greater mean change in scores from baseline to 12 months in the DHA group than in the placebo group for MMSE subitem 5 "Recall: ask for the 3 objects repeated above" (Fig. 2A) and HDS-R subitem 7 "Recall: recall 3 words for each word" (Fig. 2B). In contrast, there were no significantly different changes in the other subitems of the MMSE, HDS-R, and MoCA-J between the two groups.

Fig. 2



Changes in subitem test scores. Values are means ± SD. *p < 0.05 compared with the placebo value. Placebo group, n = 36; DHA group, n = 43. (A) MMSE subitem 5 "Recall: ask for the 3 objects repeated above". (B) HDS-R subitem 7 "Recall: recall 3 words for each word". DHA, docosahexaenoic acid; HDS-R, Hasegawa's Dementia Scale-Revised; MMSE, Mini-Mental State Examination; SD, standard deviation.

3.3 Erythrocyte plasma membrane profiles

No differences were observed in the baseline values of any fatty acid component between the two groups (Table 5). Two-way ANOVA showed significant interaction effects of month of measurement and group on EPA ($p < 0.0001$), DHA ($p = 0.016$), docosapentaenoic acid ($p = 0.031$), n-6/n-3 ratio ($p = 0.01$), DHA/AA ($p = 0.006$), and EPA/AA ($p = 0.002$) levels of the erythrocyte plasma membrane (Table 5). After 6 and 12 months of dairy beverage intake, the DHA group exhibited higher EPA, docosapentaenoic acid, and DHA concentrations as well as higher DHA/arachidonic acid (AA) and EPA/AA ratios, but lower n-6/n-3 ratio compared with baseline (Table 5). After 6 months of dairy beverage intake, the placebo group displayed lower EPA/AA ratios compared with baseline (Table 5).

Table 5

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Fatty acid profiles (mol%) of erythrocyte plasma membrane at baseline, 6 months, and 12 months in the placebo and DHA groups.

	Baseline		6 months		12 months	
	Placebo	DHA	Placebo	DHA	Placebo	DHA
Palmitic acid	22.8 ± 1.30	22.6 ± 1.05	22.0 ± 1.28	21.9 ± 1.31	22.0 ± 0.16	22.2 ± 0.94
Palmitoleic acid	0.5 ± 0.33	0.5 ± 0.19	0.5 ± 0.24	0.4 ± 0.17	0.5 ± 0.06	0.5 ± 0.17
Stearic acid	17.2 ± 1.57	17.4 ± 1.34	17.2 ± 0.87	17.0 ± 1.02	17.0 ± 0.11	17.0 ± 0.83
Oleic acid	15.9 ± 1.41	16.1 ± 1.18	14.7 ± 1.19	14.7 ± 1.15	14.7 ± 0.16	14.8 ± 1.05
Linoleic acid	11.8 ± 1.48	11.5 ± 1.71	11.4 ± 1.59	10.4 ± 1.46	11.8 ± 0.27	11.2 ± 1.65
α-Linolenic acid	0.2 ± 0.09	0.2 ± 0.05	0.2 ± 0.07	0.2 ± 0.05	0.2 ± 0.01	0.2 ± 0.07
Arachidonic acid	12.9 ± 1.77	12.9 ± 1.54	12.9 ± 1.65	12.2 ± 1.88	12.9 ± 0.26	12.3 ± 1.36
Eicosapentaenoic acid	1.9 ± 0.78	1.9 ± 0.73	1.8 ± 0.63	2.5 ± 0.73*	2.2 ± 0.14	2.7 ± 0.83*
Docosapentaenoic acid	1.7 ± 0.31	1.7 ± 0.24	1.8 ± 0.26	2.0 ± 0.33*	1.8 ± 0.04	1.9 ± 0.25*
Lignoceric acid	3.9 ± 0.49	3.9 ± 0.43	5.0 ± 0.46	5.0 ± 0.56	4.7 ± 0.07	4.6 ± 0.50
Docosahexaenoic acid	8.0 ± 1.30	8.0 ± 1.34	8.7 ± 1.36	9.7 ± 1.44*	8.5 ± 0.17	9.1 ± 0.98*
n-6/n-3	2.2 ± 0.49	2.1 ± 0.47	2.0 ± 0.41	1.6 ± 0.30*	2.0 ± 0.07	1.7 ± 0.31*
DHA/AA	0.6 ± 0.14	0.6 ± 0.15	0.7 ± 0.15	0.8 ± 0.17*	0.7 ± 0.02	0.8 ± 0.13*
EPA/AA	0.2 ± 0.08	0.2 ± 0.08	0.1 ± 0.06*	0.2 ± 0.08	0.2 ± 0.01	0.2 ± 0.09

Values are means ± SD. *, significant difference compared with baseline in each group at $p < 0.05$. Placebo group, n = 36; DHA group, n = 43. AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SD, standard deviation.

4 Discussion

We aimed to determine the effects of DHA-enriched milk beverage intake on cognitive function, RBC-PM fatty acid profile, serum lipid profiles, and serum hepatic and renal function biomarkers in healthy elderly Japanese. To our knowledge, this study is the first long-term, randomized, double-blind, placebo-controlled trial to evaluate the effects of DHA-enriched milk beverage intake on cognitive function and health in healthy elderly Japanese. DHA-enriched milk beverage intake for 12 months daily significantly increased RBC-PM DHA levels and concomitantly protected against age-related cognitive decline. Intake did not affect health, including blood pressure, serum lipid profiles, or serum hepatic and renal function biomarkers. In addition, no adverse events were reported in either group, indicating that long-term intake of the DHA-enriched milk beverage is safe.

This investigation clearly showed that mean changes in the MMSE subitem “Recall” and HDS-R subitem “Recall” scores, which were categorized as short-term memory function, from baseline to 12 months were significantly larger in the DHA group than in the placebo group (Fig. 2). These results are partially consistent with our two previous interventional studies that found greater changes in MMSE subitem scores for “Language: coping” (Hashimoto et al., 2012) and “Registration” (Hashimoto, Kato et al., 2017) from baseline to 12 months in a DHA-enriched food group than in a placebo group. The recall of three objects has been reported to be a specific and age-sensitive item on the MMSE (Anthony, LeResche, Niaz, Von Korff, & Folstein, 1982). This subitem has also been demonstrated to be affected in patients with AD (Small, Viitanen, & Backman, 1997). A meta-analysis also showed that low levels of plasma ω-3 polyunsaturated fatty acids were associated with development of dementia (Lin, Chiu, Huang, & Su, 2012). An observational study conducted in 6158 individuals aged >65 years found that high fish consumption had a protective effect on cognitive decline (Yurko-Mauro et al., 2015). In another trial with 485 participants with mild memory complaints, memory was found to improve after intake of 0.9 g/day of DHA for 24 weeks (Yurko-Mauro et al., 2010). Furthermore, analysis of human cadaver brains found less DHA in the frontal lobe and hippocampus in people with AD compared with unaffected individuals (De Mel & Suphioglu, 2014). These investigations are consistent with our finding that DHA-enriched milk beverage intake might also protect against age-related cognitive decline in elderly Japanese particpants with very mild cognitive impairment and that the effect is similar to that of ingesting fish sausage containing a higher amount of DHA (0.3 vs. 1.7 g DHA) (Bo et al., 2017).

Although we found positive effects of DHA-enriched milk beverage intake on age-related cognitive decline in the elderly, the mechanism underlying the role of DHA in this cognitive decline remains largely unknown. DHA is indicated to protect against memory impairment in amyloid β (Aβ)-peptide-infused AD model rats (Hashimoto et al., 2002; Hashimoto, Tanabe et al., 2005) and DHA-deficient elderly rats (Gamoh et al., 1999). Furthermore, DHA-induced neuroprotection was accompanied with increases in DHA/AA molar ratios, which can serve as an anti-oxidative indicator (Gamoh et al., 1999; Hashimoto et al., 2002; Hossain, Hashimoto, Gamoh, & Masumura, 1999). This may relate to the in vivo anti-oxidative effect of DHA (Hossain et al., 1999).

Moreover, DHA is reported to decrease amyloid burden in the brains of AD rats (Hashimoto et al., 2002; Hashimoto et al., 2005) by inhibiting Aβ oligomerization, Aβ-fibrillation, and Aβ-induced neurodegeneration (Hashimoto et al., 2008, Hossain et al., 2009). DHA improves learning and memory by facilitating the formation of pre- and postsynaptic proteins that enable synaptic transmission and long-term potentiation (Cao et al., 2009, Hashimoto et al., 2018). Moreover, EPA and DHA derivatives, such as resolvins and protectins, are involved in the resolution of inflammation processes (Bazan, Molina, & Gordon, 2011). The activities of these lipid mediators shut off inflammatory reactions and might inhibit Aβ-induced oxidative stress and inflammation. Long-term DHA and EPA administration is suggested to counteract age-related increases in oxidative stress, with subsequent positive effects of DHA-derived docosanoids on synaptic plasticity and cognition in aged rats (Hashimoto et al., 2015). Furthermore, low levels of DHA-derived lipid mediators, including neuroprotectin D1, which protects neurons, are seen in AD brains (Lukiw et al., 2005). Also, DHA stimulates in vitro neurogenesis and the in vivo adult brain (Kawakita, Hashimoto, & Shido, 2006). In contrast to cognitive function, significantly high levels of RBC-PM DHA and EPA were observed in the DHA group at 6 months. Increasing the RBC-PM DHA and EPA levels before a change in cognitive function leads to early induction of DHA mediators. EPA has stronger anti-inflammatory properties compared with DHA (Schunck, 2016). In addition, clinical trials have shown that EPA has stronger mood-regulating and antidepressant effects compared with DHA (Milte et al., 2011; Su et al., 2014). However, EPA levels are commonly reported to be several orders of magnitude lower in the brains of humans and rodents due to its rapid and extensive beta-oxidation (Chen and Bazinet, 2015). Indeed, DHA accounts for about 60% of polyunsaturated fatty acid in the human postmortem brain, whereas EPA accounts for 0.1% or less (Hamazaki, Hamazaki, & Inadera, 2013). Thus, the long-term effects of DHA and DHA-derived mediators might directly prevent age-related cognitive decline in the elderly.

In previous interventional studies, participants consumed DHA-enriched solid food (fish sausages) containing 1.7 g DHA and 0.4 g EPA per day (Hashimoto et al., 2012). However, the participants in the present study consumed DHA-

enriched milk beverage containing 0.3 g DHA and 0.15 g EPA per day. The DHA-enriched milk beverage was determined to protect against age-related decline in cognitive function in these healthy Japanese elderly participants. The milk beverage had much lower levels of DHA than the fish sausage, but had the same effect on cognitive function, with associated increases in DHA and EPA levels of RBC-PM. Previous reports showed that pre-emulsification of long-chain polyunsaturated fatty acids such as DHA and EPA promoted fatty acid absorption (Garaiova et al., 2007; Raatz, Redmon, Wimmergren, Donadio, & Bibus, 2009). Sausage is a solid food, whereas milk beverage is a natural emulsion. After ingestion, the sausage must first be mechanically digested and then emulsified in the intestine. Later, the sausage-derived emulsion undergoes enzymatic degradation (de-esterification), and the lipid emulsion is absorbed in the jejunum. However, the advantage of the milk beverage emulsion is that it spends less time and/or leaves less residue in the stomach than the solid sausage. Thus, the milk beverage may help to absorb more fatty acids than the sausage. This may partially explain why the smaller amount of DHA in the milk beverage had a similar effect on RBC-PM DHA profile and short-term memory.

5 Conclusion

We investigated the effect of DHA-enriched milk beverage intake on cognitive function in healthy elderly Japanese. Although the amount of DHA was lower than that previously reported, the long-term intake of the DHA emulsion increased DHA levels in the RBC-PM and prevented age-related cognitive decline. Limitations of this study include its relatively small sample size and localized sample population (i.e., a cohort of only Japanese individuals from one town). Furthermore, the preventive mechanism by the small amount of DHA (297 mg per day) remains unknown. Thus, further investigation of the effect of DHA in a larger and more diverse population, and the mechanism underlying its role in cognitive functions is needed.

6 Ethics Statement

The study was carried out in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The protocol was approved by the Ethics Committee of Shimane University (study number: 2899) and all participants provided written informed consent before participation.

CRediT authorship contribution statement

Takashi Ichinose: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft. **Masaharu Kato:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft. **Kentaro Matsuzaki:** Formal analysis, Investigation, Methodology, Writing - original draft. **Yoko Tanabe:** Data curation, Formal analysis, Investigation, Methodology, Validation. **Nobuhiko Tachibana:** Data curation, Methodology. **Miwako Morikawa:** Data curation, Investigation, Methodology. **Setsushi Kato:** Supervision. **Shuuzo Ohata:** Data curation. **Miho Ohno:** Data curation, Methodology. **Harumi Wakatsuki:** Data curation, Formal analysis, Investigation, Methodology. **Shahdat Hossain:** Data curation, Validation. **Osamu Shido:** Supervision. **Michio Hashimoto:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are very grateful to the participants of this study. We also thank ThinkSCIENCE, Inc., for language editing assistance.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Appendix A Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104195.

References

Alzheimer's Disease International. World Alzheimer Report 2015. (2015). https://www.alz.co.uk/research/WorldAlzheimerReport2015.pdf.

Anthony, J.C., LeResche, L., Niaz, U., Von Korff, M.R., & Folstein, M.F. (1982). Limits of the Mini-Mental State as a screening test for dementia and delirium among hospital patients. Psychological Medicine, 12, 397–408. doi:10.1017/s0033291700046730.

Bazan, N.G., Molina, M.F., & Gordon, W.C. (2011). Docosahexaenoic acid signalolipidomics in nutrition: Significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. Annual Review of Nutrition, 31, 321–351. doi:10.1146/annurev.nutr.012809.104635.

Bo, Y., Zhang, X., Want, Y., You, J., Cui, H., Zhu, Y., … Lu, Q. (2017). The n-3 polyunsaturated fatty acids supplementation improves the cognitive function in the Chinese elderly with mild cognitive impairment: A double-blind randomized controlled trial pii:E54. Nutrients, 9(1). doi:10.3390/nu9010054.

Cao, D., Kevala, K., Kim, J., Moon, H.S., Jun, S.B., Lovinger, D., & Kim, H.Y. (2009). Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. Journal of Neurochemistry, 111, 510–521. doi:10.1111/j.1471-4159.2009.06335.x.

Chen, I.S., Hotta, S.S., Ikeda, I., Cassidy, M.M., Sheppard, A.J., & Vahouny, G.V. (1987). Digestion, absorption and effects on cholesterol absorption of menhaden oil, fish oil concentrate and corn oil by rats. The Journal of Nutrition, 117, 1676–1680. doi:10.1093/jn/117.10.1676.

Chen, C.T., & Bazinet, R.P. (2015) β-oxidation and rapid metabolism, but not uptake regulate brain eicosapentaenoic acid levels. Prostaglandins, Leukotrienes & Essential Fatty Acids, 92, 33–40. 10.1016/j.plefa.2014.05.007. Epub 2014 Jun 5. Prostaglandins Leukot Essent Fatty Acids. 2015. PMID: 24986271

Dacks, P.A., Shineman, D.W., & Fillit, H.M. (2013). Current evidence for the clinical use of long-chain polyunsaturated n-3 fatty acids to prevent age-related cognitive decline and Alzheimer's disease. The Journal of Nutrition, Health & Aging, 17, 240–251. doi:10.1007/s12603-012-0431-3.

De Mel, D., & Suphioglu, C. (2014). Fishy business: Effect of omega-3 fatty acids on zinc transporters and free zinc availability in human neuronal cells. Nutrients, 6, 3245–3258. doi:10.3390/nu6083245.

EFSA Panel on Dietetic Products, Nutrition and Allergies. (2014). Scientific opinion on the essential composition of infant and follow-on formulae. EFSA Journal, 12, 3760. 10.2903/j.efsa.2014.3760.

Folstein, M.F., Folstein, S.E., & McHugh, P.R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of Psychiatric Research, 12, 189–198. doi:10.1016/0022-3956(75)90026-6.

Fujiwara, Y., Suzuki, H., Yasunaga, M., Sugiyama, M., Ijuin, M., Sakuma, N., … Shinkai, S. (2010). Brief screening tool for mild cognitive impairment in older Japanese: Validation of the Japanese version of the Montreal Cognitive Assessment. Geriatrics & Gerontology International, 10, 225–232. doi:10.1111/j.1447-0594.2010.00585.x.

Gamoh, S., Hashimoto, M., Sugioka, K., Shahdat Hossain, M., Hata, N., Misawa, Y., & Masumura, S. (1999). Chronic administration of docosahexaenoic acid improves reference memory-related learning ability in young rats. Neuroscience, 93, 237–241. doi:10.1016/s0306-4522(99)00107-4.

Garaiova, I., Guschina, I.A., Plummer, S.F., Tang, J., Wang, D., & Plummer, N.T. (2007). A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification. Nutrition Journal, 6, 4. doi:10.1186/1475-2891-6-4.

Hamazaki, K., Hamazaki, T., & Inadera, H. (2013). Abnormalities in the fatty acid composition of the postmortem entorhinal cortex of patients with schizophrenia, bipolar disorder, and major depressive disorder. Psychiatry Research, 210(1), 346–350. doi:10.1016/j.psychres.2013.05.006.

Hashimoto, M., Hossain, S., Agdul, H., & Shido, O. (2005). Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid beta-infused rats relates to the decreases of amyloid beta and cholesterol levels in detergent-insoluble membrane fractions. Biochimica et Biophysica Acta, 1738, 91–98. doi:10.1016/j.bbalip.2005.11.011.

Hashimoto, M., Hossain, S., Al Mamun, A., Matsuzaki, K., & Arai, H. (2017). Docosahexaenoic acid: One molecule diverse functions. Critical Reviews in Biotechnology, 37, 579–597. doi:10.1080/07388551.2016.1207153.

Hashimoto, M., Hossain, S., Katakura, M., Mamun, A.A., & Shido, O. (2018). Docosahexaenoic acid helps to lessen extinction memory in rats. Molecules, 23, 451. doi:10.3390/molecules23020451.

Hashimoto, M., Hossain, S., Shimada, T., Sugioka, K., Yamasaki, H., Fujii, Y., … Shido, O. (2002). Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. Journal of Neurochemistry, 81, 1084–1091. doi:10.1046/j.1471-4159.2002.00905.x.

Hashimoto, M., Katakura, M., Tanabe, Y., Al Mamun, A., Inoue, T., Hossain, … Shido, O. (2015). n-3 fatty acids effectively improve the reference memory-related learning ability associated with increased brain docosahexaenoic acid-derived docosanoids in aged rats. Biochimica et Biophysica Acta, 1851, 203–209. 10.1016/j.bbalip.2014.10.009.

Hashimoto, M., Kato, S., Tanabe, Y., Katakura, M., Mamun, A.A., Ohno, M., … Shido, O. (2017). Beneficial effects of dietary docosahexaenoic acid intervention on cognitive function and mental health of the oldest elderly in Japanese care facilities and nursing homes. Geriatrics & Gerontology International, 17, 330–337. doi:10.1111/ggi.12691.

Hashimoto, M., Matsuzaki, K., Kato, S., Hossain, S., Ohno, M., & Shido, O. (2020). Twelve-month studies on perilla oil intake in Japanese adults – Possible supplement for mental health. Foods, 9, E530. doi:10.3390/foods9040530.

Hashimoto, M., Shahdat, H.M., Yamashita, S., Katakura, M., Tanabe, Y., Fujiwara, H., … Shido, O. (2008). Docosahexaenoic acid disrupts in vitro amyloid beta(1–40) fibrillation and concomitantly inhibits amyloid levels in cerebral cortex of Alzheimer's disease model rats. Journal of Neurochemistry, 107, 1634–1646. doi:10.1111/j.1471-4159.2008.05731.x.

Hashimoto, M., Shinozuka, K., Gamoh, S., Tanabe, Y., Hossain, S., Kwon, Y.M., … Masumura, S. (1999). The hypotensive effect of docosahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats. The Journal of Nutrition, 129, 70–76. doi:10.1093/jn/129.1.70.

Hashimoto, M., Tanabe, Y., Fujii, Y., Kikuta, T., Shibata, H., & Shido, O. (2005). Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid β-infused rats. The Journal of Nutrition, 135, 549–555. doi:10.1093/jn/135.3.549.

Hashimoto, M., Yamashita, K., Kato, S., Tamai, T., Tanabe, Y., Mitarai, M., … Ohno, M. (2012). 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. Journal of Aging Research & Clinical Practice, 1, 193–201.

Hossain, M.S., Hashimoto, M., Gamoh, S., & Masumura, S. (1999). Antioxidative effects of docosahexaenoic acid in the cerebrum versus cerebellum and brainstem of aged hypercholesterolemic rats. Journal of Neurochemistry, 72, 1133–1138. doi:10.1046/j.1471-4159.1999.0721133.x.

Hashimoto, M., Tanabe, Y., Hossain, S., Matsuzaki, K., Ohno, M., Kato, S., … Shido, O. (2020). Intake of alpha-linolenic acid-rich perilla frutescens leaf powder decreases home blood pressure and serum oxidized low-density lipoprotein in Japanese adults. Molecules, 25, 2099.

Hossain, S., Hashimoto, M., Katakura, M., Miwa, K., Shimada, T., & Shido, O. (2009). Mechanism of docosahexaenoic acid-induced inhibition of in vitro Abeta1-42 fibrillation and Abeta1-42-induced toxicity in SH-S5Y5 cells. Journal of Neurochemistry., 111, 568–579. doi:10.1111/j.1471-4159.2009.06336.x.

Ikeda, I., Sasaki, E., Yasunami, H., Nomiyama, S., Nakayama, M., Sugano, M., … Yazawa, K. (1995). Digestion and lymphatic transport of eicosapentaenoic and docosahexaenoic acids given in the form of triacylglycerol, free acid and ethyl ester in rats. Biochimica et Biophysica Acta, 1259, 297–304. doi:10.1016/0005-2760(95)00180-8.

Imai, Y., & Hasegawa, K. (1994). The revised Hasegawa's dementia scale (HDS-R)-Evaluation of its usefulness as a screening test for dementia. Journal of the Hong Kong College of Psychiatrists, 4(Suppl 2), 20–24.

Ishibashi, T. (1999). Suigaibunnpikinou no karei niyoru henka [Aging and exocrine pancreatic function evaluated by endoscopic retrograde aspiration of pure pancreatic juice]. Okayama Igakukai Zasshi, 111, 61–69.

Kawakita, E., Hashimoto, M., & Shido, O. (2006). Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. Neuroscience, 139, 991–997. doi:10.1016/j.neuroscience.2006.01.021.

Kobayashi, S., Murakami, K., Sasaki, S., Okubo, H., Hirota, N., Notsu, A., … Date, C. (2011). Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history

questionnaires against 16 d dietary records in Japanese adults. Public Health Nutrition, 14, 1200–1211. doi:10.1017/S1368980011000504.

Liddle, R.A., Goldfine, I.D., Rosen, M.S., Taplitz, R.A., & Williams, J.A. (1985). Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. The Journal of Clinical Investigation, 75, 1144–1152. doi:10.1172/JCI111809.

Lin, P.Y., Chiu, C.C., Huang, S.Y., & Su, K.P. (2012). A meta-analytic review of polyunsaturated fatty acid compositions in dementia. The Journal of Clinical Psychiatry, 73, 1245–1254. doi:10.4088/JCP.11r07546.

Lukiw, W.J., Cui, J., Marcheselli, V.L., Bodker, M., Botkjaer, A., Gotlinger, K., … Bazan, N.G. (2005). A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. The Journal of Clinical Investigation, 115, 2774–2783. doi:10.1172/JCI25420.

Milte, C.M., Sinn, N., Buckley, J.D., Coates, A.M., Young, R.M., & Howe, P.R. (2011). Polyunsaturated fatty acids, cognition and literacy in children with ADHD with and without learning difficulties. Journal of Child Health Care, 15, 299–311.

Noel, K., Hoffman, J., Ellis, L., Yurko-Mauro, K., Cella, C., Sercus, B., & Nalysnyk, L. (2006). DHA and cognitive function in the elderly: A systematic review of the literature. Research and Practice in Alzheimer's Disease, 11, 381–387.

Raatz, S.K., Redmon, J.B., Wimmergren, N., Donadio, J.V., & Bibus, D.M. (2009). Enhanced absorption of n-3 fatty acids from emulsified compared with encapsulated fish oil. Journal of the American Dietetic Association, 109, 1076–1081. doi:10.1016/j.jada.2009.03.006.

Sakaguchi, H., Haraguchi, N., Oda, Y., & Ueda, F. (2013). Development of self-emulsifying DHA formulation. Fujifilm Research & Development, 58, 25–27.

Schunck, W.H. (2016). EPA and/or DHA? A test question on the principles and opportunities in utilizing the therapeutic potential of omega-3 fatty acids. Journal of Lipid Research, 57, 1608–1611. doi:10.1194/jlr.C071084.

Sinclair, A.J. (1975). Long-chain polyunsaturated fatty acids in the mammalian brain. The Proceedings of the Nutrition Society, 34, 287–291. doi:10.1079/pns19750051.

Small, B.J., Viitanen, M., & Backman, L. (1997). Mini-mental State Examination item scores as predictors of Alzheimer's disease: Incidence data from the Kungsholmen Project, Stockholm. The Journals of Gerontology Series A, Biological Sciences and Medical Sciences, 52, M299–M304. doi:10.1093/gerona/52a.5.m299.

Söderberg, M., Edlund, C., Kristensson, K., & Dallner, G. (1991). Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids, 26, 421–425. doi:10.1007/bf02536067.

Solfrizzi, V., Frisardi, V., Capurso, C., D'Introno, A., Colacicco, A.M., Vendemiale, G., … Panza, F. (2009). Dietary fatty acids and predementia syndromes. The Scientific World Journal, 9, 792–810. doi:10.1100/tsw.2009.82.

Su, K.P., Lai, H.C., Yang, H.T., Su, W.P., Peng, C.Y., Chang, J.P., … Pariante, C.M. (2014). Omega-3 fatty acids in the prevention of interferon- alpha induced depression: Results from a randomized, controlled trial. Biological Psychiatry, 76, 559–566.

Sumiyoshi, E., Matsuzaki, K., Sugimoto, N., Tanabe, Y., Hara, T., Katakura, M., … Shido, O. (2019). Sub-chronic consumption of dark chocolate enhances cognitive function and releases nerve growth factors: A parallel-group randomized trial. Nutrients, 11, 2800.

Visioli, F., Risé, P., Barassi, M.C., Marangoni, F., & Galli, C. (2003). Dietary intake of fish vs. formulations leads to higher plasma concentrations of n−3 fatty acids. Lipids, 38, 415–418. doi:10.1007/s11745-003-1077-x.

Weiser, M.J., Butt, C.M., & Mohajeri, M.H. (2016). Docosahexaenoic acid and cognition throughout the lifespan. Nutrients, 8, 99. doi:10.3390/nu8020099.

Yurko-Mauro, K., Alexander, D.D., & Van Elswyk, M.E. (2015). Docosahexaenoic acid and adult memory: A systematic review and meta-analysis. PLoS One, 10, e0120391. doi:10.1371/journal.pone.0120391.

Yurko-Mauro, K., McCarthy, D., Rom, D., Nelson, E.B., Ryan, A.S., Blackwell, A., … Stedman, M. (2010). Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. Alzheimer's & Dementia, 6, 456–464. doi:10.1016/j.jalz.2010.01.013.