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Arachidonic or Docosahexaenoic Acid Diet Prevents Memory Impairment in Tg2576 Mice

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Abstract. It is believed that the amyloid β-protein (Aβ) plays a causative role in the development of Alzheimer's disease (AD). The amyloid-β protein precursor (AβPP), a substrate of Aβ, and β-secretase and γ-secretase complex proteins, which process AβPP to generate Aβ, are all membrane proteins. Thus, it is reasonable to assume that alterations in brain lipid metabolism modulate AβPP and/or Aβ metabolism. However, the role of cellular polyunsaturated fatty acids in AβPP processing has not been completely understood yet. We report here that 4 months of treatment of Tg2576 mice with an arachidonic acid (ARA)- or a docosahexaenoic acid (DHA)-containing (ARA+ or DHA+) diet prevented memory impairment at 13 months of age. Although, AβPP processing to generate soluble AβPP and induce Aβ synthesis was enhanced, $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio decreased in 14-month-old Tg2576 mice fed with the ARA+ or DHA+ diet. The ARA+ or DHA+ diet did not alter the AβPP levels and the expression levels of Aβ-degrading enzymes. In cortical primary neuron cultures, ARA or DHA treatment also increased soluble AβPP and $A\beta_{1\text{-}40}$ levels, and decreased $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio, which are similar to what were observed in Tg2576 mice fed with ARA+ or DHA+ diet. These findings suggest that not only the DHA+ diet, but also the ARA+ diet could prevent cognitive dysfunction in Tg2576 mice through the alteration of AβPP processing.

Keywords: Alzheimer's disease, amyloid β-protein, amyloid β-protein precursor, arachidonic acid, docosahexaenoic acid, memory impairment

INTRODUCTION

The deposition of senile plaques in the brain is one of the pathological hallmarks of Alzheimer's disease (AD). Amyloid β-protein (Aβ) is the major component of senile plaques and is hypothesized to initiate

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a pathologic cascade that eventually leads to AD, and several lines of evidence have converged recently to demonstrate the soluble oligomers of Aβ, but not monomers or insoluble amyloid fibrils, may be responsible for synaptic dysfunction in the brains of AD patients and AD animal models [1]. Aβ is generated from the amyloid-β protein precursor (AβPP) through its sequential proteolytic cleavage catalyzed by β- and γ-secretases. β-Site AβPP cleaving enzyme (BACE) is a typical aspartyl protease, and γ-secretase is an aspartyl protease complex composed of presenilin, nicastrin, APH-1, and PEN-2, with presenilin carrying the protease active site and working intramembrane site [2]. In addition, there is a nonamyloidgenic pathway, which is mediated by α-secretase, which is a member of the disintegrin and metalloprotease (ADAM) family. This secretase produces a soluble fragment of AβPP (sAβPPα), and sAβPP is considered to have neurotrophic and neuroprotective properties [3].

It has been shown that the proteolytic activity of β-secretase and γ-secretase is modulated by membrane lipids, neutral glycosphingolipids, anionic glycerophospholipids, and cholesterol [4–6]. This may be because AβPP, BACE, and proteins consisting of the γ-secretase complex are the membrane proteins, and thus, AβPP metabolism could be modulated by membrane lipid composition. It is also known that neural tissues of mammals contain the highest concentrations of polyunsaturated fatty acids (PUFAs), including arachidonic acid (ARA) and docosahexaenoic acid (DHA) and both ARA and DHA are major constituents of cell membranes [7, 8]. Previous studies have shown that ARA and DHA play important roles in the development and maintenance of the central nervous system and the preservation of physiological and psychological function [9–11].

In the brain membrane of AD patients, the levels of ARA and DHA decreased compared with those of normal controls [12]. There have been lines of studies showing that DHA has potential neuroprotective properties [13–15], and that dietary intake of n-3 PUFAs prevents cognitive decline [16–18]. In addition, dietary intake of DHA decreases brain Aβ levels in AβPP transgenic mice [11, 13, 19, 20]. It has been reported that a lower brain uptake of docosahexaenoic acid (DHA) in APOE4 than in APOE2 mice that may limit the biodistribution of DHA in cerebral tissues [21]. It has been shown that serum EPA and DHA compositions were higher, while ARA decreased with age, and these associations remained consistent even after adjusting for corresponding fatty-acid intake [22]. This suggests that ARA may be involved in impaired functions developed with aging. However, the effect of another group of fatty acids, n-6 PUFAs, particularly ARA, on the impairment of learning memory and pathophysiology of AD remains unclarified.

In this study, we examined the effects of dietary supplementation with ARA or DHA on the cognitive function of Tg2576 mice. In addition, we determined whether the ARA+ or DHA+ diet alters Aβ levels and AβPP processing *in vivo* and *in vitro*. Here, we report that not only the DHA+ diet but also the ARA+ diet could prevent cognitive dysfunction in Tg2576 mice.

MATERIALS AND METHODS

Diets and animals

We prepared three different diets: a modified AIN-76 rodent diet, containing ARA or DHA with a constant n-6/n-3 ratio and a constant proportion of polyunsaturated, monounsaturated, and saturated fatty acids (PMS). The control diet contained 5% fat prepared by mixing palm oil, soybean oil, and linseed oil, without ARA or DHA. The ARA- or DHA-containing diet contained 5% fat prepared by mixing ARA- or DHA-enriched triacylglycerol with the oils described above. The fatty acid composition, n-6/n-3 ratio, and proportion of PMS of each diet are shown in Table 1. Dietary foods with ARA or DHA were stored at 4°C and protected from light to prevent oxidation. Female Tg2576 mice, an animal model of AD overexpressing human $A\beta PP_{695}$ with the Swedish mutation (K670N/M671L), were obtained from Taconic (Germantown, NY). All experiments were performed in accordance with Guidelines for Animal Experiments of the Animal Experimentation Committee of National Center for Geriatrics and Gerontology and that of Meijo University. Mice at 9 months of age were fed with PUFA-containing diet or control diet *ad libitum*. There were 12–14 animals in each group. There were no significant differences in the amount of feed consumed or in the weight of the mice within or between treatment groups (Supplementary Fig. 1). Cognitive dysfunctions in Tg2576 mice start to appear at around 6 months of age and are stable until 13 months of age [23]. Thus, we evaluated the effects of ARA- and DHA-containing diet on cognitive function at the age of 13 months. Behavioral analyses were carried out sequentially in accordance with the experimental schedule shown in Fig. 1A. 14-month-old Tg2576 mice were killed by inhalation of CO_2. The brain

Table 1
Fatty acid composition of experimental diets

	Control	4% ARA	4% DHA
Fatty acid (g/100 g diet)			
16:0 palmitic acid	1.33	1.32	1.37
18:0 stearic acid	0.20	0.22	0.20
18:1 oleic acid	1.51	1.43	1.53
18:2 linoleic acid	1.25	1.00	1.21
18:3 α-linolenic acid	0.62	0.63	0.36
20:3 Dihomo- γ-linolenic acid	0	0.02	0
20:4 ARA	0	0.22	0
22:6 DHA	0	0	0.24
others	0.09	0.17	0.10
Fatty acid composition (%)			
Saturated	32.42	34.22	33.32
Monounsaturated	30.24	28.52	30.53
Polyunsaturated	37.34	37.26	36.15
n-6 to n-3 ratio	2.01	1.97	2.02

was separated into the cerebral cortex, hippocampus, cerebellum, and brainstem, which were then rapidly frozen in liquid nitrogen and stored at –80°C until analysis.

Behavioral analysis

Spontaneous alternation in Y-maze test

The Y-maze test was performed, in accordance with a previous report [23]. The maze was made of gray painted plastics; each arm was 40 cm long, 12 cm high, 3 cm wide at the bottom, and 10 cm wide at the top. The arms converged at an equilateral triangular central area that was 4 cm at its longest axis. Each mouse was placed at the center of the apparatus and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded using video camera. Alternation was defined as successive entry into the three arms, on overlapping triplet sets. Alternation behavior (%) was calculated as the ratio of actual alternations to possible alternations (defined as the number of arm entries minus two), multiplied by 100.

Novel-object recognition test

The novel-object recognition test was performed, in accordance with a previous report [24]. The test procedure consisted of three sessions: habituation, training, and retention. Each mouse was individually habituated to the box (30 cm × 30 cm × 35 cm high), with 10 min of exploration in the absence of objects for 3 days (habituation session). During the training session, two objects were placed in the back corner of the box. A mouse was then placed midway at the front of the box and the total time spent exploring the two objects was recorded for 10 min. During the retention session, the animals were placed back into the same box 24 h after the training session, in which one of the familiar objects used during training was replaced with a novel object. The animals were then allowed to explore freely for 10 min and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. Preference index, that is, the ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) to the total amount of time spent exploring both objects, was used to measure cognitive function.

Cued and contextual fear conditioning tests

Cued and contextual fear conditioning tests were performed, in accordance with previous reports [25]. To measure basal levels of the freezing response (preconditioning phase), mice were individually placed in a neutral cage (17 cm × 27 cm × 12.5 cm high) for 1 min, and then in the conditioning cage (25 cm × 31 cm × 11 cm high) for 2 min. For training (conditioning phase), mice were placed in the conditioning cage, and then a 15-s tone (80 dB) was delivered as a conditioned stimulus. During the last 5 s of the tone stimulus, a foot shock of 0.6 mA was delivered as an unconditioned stimulus through a shock generator (Neuroscience Idea Co., Ltd., Osaka, Japan). This procedure was repeated four times at 15-s intervals. Cued and contextual tests were carried out 1 day after fear conditioning. For the cued test, the freezing response was measured in the neutral cage for 1 min in the presence of a continuous-tone stimulus identical to the conditioned stimulus. For the contextual test, mice were placed in the conditioning cage, and the freezing response was measured for 2 min in the absence of the conditioned stimulus.

Fatty acid analysis

Total lipids in red blood cells, the liver, and the cortex were extracted using chloroform and methanol [26]. The phospholipid fraction was separated from the extracted lipids by thin-layer chromatography using silica gel 60 (Merck, Darmstadt, Germany). The solvent system used was n-hexane/diethyl ether (70/30, v/v). The fatty acid residues in the phospholipid fraction were analyzed [27]. In brief, an

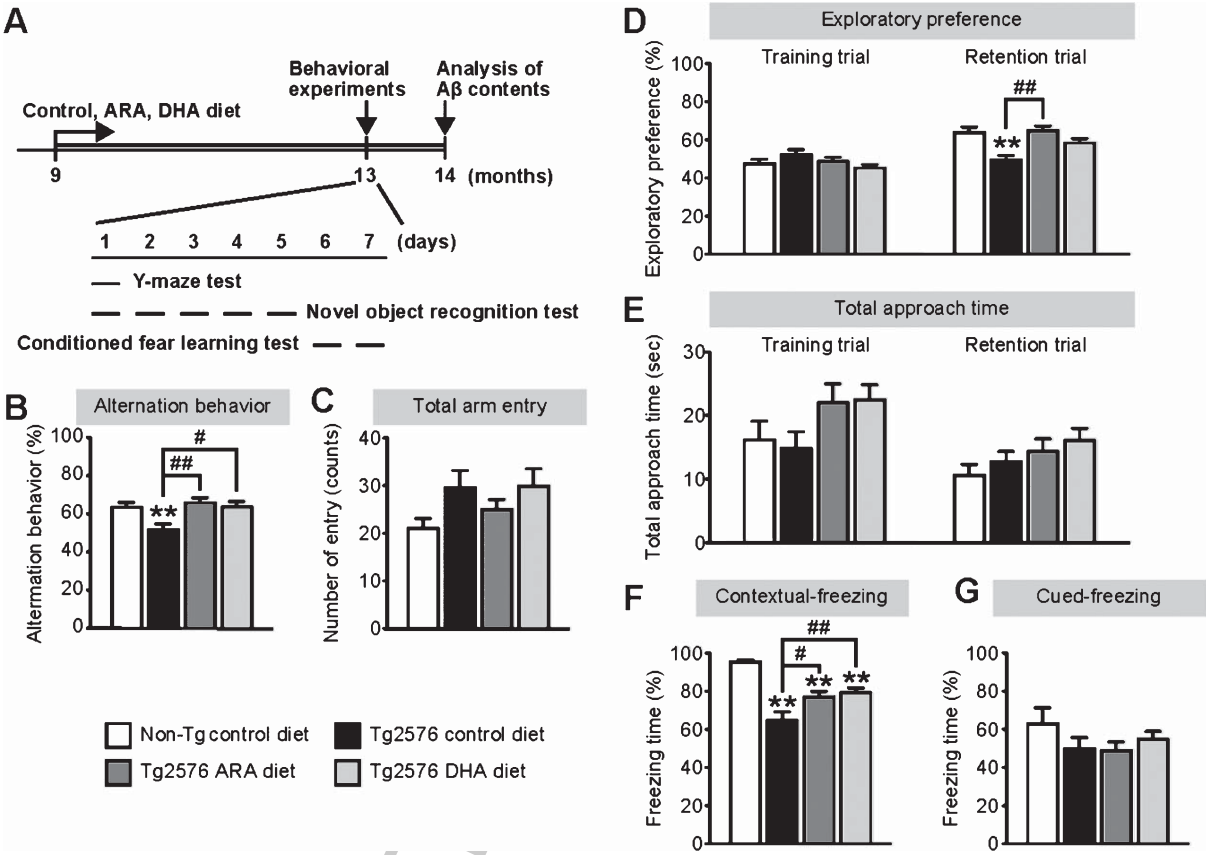

Fig. 1. Effects of ARA+ or DHA+ diet on performances of Tg2576 mice in Y-maze test, novel-object recognition test and conditioned-fear learning tests. A) Experimental schedule for Y-maze, novel-object recognition, and conditioned-fear learning tests. B, C) Effects of ARA- and DHA-containing diet on short-term memory in Y-maze test in Tg2576 mice. Spontaneous alternation behavior (B) and total arm entries (C) during an 8-min session in the Y-maze task were measured. Values indicate the mean ± SEM. One-way ANOVA showed the following: alternation behavior (B): $F(3,69) = 5.35$, $p < 0.01$, total arm entries (C): $F(3,69) = 1.84$, $p = 0.15$, $^{**}p < 0.01$ versus Control-diet-fed non-Tg mice. $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ versus Control-diet-fed Tg2576 mice. D, E) Effect of ARA- and DHA-containing diet on object recognition memory in novel-object recognition test in Tg2576 mice. One-way ANOVA showed the following: exploratory preference (D): animal group; $F(3,69) = 2.72$, $p = 0.05$, trial; $F(1,69) = 46.98$, $p < 0.01$, interaction of animal group with trial; $F(3,69) = 8.44$, $p < 0.01$, total approach time (E): animal group; $F(3,69) = 2.07$, $p = 0.11$, trial; $F(1,69) = 23.02$, $p < 0.01$, interaction of animal group with trial; $F(3,69) = 1.13$, $p = 0.34$, $^{**}p < 0.01$ verss Control-diet-fed non-Tg mice, $^{\#\#}p < 0.01$ versus Control-diet-fed Tg2576 mice. F, G) Effect of the ARA+ or DHA+ diet on contextual- and cue-dependent associative learning of Tg2576 mice in conditioned-fear learning test. The postconditioning session was carried out 24 h after the preconditioning. Context- and cue-dependent freezing times were measured for 2 and 1 min, respectively. One-way ANOVA showed the following: context-dependent test (F): $F(3,69) = 14.56$, $p < 0.01$, cue-dependent test (G): $F(3,69) = 1.22$, $p = 0.31$, $^{**}p < 0.01$ versus Control-diet-fed non-Tg mice, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ verus Control-diet-fed Tg2576 mice.

internal standard (pentadecanoic acid) was added to each sample and it was incubated in methanolic HCl at 50°C for 3 h to induce transmethylation of fatty acid residues. Fatty acid methyl esters were extracted with n-hexane and analyzed by capillary gas-liquid chromatography. Analytical conditions were as follows: 1) apparatus, Agilent 6890 (Agilent Technologies Inc., Wilmington, DE); 2) column, SP-2330 (30 m × 0.32 mm × 0.2 mm, Supelco, Inc., Bellefonte, PA); 3) carrier, He (30 cm/s); 4) column temperature, 180°C for 2 min, and increased to 220°C at a rate of +2°C/min.

Aβ ELISA

The frozen mouse cortex and hippocampus were first homogenized in 30 volumes of Tris-buffered saline (TBS; 10 mM Tris and 150 mM NaCl, pH 7.5) containing a protease inhibitor cocktail (Protease Inhibitor Cocktail, Roche, Mannheim, Germany) and then centrifuged at 450,000× *g* for 20 min at 4°C, as described elsewhere with some modifications [28]. Supernatants were saved for soluble Aβ analyses. Pellets were resuspended and further homogenized in 30 volumes of 2% SDS with the same protease inhibitors, followed by centrifugation at 15,000× *g* for 20 min. SDS

supernatants were used for insoluble Aβ determination. $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were assayed using ELISA kits (Wako Pure Chemical Industries, Osaka, Japan).

Dot blot analysis

Protein extracts were prepared from the cortex and hippocampus using TBS solution as described in the Aβ ELISA section. Samples were spotted onto a nitrocellulose membrane and blocked for 1 h in 5% (w/v) nonfat dry milk in TBS supplemented with 0.1% Tween-20. Membranes were labeled with a rabbit anti-oligomer (A11) antibody (Invitrogen, Carlsbad, CA) overnight at 4°C and incubated with horseradish peroxidase-linked anti-rabbit antibody for 1 h at room temperature. SuperSignal West Dura (Thermo Fisher Scientific, Waltham, MA) was used for chemiluminescence detection of proteins. The bands of interest were analyzed using a luminescent image analyzer LAS-3000 mini (Fujifilm, Tokyo, Japan), and signals were quantified using Multi Gauge software (Fujifilm).

Immunoblots

Protein extracts were prepared from the cortex using TBS and SDS solutions as described in the Aβ ELISA section. Protein concentration was determined by the BCA method. Equal amounts of protein samples (3 μg/well) were subjected to SDS-PAGE, and separated proteins were transferred to 0.45 μm Immobilon-P membranes (Millipore, Billerica, MA) and blocked for 1 h in 5% (w/v) nonfat dry milk in TBS supplemented with 0.1% Tween-20. The following antibodies were used: anti-ADAM 10 (Millipore), anti-BACE1 (Cell Signaling Technology, Danvers, MA), anti-nicastrin (Epitomics, Burlingame, CA), anti-presenilin 1 (PS-1, Millipore), anti-N-terminal AβPP (22C11, Millipore), anti-human sAβPPβ with Swedish mutation (IBL, Gunma, Japan), UT-18 (C-terminal fragment of AβPP antibody [29], anti-neprilysin (R&D systems), anti-insulin-degrading enzyme (IDE, Covance, Princeton, NJ), and anti-actin (Cell Signaling Technology) antibody. Membranes were labeled with a primary antibody overnight at 4°C, and incubated with a horseradish peroxidase-linked secondary antibody for 1 h at room temperature. Signal detection and quantification were described in the dot blot analysis section.

Rat primary cortical neuron culture

Cerebral cortical neuronal cultures were prepared from Sprague Dawley rats on embryonic day 17 as described previously [30]. Cortical fragments were incubated in 0.125% trypsin (Invitrogen) and 0.12 mg/ml DNase I (Roche) in Hanks' balanced salt solution at 37°C for 20 min. The fragments were then dissociated into single cells by pipetting. The dissociated single cells were suspended in a feeding medium and plated onto poly $_D$-lysine-coated 6-well plates at a cell density of 1.5×10^6/well. The feeding medium consisted of Dulbecco's modified Eagle's medium/F12 (Invitrogen) containing 0.1% bovine albumin fraction V solution (Invitrogen) and N2 supplements. The medium was changed in twice a week. Seven day after plating, ARA or DHA was added to neuronal cultures. 48 h following the commencement of the treatment, cell and medium samples were collected.

Statistical analysis

All results were expressed as the mean ± SEM for each group. Statistical analysis was performed using a statistical package, GraphPad prism software (GraphPad Software, San Diego, CA). In Fig. 1, the difference between groups was analyzed by one-way or repeated analysis of variance (ANOVA), followed by Bonferroni's test. In Figs. 2–4, one-way ANOVA and *post-hoc* Dunnett's test were carried out to compare the means of the test diet groups with those of the control diet group.

RESULTS

Effects of ARA+ or DHA+ diet on body weight and fatty acid profiles in Tg2576 mice

Previous reports have shown the effects of n-6 and n-3 PUFA in diets on the development of several diseases including AD; however, the n-6/n-3 ratio in their diets used varied. Because n-6/n-3 fatty acid ratio affects AD pathophysiology and memory function, we fixed the ratio in an experimental diet with or without PUFA (ARA or DHA), and thus n-6/n-3 fatty acid ratio and proportions of PMS were maintained to be similar. All diets contained 5 g of fat per 100 g of chow. An experimental diet had 0.22 or 0.24 g of ARA or DHA per 5 g of fat (n-6/n-3 ratio = 2:1). The fatty acid composition of each diet is shown in Table 1. 9-month-old Tg2576 mice were fed the three different diets (control diet, and ARA+ and DHA+ diets). After 4 months of dietary PUFA supplementation (13 months of age), the mice were subjected to sequential behavioral tests and their cognitive functions were evaluated (Fig. 1A). After 5 months of dietary PUFA supplementation (14 month of age), the mice were killed and brain tissue samples were collected. The mean body weights of Tg2576 mice at 14 months of age did not differ across the diet groups (Supplementary Fig. 1).

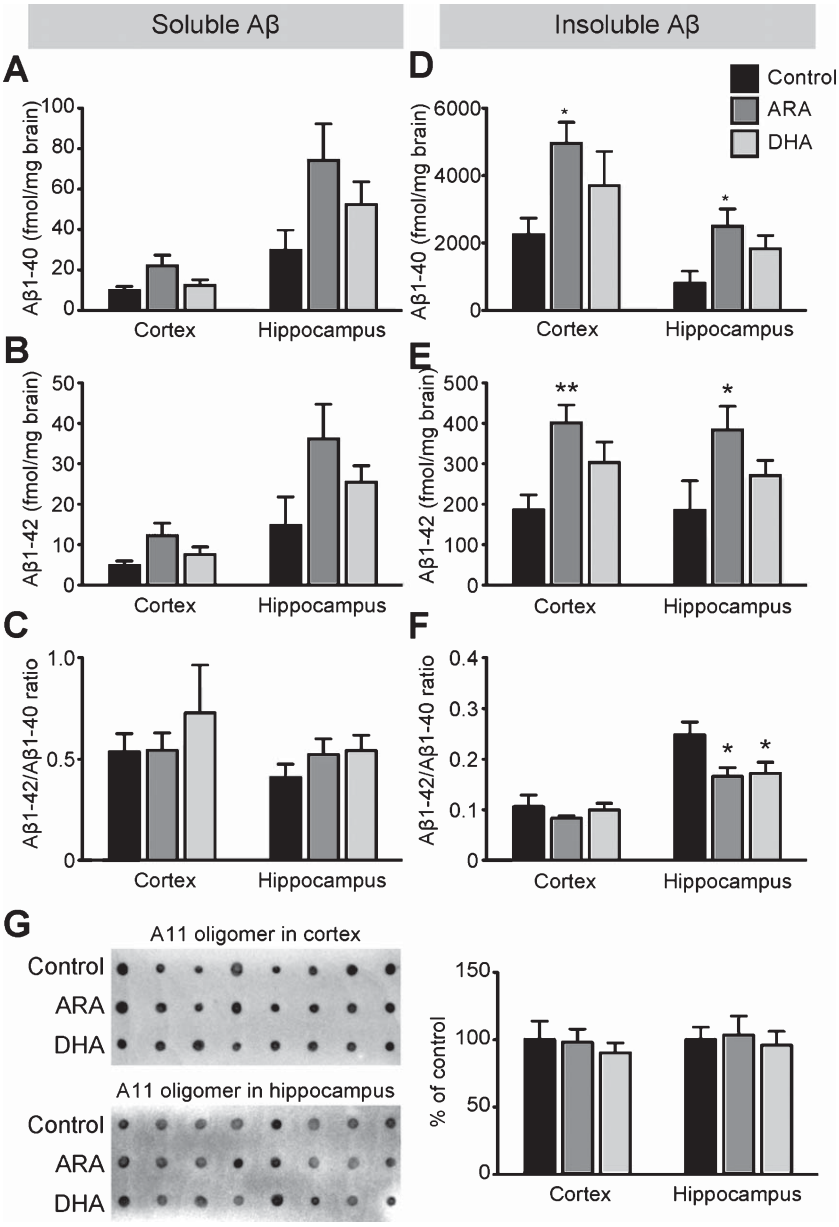

Fig. 2. Effects of ARA+or DHA+ diet on $A\beta_{40}$, $A\beta_{42}$, and oligomer Aβ levels in Tg2576 mouse brain. Soluble and insoluble Aβ levels in Tg2576 mice fed with the ARA+or DHA+ diet. Soluble $A\beta_{40}$ (A) and $A\beta_{42}$ (B), insoluble $A\beta_{40}$ (D) and $A\beta_{42}$ (E) levels, and $A\beta_{42}/A\beta_{40}$ ratio (C and F). Aβ oligomer levels in the cortex and hippocampus were determined by dot blot analysis using the anti-oligomer (A11) antibody, and the intensities of signals were determined (G). $n = 8$, each. $^{*}p < 0.05$ versus control diet.

Effects of ARA+ or DHA+ diet on impaired learning and memory in Tg2576 mice

[1] Spontaneous alternation in Y-maze test

We evaluated short-term memory in Tg2576 mice in a Y-maze test. The mice fed with the control diet showed significantly reduced spontaneous alternation behavior in the Y-maze test compared with control-diet-fed non-Tg mice (Fig. 1B), indicating an impairment of short-term memory in Tg2576 mice. The alternation behavior of Tg2576 mice fed with the ARA+ and DHA+ diets was significantly increased compared with that of Tg2576 mice fed with the control diet (Fig. 1B). These results indicate that dietary ARA and DHA prevent the impairment of short-term memory. There was no significant difference in the number

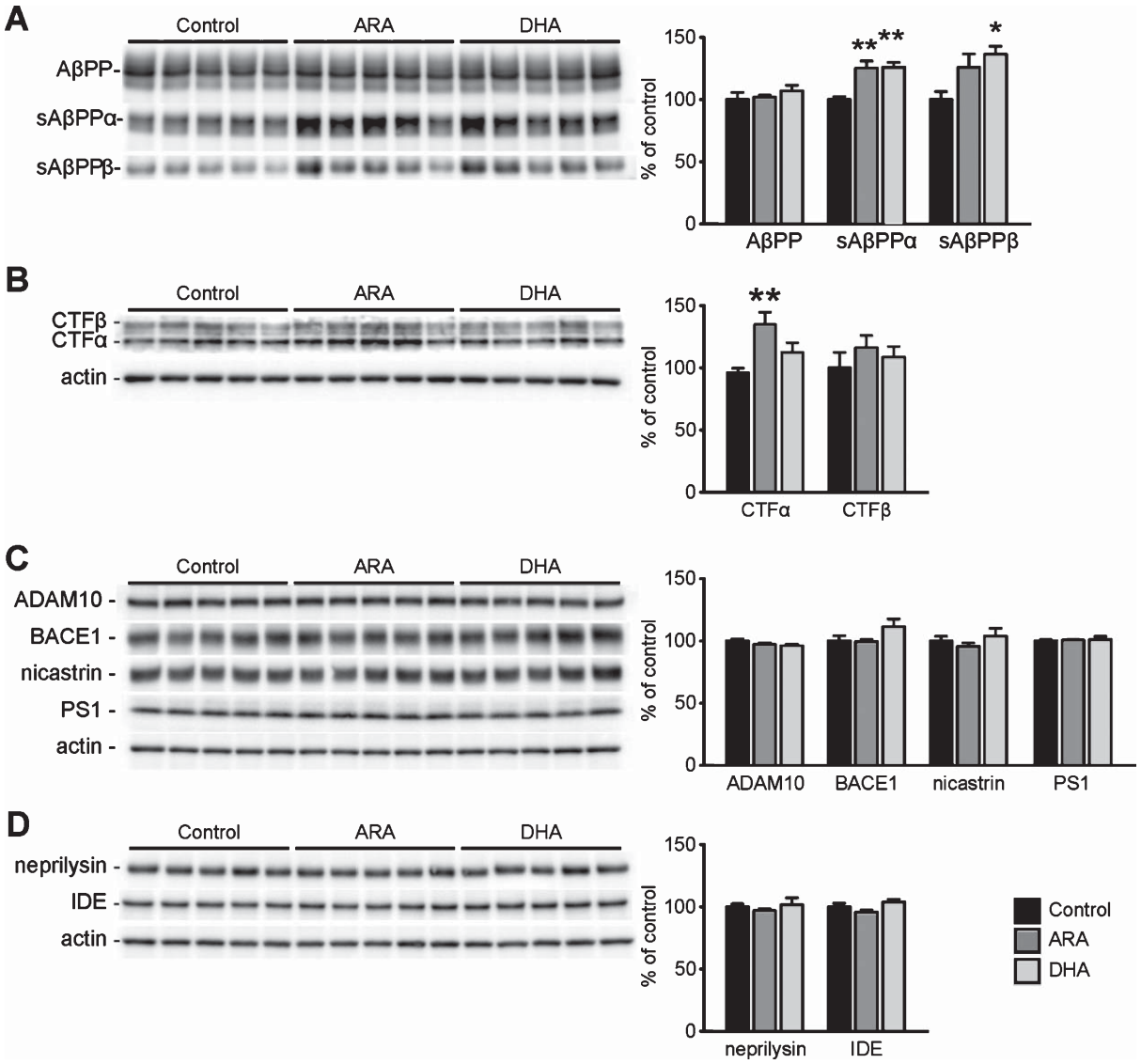

Fig. 3. Effects of the ARA+or DHA+ diet on AβPP, AβPP metabolite, AβPP metabolizing enzyme, and Aβ-degrading enzyme expression levels in Tg2576 mouse brain. Protein extracts from the cortex of 14-month-old control-fed, and mice fed with ARA+ and DHA+ diet are analyzed by western blotting. A) Expression levels of cellular AβPP and secreted AβPP fragments, sAβPPα and sAβPPβ. B) Expression levels of AβPP C-terminal fragments, CTF-α and CTF-β. C) Expression levels of ADAM10, BACE1, PS-1, and nicastrin. D) Expression levels of neplyrisin and IDE. Protein levels quantified from blots were normalized to β-actin level as a loading control. $^{*}p < 0.05$ versus control diet. $^{**}p < 0.01$ versus control diet.

of arm entries among the groups (Fig. 1C), suggesting that all mice have the same levels of motivation, curiosity, and motor function.

[2] Object recognition in novel-object recognition test

We next evaluated visual recognition memory by the novel-object recognition test. During the training session, there were no significant differences in exploratory preference between the two objects and total exploratory time among the groups (Fig. 1D, E), suggesting that all groups of mice have the similar levels of motivation, curiosity, and interest in exploring novel objects.

For the retention session, the level of exploratory preference for the novel objects in control-diet-fed Tg2576 mice was significantly decreased compared with that in the control-diet-fed non-Tg mice

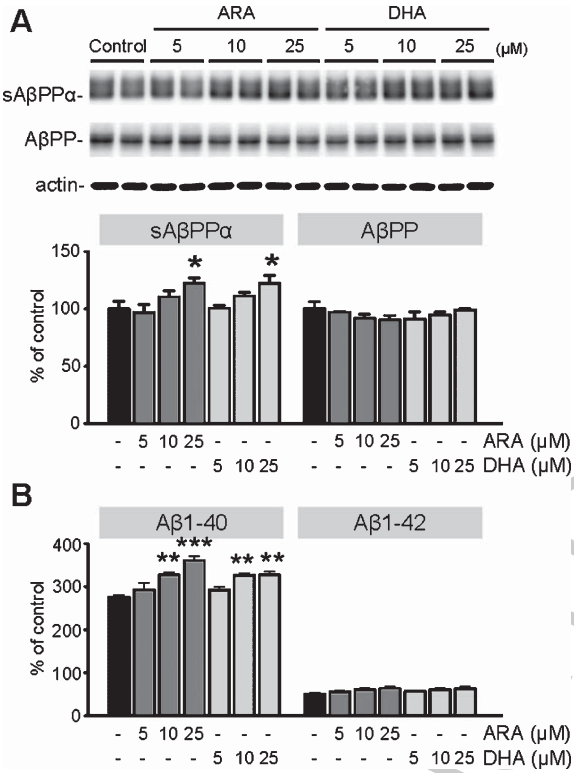

Fig. 4. Effects of ARA+ or DHA+ diet on AβPP, AβPP metabolite and Aβ levels in rat cortical primary neurons. Primary cortical neuron cultured for 7 days were treated with the indicated concentration of ARA or DHA. After 48 h treatment, cells and culture medium were harvested. A) Expression levels of cellular AβPP (cell lysate) and AβPP fragment, sAβPPα (medium). B) $Aβ_{40}$ and $Aβ_{42}$ levels in the culture medium. $^{*}p < 0.05$ versus control. $^{**}p < 0.01$ versus control.

(Fig. 1D), indicating an impairment of visual recognition memory in control-diet-fed Tg2576 mice. The ARA-containing-diet-fed Tg2576 mice spent a significantly longer time in exploring the novel object than the control-diet-fed Tg2576 mice. The DHA-containing-diet-fed Tg2576 mice also showed a tendency to spend a longer exploring time ($p = 0.08$), but this difference was not significant (Fig. 1D). These results indicate that the ARA diet prevented the development of impairment of recognition memory.

[3] Associative learning in cued and contextual fear conditioning tests

Lastly, we evaluated associative learning by the conditioned fear-learning test. In the preconditioning phase (training), the mice hardly showed any freezing response. There were no significant differences in basal levels of the freezing response between the groups (data not shown). In the contextual learning test, the control-diet-fed non-Tg mice showed a markedly strong contextual freezing response 24 h after fear conditioning. The control-diet-fed Tg2576 mice exhibited a weaker freezing response in the contextual tests than the control-diet-fed non-Tg mice (Fig. 1F), indicating an impairment of associative learning in the control-diet-fed Tg2576 mice. Although Tg2576 mice fed with the ARA+ or DHA+ diet also exhibited a weaker freezing response than control-diet-fed non-Tg mice, these mice showed an enhanced contextual freezing response compared with the control-diet-fed Tg2576 mice (Fig. 1F), indicating that dietary ARA and DHA subtly but significantly improved the impairment of associative learning. In the cued learning test, there was no significant difference in the level of the cued freezing response 24 h after fear conditioning among the groups (Fig. 1G). No alterations of the nociceptive response were found in any of the mutant mice: there was no significant difference in the minimal current required to elicit flinching or jumping among the mice (data not shown).

Effects of ARA+ or DHA+ diet on Aβ levels

In the "amyloid cascade hypothesis", it is proposed that assemblies of Aβ initiate a process leading to neuronal dysfunction, neuronal loss, and cognitive failure [31]. Therefore, we next determined whether the ARA- or DHA-containing diet affects Aβ levels in the 14-month-old Tg2576 mouse brain. The soluble levels of $Aβ_{40}$ and $Aβ_{42}$, and $Aβ_{42}/Aβ_{40}$ ratio in the ARA-containing-diet-fed mice were not significantly altered in the cortex and hippocampus (Fig. 2A-C). An unexpected finding was that the insoluble $Aβ_{40}$ and $Aβ_{42}$ levels were significantly 2-3 times higher in the ARA-containing-diet-fed mice than in the control-diet-fed mice (Fig. 2D, E). The $Aβ_{42}/Aβ_{40}$ ratio was significantly decreased in the hippocampus of mice fed with ARA+ or DHA+ diet compared with those of mice fed with the control diet (Fig. 2F). Recent studies showed that soluble Aβ oligomers are more neurotoxic and can impair cognitive function [32–35]. We next evaluated the levels of Aβ oligomers by dot blot analysis using the A11 anti-oligomeric antibody [36]. No significant differences were found between mouse groups fed the ARA+ or DHA+ diet and control diet (Fig. 2G), suggesting that the levels of soluble Aβ oligomers are not increased in the cortex and hippocampus by the ARA- or DHA-containing diet.

ARA + or DHA+diet increases levels of sAβPPs and C-terminal fragments (CTFs), but AβPP processing enzyme and Aβ-degrading enzyme expression levels are not affected

Previous reports have shown that the sAβPP fragments have neurotrophic properties and enhance synaptic plasticity and memory [37–42], and that intracellular CTFs regulate gene transcription, calcium signaling, synaptic plasticity, and memory [16, 43–46]. Thus, we first measured the expression levels of AβPP metabolites, secreted forms of AβPPs (sAβPPα and sAβPPβ), and CTFs of AβPP (CTF-α and CTF-β). The sAβPPα and sAβPPβ expression levels were higher in the cortex of mice fed with ARA+ or DHA+ diet than in the control-diet-fed-mouse cortex (Fig. 3A). The cellular AβPP level was unchanged in the group fed with ARA+ or DHA+ diet. CTF-α, but not CTF-β, level was also increased in the cortex of mice fed with ARA+ diet (Fig. 3B). Because AβPP processing was increased in cortex of mice fed with the ARA+ or DHA+ diet, we next examined whether the ARA+ or DHA+ diet had any effects on AβPP processing enzymes. We measured α-, β-, and γ-secretase (ADAM10, BACE1, PS-1, and nicastrin) expression levels in the cortex of Tg2576 mice fed with the ARA+ or DHA+ diet. The expression levels of these secretases were unchanged by ARA or DHA supplementation (Fig. 3C). Because Aβ levels are also increased by degradation of Aβ peptides, we measured the expression levels of the Aβ-degrading enzymes neprilysin and IDE. The expression levels of these enzymes were unchanged by the dietary ARA or DHA supplementation (Fig. 3D). These results suggest that the ARA+ or DHA+ diet increase Aβ, sAβPPα, sAβPPβ, and CTF-α levels by enhancing AβPP processing.

Effects of ARA+ or DHA+ diet on Aβ levels in primary rat cortical neurons in culture

Because the ARA+ or DHA+ diet enhanced AβPP processing, we determined whether the ARA+ or DHA+ diet directly enhanced AβPP processing *in vitro*. We prepared rat cortical neuron cultures and treated them with ARA or DHA for 48 h. Western blot analysis showed that ARA or DHA treatment increased sAβPPα secretion level in a dose-dependent manner (Fig. 4A). ARA or DHA treatment did not affect total AβPP content in cell lysate (Fig. 4A). $A\beta_{40}$ but not $A\beta_{42}$ level in the medium was also increased by ARA or DHA treatment (Fig. 4B). Consistent with the *in vivo* data, ARA or DHA enhanced AβPP processing.

DISCUSSION

A Western diet contains high amounts of n-6 PUFA and low amounts of n-3 PUFA, and the resulting high n-6/n-3 ratio is considered to contribute to cardiovascular disease, inflammation, and cancer [47]. It has been shown that a high n-6/n-3 ratio diet accelerates atherosclerosis and inflammation in several mouse disease models [48, 49]. Calon et al. reported that cognitive deficits in Tg2576 mice are worsened by a higher n-6/n-3 ratio diet (85:1) rather than by a lower ratio (5:1) [19]. The n-6/n-3 ratio also affects AD pathophysiology and pathology [17]; a high ratio (72:1) induces large amounts of Aβ deposition compared with a low ratio (4:1) [50]. However, the n-6/n-3 ratio in diet used in their study was varied, thus, it seems difficult to determine the real effect of target fatty acids such as DHA on AD pathologies. Therefore, in this study, we used the same n-6/n-3 ratio (2:1) in all experimental diets in order to estimate the specific effects of the ARA+ or DHA+ diet on AD pathophysiology, removing the effect of difference in n-6/n-3 fatty acid ratio. Unexpectedly, we found that not only the DHA+ diet but also the ARA+ diet improved or ameliorated cognitive deficits.

Tg2576 mice show age-related Aβ deposition and progressive memory deficits [23, 51]. DHA+ diet prevented memory impairment in aged rats [52, 53]. In agreement with these studies, Tg2576 mice showed impairments of short-term and recognition memory at the age of 13 months; however, when these mice were fed with the ARA+ or DHA+ diet from the age of 9 months, impairments of short-term memory and recognition memory in the novel-object recognition test were attenuated, suggesting that the ARA+ or DHA+ diet rescued hippocampal, perientorhinal, and prefrontal cortical dysfunctions. It has been shown that cued fear conditioning depends on the amygdala, whereas contextual fear conditioning depends on both the hippocampus and amygdala [54]. Our finding of abnormal responses in contextual but not cued fear conditioning suggests that the hippocampus-dependent associative learning is impaired in Tg2576 mice and the ARA+ or DHA+ diet prevented functional impairment of the hippocampus.

Regarding the molecular mechanism by which the ARA+ or DHA+ diet attenuates memory impairments in Tg2576 mice, previous reports have shown that cognitive dysfunction was caused by the extracellular accumulation of soluble Aβ assemblies [35, 55], and that Aβ oligomers and Aβ-derived diffusible ligands may play the crucial role of being the earliest effec-

tor that causes synaptic dysfunction and early memory loss associated with dementia in AD [55, 56]. In our study, the ARA- or DHA-containing diet increased insoluble Aβ levels in the Tg2576 mouse brain; however, toxic soluble Aβ oligomer levels were unchanged by these diet supplements. One of possible mechanism underlying the protective effect of the ARA+ or DHA+ diet on memory impairment may be the positive role(s) of sAβPPα, whose levels were increased in the brains of mice fed with the ARA+ or DHA+ diet. The positive roles of AβPP and its metabolites in synaptic plasticity and memory in a normal brain are supported by the observation that AβPP knockout mice show long-term potentiation (LTP) and memory impairment [57]. Studies on AβPP fragment function have demonstrated that the sAβPP fragments may have neurotrophic properties and enhance synaptic plasticity and memory [37, 39, 40, 58], and intracellular CTFs, whose expression levels were also increased in the brains of mice fed with the ARA+ or DHA+ diet in our study, may regulate gene transcription, calcium signaling, synaptic plasticity, and memory [11, 43, 46]. The secreted sAβPPα domain is sufficient to rescue abnormalities of AβPP knockout mice, including attenuations of impairments in spatial learning and LTP [23]. Likewise, suppression of β-secretase function in BACE1 knockout mice also impairs synaptic plasticity and memory [59]. These lines of evidence suggest that induction of sAβPP secretion by the ARA+ or DHA+ diet prevents recognition memory impairment in Tg2576 mice.

Another possible explanation may be that although the levels of Aβ oligomers remained unchanged, which was determined by dot blot analysis, the $A\beta_{42}/A\beta_{40}$ ratio was decreased in the brains of Tg2576 mice fed with the ARA+ or DHA+ diet. A high $A\beta_{42}/A\beta_{40}$ ratio is a major determinant for AD development in familial AD with presenilin mutations [60–62]. Our previous studies have shown that monomeric $A\beta_{40}$ has neuroprotective effects against metal-induced oxidative damage and $A\beta_{42}$-induced neuronal death, whereas $A\beta_{42}$ is highly amyloidogenic and thus exerting a strong neurotoxicity [32, 63]. These lines of evidence suggest that a lower $A\beta_{42}/A\beta_{40}$ ratio in Tg2576 mice fed with the ARA+ or DHA+ diet may attenuate impaired neuronal/brain functions. In support of *in vivo* data, the levels of $A\beta_{40}$ in neuronal cultures significantly increased, whereas $A\beta_{42}$ remained unchanged, indicating that the $A\beta_{42}/A\beta_{40}$ ratio decreased by the treatment with ARA or DHA. These results suggest that PUFAs alter AβPP processing to decrease $A\beta_{42}/A\beta_{40}$ ratio. One may say that the dot blot analysis data do not seem to be suitable for quantitative analysis, and future experiments may be needed to carry out in more precise manner for the quantification.

ARA is metabolized to eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes, all of which are potent mediators of inflammation. Cyclooxygenase is essential for the synthesis of prostaglandin E_2 (PGE_2), which stimulates the production of Aβ through internalization of PGE_2 receptors [64]. It has been shown that elevated expression levels of PGE_2 are observed in the brains of AD patients [65–67], and phospholipase A_2 reduction ameliorates cognitive deficits in a mouse model of AD [68–70]. However, the protective effects of ARA on brain and cognitive function also have been reported, that are, ARA-containing diet preserves synaptic plasticity in aged rats [71] and dietary supplementation of ARA and DHA improves cognitive dysfunction [72, 73]. In this study, we also observed that ARA+ diet increased Aβ levels *in vivo* independent of secretase expression levels. Most recently, it has been demonstrated that exposing differentiated neuronal cells to type III secretory phospholipase A2 and ARA increases sAβPPα secretion level and decreases the levels of $A\beta_{1-42}$ in SH-SY5Y cells and that these changes are accompanied by an increased membrane fluidity [16, 74]. Because higher levels of PUFAs in the membrane provide greater membrane fluidity [75, 76], the ARA+ diet may increase the fluidity of phospholipid membranes and influence Aβ production by alteration of activities of enzymes responsible for Aβ production. In support of this notion, previous studies have shown that reduced cholesterol levels in the call membrane reduces Aβ synthesis [6] and altered sphingomyelin level had an effect on AβPP processing and Aβ synthesis [5]. In the present study, we had no chance to determine the level of ARA and DHA in mouse brains. However, our previous studies have shown that the ARA+ and DHA+ diet had an effect on ARA and DHA levels in the mouse and rat brains [77, 78], suggesting that altered ARA and DHA levels have an effect on AβPP processing and Aβ synthesis.

There is a discrepancy between previous studies and our study in terms of the effects of the DHA+ diet on Aβ levels. In previous studies, the DHA+ diet attenuates AD pathology, decreases Aβ deposition levels, and improves cognitive dysfunction [13, 19, 20, 52, 53]. Previous study showed a protective effect associated with increased fish consumption [79]. In agreement with these lines of evidence, our study also showed that the DHA+ diet protected Tg2576 mice from memory impairment. However, unexpectedly, the DHA diet

tended to increase Aβ levels. This may be because the n-3/n-6 ratios in the diets in our study and those in previous studies are quite different. In previous studies, DHA diets contained high doses of DHA (15.0–25.4% of total fat), and diets containing various different n-6/n-3 ratios were used [19, 20]. In contrast, in our study, we used a low-dose DHA diet (4.0% of total fat) and kept the n-6/n-3 ratio constant (2:1) across all experimental diets, and we could not detect decreases in Aβ levels by the DHA+ diet. These lines of evidence suggest that not only the difference in DHA dose but also n-3/n-6 ratio may affect membrane profiles and functions of proteins associated with the membrane.

In summary, our data show that ARA or DHA, which is an essential PUFA, is required for attenuating the impairment of memory, independent of Aβ accumulation in Tg2576 mice. PUFA supplementation in diet might be a new strategy for AD therapy.

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SUPPLEMENTARY MATERIAL

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REFERENCES

[1] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **297**, 353-356.

[2] Haass C (2004) Take five–BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *EMBO J* **23**, 483-488.

[3] Thornton E, Vink R, Blumbergs PC, Van Den Heuvel C (2006) Soluble amyloid precursor protein alpha reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Res* **1094**, 38-46.

[4] Grimm MO, Grimm HS, Patzold AJ, Zinser EG, Halonen R, Duering M, Tschape JA, De Strooper B, Muller U, Shen J, Hartmann T (2005) Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol* **7**, 1118-1123.

[5] Sawamura N, Ko M, Yu W, Zou K, Hanada K, Suzuki T, Gong JS, Yanagisawa K, Michikawa M (2004) Modulation of amyloid precursor protein cleavage by cellular sphingolipids. *J Biol Chem* **279**, 11984-11991.

[6] Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci U S A* **95**, 6460-6464.

[7] Diau GY, Hsieh AT, Sarkadi-Nagy EA, Wijendran V, Nathanielsz PW, Brenna JT (2005) The influence of long chain polyunsaturate supplementation on docosahexaenoic acid and arachidonic acid in baboon neonate central nervous system. *BMC Med* **3**, 11.

[8] Bazinet RP, Laye S (2014) Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci* **15**, 771-785.

[9] Uauy R, Dangour AD (2006) Nutrition in brain development and aging: Role of essential fatty acids. *Nutr Rev* **64**, S24-33; discussion S72-S91.

[10] Janssen CI, Kiliaan AJ (2014) Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: The influence of LCPUFA on neural development, aging, and neurodegeneration. *Prog Lipid Res* **53**, 1-17.

[11] Peters BD, Voineskos AN, Szeszko PR, Lett TA, DeRosse P, Guha S, Karlsgodt KH, Ikuta T, Felsky D, John M, Rotenberg DJ, Kennedy JL, Lencz T, Malhotra AK (2014) Brain white matter development is associated with a human-specific haplotype increasing the synthesis of long chain fatty acids. *J Neurosci* **34**, 6367-6376.

[12] Prasad MR, Lovell MA, Yatin M, Dhillon H, Markesbery WR (1998) Regional membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res* **23**, 81-88.

[13] Hooijmans CR, Van der Zee CE, Dederen PJ, Brouwer KM, Reijmer YD, van Groen T, Broersen LM, Lutjohann D, Heerschap A, Kiliaan AJ (2009) DHA and cholesterol containing diets influence Alzheimer-like pathology, cognition and cerebral vasculature in APPswe/PS1dE9 mice. *Neurobiol Dis* **33**, 482-498.

[14] Bahety P, Tan YM, Hong Y, Zhang L, Chan EC, Ee PL (2014) Metabotyping of docosahexaenoic acid - treated Alzheimer's disease cell model. *PLoS One* **9**, e90123.

[15] Freund-Levi Y, Vedin I, Hjorth E, Basun H, Faxen Irving G, Schultzberg M, Eriksdotter M, Palmblad J, Vessby B, Wahlund LO, Cederholm T, Basu S (2014) Effects of supplementation with omega-3 fatty acids on oxidative stress and inflammation in patients with Alzheimer's disease: The OmegAD study. *J Alzheimers Dis* **42**, 823-831.

[16] Fukaya T, Gondaira T, Kashiyae Y, Kotani S, Ishikura Y, Fujikawa S, Kiso Y, Sakakibara M (2007) Arachidonic acid preserves hippocampal neuron membrane fluidity in senescent rats. *Neurobiol Aging* **28**, 1179-1186.

[17] Torres M, Price SL, Fiol-Deroque MA, Marcilla-Etxenike A, Ahyayauch H, Barcelo-Coblijn G, Teres S, Katsouri L, Ordinas M, Lopez DJ, Ibarguren M, Goni FM, Busquets X, Vitorica J, Sastre M, Escriba PV (2014) Membrane lipid modifications and therapeutic effects mediated by hydroxydocosahexaenoic acid on Alzheimer's disease. *Biochim Biophys Acta* **1838**, 1680-1692.

[18] Lorente-Cebrian S, Costa AG, Navas-Carretero S, Zabala M, Laiglesia LM, Martinez JA, Moreno-Aliaga MJ (2015)

An update on the role of omega-3 fatty acids on inflammatory and degenerative diseases. *J Physiol Biochem* **71**, 341-349.

[19] Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, Rostaing P, Triller A, Salem N Jr, Ashe KH, Frautschy SA, Cole GM (2004) Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* **43**, 633-645.

[20] Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L, LaFerla FM (2007) Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-beta and tau pathology via a mechanism involving presenilin 1 levels. *J Neurosci* **27**, 4385-4395.

[21] Vandal M, Alata W, Tremblay C, Rioux-Perreault C, Salem N Jr, Calon F, Plourde M (2014) Reduction in DHA transport to the brain of mice expressing human APOE4 compared to APOE2. *J Neurochem* **129**, 516-526.

[22] Otsuka R, Kato Y, Imai T, Ando F, Shimokata H (2013) Higher serum EPA or DHA, and lower ARA compositions with age independent fatty acid intake in Japanese aged 40 to 79. *Lipids* **48**, 719-727.

[23] Mouri A, Noda Y, Hara H, Mizoguchi H, Tabira T, Nabeshima T (2007) Oral vaccination with a viral vector containing Abeta cDNA attenuates age-related Abeta accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. *FASEB J* **21**, 2135-2148.

[24] Koseki T, Mouri A, Mamiya T, Aoyama Y, Toriumi K, Suzuki S, Nakajima A, Yamada T, Nagai T, Nabeshima T (2012) Exposure to enriched environments during adolescence prevents abnormal behaviours associated with histone deacetylation in phencyclidine-treated mice. *Int J Neuropsychopharmacol* **15**, 1489-1501.

[25] Mouri A, Noda Y, Shimizu S, Tsujimoto Y, Nabeshima T (2010) The role of cyclophilin D in learning and memory. *Hippocampus* **20**, 293-304.

[26] Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497-509.

[27] Sakuradani E, Kobayashi M, Shimizu S (1999) Delta 9-fatty acid desaturase from arachidonic acid-producing fungus. Unique gene sequence and its heterologous expression in a fungus, Aspergillus. *Eur J Biochem* **260**, 208-216.

[28] Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG (2001) Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci* **21**, 372-381.

[29] Tomita S, Ozaki T, Taru H, Oguchi S, Takeda S, Yagi Y, Sakiyama S, Kirino Y, Suzuki T (1999) Interaction of a neuron-specific protein containing PDZ domains with Alzheimer's amyloid precursor protein. *J Biol Chem* **274**, 2243-2254.

[30] Michikawa M, Yanagisawa K (1998) Apolipoprotein E4 induces neuronal cell death under conditions of suppressed de novo cholesterol synthesis. *J Neurosci Res* **54**, 58-67.

[31] Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* **8**, 101-112.

[32] Zou K, Kim D, Kakio A, Byun K, Gong JS, Kim J, Kim M, Sawamura N, Nishimoto S, Matsuzaki K, Lee B, Yanagisawa K, Michikawa M (2003) Amyloid beta-protein (Abeta)1-40 protects neurons from damage induced by Abeta1-42 in culture and in rat brain. *J Neurochem* **87**, 609-619.

[33] Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005) Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci* **8**, 79-84.

[34] Klyubin I, Walsh DM, Lemere CA, Cullen WK, Shankar GM, Betts V, Spooner ET, Jiang L, Anwyl R, Selkoe DJ, Rowan MJ (2005) Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity *in vivo*. *Nat Med* **11**, 556-561.

[35] Lesne S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* **440**, 352-357.

[36] Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**, 486-489.

[37] Furukawa K, Sopher BL, Rydel RE, Begley JG, Pham DG, Martin GM, Fox M, Mattson MP (1996) Increased activity-regulating and neuroprotective efficacy of alpha-secretase-derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. *J Neurochem* **67**, 1882-1896.

[38] Ishida A, Furukawa K, Keller JN, Mattson MP (1997) Secreted form of beta-amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. *Neuroreport* **8**, 2133-2137.

[39] Mattson MP (1994) Secreted forms of beta-amyloid precursor protein modulate dendrite outgrowth and calcium responses to glutamate in cultured embryonic hippocampal neurons. *J Neurobiol* **25**, 439-450.

[40] Meziane H, Dodart JC, Mathis C, Little S, Clemens J, Paul SM, Ungerer A (1998) Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice. *Proc Natl Acad Sci U S A* **95**, 12683-12688.

[41] Mucke L, Masliah E, Johnson WB, Ruppe MD, Alford M, Rockenstein EM, Forss-Petter S, Pietropaolo M, Mallory M, Abraham CR (1994) Synaptotrophic effects of human amyloid beta protein precursors in the cortex of transgenic mice. *Brain Res* **666**, 151-167.

[42] Smith-Swintosky VL, Pettigrew LC, Craddock SD, Culwell AR, Rydel RE, Mattson MP (1994) Secreted forms of beta-amyloid precursor protein protect against ischemic brain injury. *J Neurochem* **63**, 781-784.

[43] Cao X, Sudhof TC (2001) A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* **293**, 115-120.

[44] Gao Y, Pimplikar SW (2001) The gamma -secretase-cleaved C-terminal fragment of amyloid precursor protein mediates signaling to the nucleus. *Proc Natl Acad Sci U S A* **98**, 14979-14984.

[45] Kimberly WT, Zheng JB, Guenette SY, Selkoe DJ (2001) The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner. *J Biol Chem* **276**, 40288-40292.

[46] Leissring MA, Murphy MP, Mead TR, Akbari Y, Sugarman MC, Jannatipour M, Anliker B, Muller U, Saftig P, De Strooper B, Wolfe MS, Golde TE, LaFerla FM (2002) A physiologic signaling role for the gamma-secretase-derived intracellular fragment of APP. *Proc Natl Acad Sci U S A* **99**, 4697-4702.

[47] Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)* **233**, 674-688.

[48] Song Z, Yang L, Shu G, Lu H, Sun G (2013) Effects of the n-6/n-3 polyunsaturated fatty acids ratio on postpran-

dial metabolism in hypertriacylglycerolemia patients. *Lipids Health Dis* **12**, 181.

[49] Wang S, Wu D, Matthan NR, Lamon-Fava S, Lecker JL, Lichtenstein AH (2009) Reduction in dietary omega-6 polyunsaturated fatty acids: Eicosapentaenoic acid plus docosahexaenoic acid ratio minimizes atherosclerotic lesion formation and inflammatory response in the LDL receptor null mouse. *Atherosclerosis* **204**, 147-155.

[50] Julien C, Tremblay C, Phivilay A, Berthiaume L, Emond V, Julien P, Calon F (2010) High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse model. *Neurobiol Aging* **31**, 1516-1531.

[51] Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99-102.

[52] Hashimoto M, Katakura M, Tanabe Y, Al Mamun A, Inoue T, Hossain S, Arita M, 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. *Biochim Biophys Acta* **1851**, 203-209.

[53] Huang S, Dai Y, Zhang Z, Hao W, Chen H (2014) Docosahexaenoic acid intake ameliorates ketamine-induced impairment of spatial cognition and learning ability in ICR mice. *Neurosci Lett* **580**, 125-129.

[54] Phillips RG, LeDoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* **106**, 274-285.

[55] Forny-Germano L, Lyra e Silva NM, Batista AF, Brito-Moreira J, Gralle M, Boehnke SE, Coe BC, Lablans A, Marques SA, Martinez AM, Klein WL, Houzel JC, Ferreira ST, Munoz DP, De Felice FG (2014) Alzheimer's disease-like pathology induced by amyloid-beta oligomers in nonhuman primates. *J Neurosci* **34**, 13629-13643.

[56] Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, Krafft GA, Klein WL (2003) Alzheimer's disease-affected brain: Presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc Natl Acad Sci U S A* **100**, 10417-10422.

[57] Dawson GR, Seabrook GR, Zheng H, Smith DW, Graham S, O'Dowd G, Bowery BJ, Boyce S, Trumbauer ME, Chen HY, Van der Ploeg LH, Sirinathsinghji DJ (1999) Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein. *Neuroscience* **90**, 1-13.

[58] Jimenez S, Torres M, Vizuete M, Sanchez-Varo R, Sanchez-Mejias E, Trujillo-Estrada L, Carmona-Cuenca I, Caballero C, Ruano D, Gutierrez A, Vitorica J (2011) Age-dependent accumulation of soluble amyloid beta (Abeta) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-alpha (sAPP(alpha)) by modulating phosphatidylinositol 3-kinase (PI3K)/Akt-GSK-3beta pathway in Alzheimer mouse model. *J Biol Chem* **286**, 18414-18425.

[59] Laird FM, Cai H, Savonenko AV, Farah MH, He K, Melnikova T, Wen H, Chiang HC, Xu G, Koliatsos VE, Borchelt DR, Price DL, Lee HK, Wong PC (2005) BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. *J Neurosci* **25**, 11693-11709.

[60] Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada CM, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio *in vitro* and *in vivo*. *Neuron* **17**, 1005-1013.

[61] Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S (1996) Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature* **383**, 710-713.

[62] Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* **2**, 864-870.

[63] Zou K, Gong JS, Yanagisawa K, Michikawa M (2002) A novel function of monomeric amyloid beta-protein serving as an antioxidant molecule against metal-induced oxidative damage. *J Neurosci* **22**, 4833-4841.

[64] Hoshino T, Namba T, Takehara M, Nakaya T, Sugimoto Y, Araki W, Narumiya S, Suzuki T, Mizushima T (2009) Prostaglandin E2 stimulates the production of amyloid-beta peptides through internalization of the EP4 receptor. *J Biol Chem* **284**, 18493-18502.

[65] Shi J, Wang Q, Johansson JU, Liang X, Woodling NS, Priyam P, Loui TM, Merchant M, Breyer RM, Montine TJ, Andreasson K (2012) Inflammatory prostaglandin E2 signaling in a mouse model of Alzheimer disease. *Ann Neurol* **72**, 788-798.

[66] Montine TJ, Sidell KR, Crews BC, Markesbery WR, Marnett LJ, Roberts LJ, 2nd, Morrow JD (1999) Elevated CSF prostaglandin E2 levels in patients with probable AD. *Neurology* **53**, 1495-1498.

[67] Akitake Y, Nakatani Y, Kamei D, Hosokawa M, Akatsu H, Uematsu S, Akira S, Kudo I, Hara S, Takahashi M (2013) Microsomal prostaglandin E synthase-1 is induced in alzheimer's disease and its deletion mitigates alzheimer's disease-like pathology in a mouse model. *J Neurosci Res* **91**, 909-919.

[68] Sanchez-Mejia RO, Newman JW, Toh S, Yu GQ, Zhou Y, Halabisky B, Cisse M, Scearce-Levie K, Cheng IH, Gan L, Palop JJ, Bonventre JV, Mucke L (2008) Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. *Nat Neurosci* **11**, 1311-1318.

[69] Fonteh AN, Chiang J, Cipolla M, Hale J, Diallo F, Chirino A, Arakaki X, Harrington MG (2013) Alterations in cerebrospinal fluid glycerophospholipids and phospholipase A2 activity in Alzheimer's disease. *J Lipid Res* **54**, 2884-2897.

[70] Fitzpatrick AL, Irizarry MC, Cushman M, Jenny NS, Chi GC, Koro C (2014) Lipoprotein-associated phospholipase A2 and risk of dementia in the Cardiovascular Health Study. *Atherosclerosis* **235**, 384-391.

[71] Kotani S, Nakazawa H, Tokimasa T, Akimoto K, Kawashima H, Toyoda-Ono Y, Kiso Y, Okaichi H, Sakakibara M (2003) Synaptic plasticity preserved with arachidonic acid diet in aged rats. *Neurosci Res* **46**, 453-461.

[72] Kotani S, Sakaguchi E, Warashina S, Matsukawa N, Ishikura Y, Kiso Y, Sakakibara M, Yoshimoto T, Guo J, Yamashima T (2006) Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. *Neurosci Res* **56**, 159-164.

[73] Kiso Y (2011) Pharmacology in health foods: Effects of arachidonic acid and docosahexaenoic acid on the age-related

decline in brain and cardiovascular system function. *J Pharmacol Sci* **115**, 471-475.

[74] Ma QL, Yang F, Rosario ER, Ubeda OJ, Beech W, Gant DJ, Chen PP, Hudspeth B, Chen C, Zhao Y, Vinters HV, Frautschy SA, Cole GM (2009) Beta-amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling: Suppression by omega-3 fatty acids and curcumin. *J Neurosci* **29**, 9078-9089.

[75] Hulbert AJ (2003) Life, death and membrane bilayers. *J Exp Biol* **206**, 2303-2311.

[76] Le Borgne F, Guyot S, Logerot M, Beney L, Gervais P, Demarquoy J (2012) Exploration of lipid metabolism in relation with plasma membrane properties of Duchenne muscular dystrophy cells: Influence of L-carnitine. *PLoS One* **7**, e49346.

[77] Tokuda H, Kontani M, Kawashima H, Kiso Y, Shibata H, Osumi N (2014) Differential effect of arachidonic acid and docosahexaenoic acid on age-related decreases in hippocampal neurogenesis. *Neurosci Res* **88**, 58-66.

[78] Hosono T, Nishitsuji K, Nakamura T, Jung CG, Kontani M, Tokuda H, Kawashima H, Kiso Y, Suzuki T, Michikawa M (2015) Arachidonic acid diet attenuates brain Abeta deposition in Tg2576 mice. *Brain Res* **1613**, 92-99.

[79] Kalmijn S, van Boxtel MP, Ocke M, Verschuren WM, Kromhout D, Launer LJ (2004) Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. *Neurology* **62**, 275-280.