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Korea Maritime University

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Intakes of Dietary Docosahexaenoic Acid Ethyl Ester and Egg Phosphatidylcholine Improve Maze-Learning Ability in Young and Old Mice[1]

Sun-Young Lim[2] and Hiramitsu Suzuki

National Food Research Institute, Kannondai, Tsukuba, Ibaraki 305-8642, Japan

ABSTRACT The effect of dietary docosahexaenoic acid (DHA) [22:6 (n-3)] ethyl ester (EE) and egg-phosphatidylcholine (PC) on maze-learning ability in young and old mice was studied. Male Crj:CD-1 mice aged either 3 wk or 14 mo were fed a diet containing 2 g DHA-EE/100 g diet plus 3 g palm oil/100 g diet (DHA-EE Group), 5 g egg-PC/100 g diet (egg-PC Group), 1 g DHA-EE/100 g diet plus 2.5 g egg-PC/100 g diet plus 1.5 g palm oil/100 g diet (DHA-EE + egg-PC Group) or 5 g palm oil/100 g diet (Control Group) for 5 mo. Maze-learning ability was assessed 4 mo after the start of the experiment. The time required to reach the maze exit and the number of times that a mouse strayed into blind alleys in the maze were measured in three trials every 4 d. In trial 2 of young mice, performed on d 4 after the first trial, the DHA-EE group required less ($P < 0.05$) time to reach the maze exit and DHA-EE and egg-PC groups strayed ($P < 0.05$) into blind alleys fewer times than the control group. In trial 2 of old mice, the DHA-EE, egg-PC and DHA-EE + egg-PC groups needed less ($P < 0.05$) time to find the exit and spent a fewer ($P < 0.05$) number of times in blind alleys than did the control group. The DHA-EE, DHA-EE + egg-PC and egg-PC groups strayed into blind alleys fewer times than the control group in trial 3 of old mice ($P < 0.05$). Our results suggest that the intake of DHA-EE and the egg-PC diet effectively enhances maze-learning ability and brain functions in old mice. J. Nutr. 130: 1629–1632, 2000.

KEY WORDS: • *age* • *docosahexaenoic acid* • *phosphatidylcholine* • *maze-learning ability* • *mice*

Docosahexaenoic acid [DHA,[3] 22:6 (n-3)] is highly enriched in brain lipids and is one of the major highly unsaturated fatty acids (HUFA) of membrane phospholipids. It has been reported that memory and learning deficits occur in animals during the normal aging process (Bartus et al. 1978, Barnes 1979). The results from an early study suggested that the concentration of HUFA in total phospholipids from the frontal brain cortex was reduced with increasing age (Bowen et al. 1973). A decrease in HUFA levels, particularly DHA and arachidonic acid [20:4 (n-6)], in total brain lipids of aged rats has also been reported (Suzuki et al. 1989). Thus, it has been proposed that changes in the fatty acid composition and the metabolism of brain lipids occur during aging, and these appear to be correlated in part with an age-related deterioration of functions of the central nervous system (Söderberg et al. 1990). For example, in the case of Alzheimer's disease, a decrease in DHA in brain phosphatidylethanolamine has been reported (Söderberg et al. 1991). The possibility of a relationship between lipid intake and learning performance in training tests has also been investigated extensively. In such studies, it was demonstrated that long-term feeding of fish oil increased and/or maintained learning ability in mice (Suzuki et al. 1998). Aged animals, fed an α-linolenic acid-rich diet, had an increased learning ability and a longer mean survival time (Umezawa et al. 1995, Yamamoto et al. 1991). These studies suggest that (n-3) polyunsaturated fatty acids (PUFA) are an essential component for maintaining and improving brain function in aged animals. However, there have been few comparative studies of the direct effect of dietary DHA on learning ability during aging.

The central cholinergic nervous system is known to play a critical role in cognitive functions such as memory and learning (Drachman and Leavitt 1974), and impaired cholinergic transmission is thought to be associated with the memory deficits that occur during aging (Caird 1966). Although the rate of acetylcholine synthesis and release decreases during aging, there are no changes in its basal concentration (Decker 1987). The use of phosphatidylcholine (PC) as a choline source has been proposed because its consumption does not give subjects a fishy odor (Jope 1982), and it causes a much greater increase in plasma choline levels than equivalent doses of choline chloride (Wurtman et al. 1977). An increase in avoidance performance in aged mice fed dietary PC in comparison with control animals has been reported (Leathwood et al. 1982). However, because the PC used in this study originated from soybean, which also contains α-linolenic acid, the effect on learning performance could be associated with the influence of DHA, which is synthesized from α-linolenic acid in vivo. There is little information on the direct effect of PC on learning ability in mice of different ages.

In this study, the effect of diet on learning behavior was

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[2] To whom correspondence should be addressed.

[3] Abbreviations used: DHA, docosahexaenoic acid; DHA-EE, docosahexaenoic acid ethyl ester; HUFA, highly unsaturated fatty acids; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acids.

studied in animals of different age. The influence of feeding DHA ethyl ester (DHA-EE) and PC from eggs on maze-learning ability in mice was examined, and the additive effect of dietary DHA and PC on the learning task was determined in young and old mice.

MATERIALS AND METHODS

Animals and diets. Male Crj:CD-1 mice aged 3 wk or 14 mo were used. All mice originated from the same colonies and were obtained from Charles River Japan (Atsugi, Kanagawa, Japan). Mice (14 mo old) were fed a nonpurified diet, MF (Oriental Yeast, Tokyo, Japan) in our laboratory for 13 mo. DHA (DHA-95E, ethyl ester derivative of all *cis*-4,7,10,13,16,19-DHA, 95% pure) was obtained from Harima Chemicals (Tsukuba, Japan). PC (PC-98S, 98% pure) was obtained from Q. P. (Tokyo, Japan). Twenty-eight 3-wk-old mice were randomly divided into four groups of seven as follows: *1*) a group fed 5 g palm oil/100 g diet (Control Group); *2*) a group fed 2 g DHA-EE/100 g diet plus 3 g palm oil/100 g diet (DHA-EE Group); *3*) a group fed 5 g egg-PC/100 g diet (egg-PC Group); and *4*) a group fed 1 g DHA-EE/100 g diet plus 2.5 g egg-PC/100 g diet plus 1.5 g palm oil/100 g diet (DHA-EE + egg-PC Group) for 5 mo. Twenty-four 14-mo-old mice were divided into four groups of six and fed the same four diets as described for the 3-wk-old mice. Each diet contained 5 g/100 g lipid sources and is presented in **Table 1**. The main fatty acid composition of lipids in each diet group is presented in **Table 2**. In the palm oil diet, the main fatty acids were palmitic acid (16:0) and oleic acid [18:1 (n-9)]. In this study, the control diet was (n-3) fatty acid deficient, and all diets were deficient in α-linolenic acid. The experimental diets contained different amounts of DHA (0.9 g/100 g fatty acids in the egg-PC group, 13.8 g/100 g fatty acids in the DHA-EE + egg-PC group and 23.7 g/100 g fatty acids for the DHA-EE group).The palm oil, DHA-EE and DHA-EE + egg-PC diets contained 5, 3 and 1.5 g/100 g triglyceride, respectively. The egg-PC diet contained no triglyceride. The egg-PC and DHA-EE + egg-PC diets contained 5 and 2.5 g/100 g phospholipid, respectively. There was no phospholipid in the palm oil and DHA-EE diets. For the levels of choline, the palm oil, DHA-EE, egg-PC and DHA-EE + egg-PC diets contained 0.03, 0.03, 0.71 and 0.37 g/100 g, respectively. The palm oil and egg-PC diets contained no ethyl ester. The diets were stored at −25°C and fresh supplies were given to the mice once every 2 d. The diet and water were given ad libitum. All mice were housed in a standard environment, in which temperature was maintained at 24 ± 0.5°C, and the relative humidity was kept at 65 ± 5% with 12-h periods of light and dark. Body weights were measured once a week. All mice were maintained according to the guidelines for experimental animals of National Food Research Institute, Japan.

Determination of maze-learning ability. To determine maze-learning ability in mice, a video tracking and motion analysis system (EMTEC, Tama, Tokyo, Japan) was used. The analysis system, measuring the rapid real-time picture acquisition, was described previously by Suzuki et al. (1998). In this study, we used a new and more complicated maze, which contains many blind alleys (**Fig. 1**). The apparatus consisted of a maze (36 × 50 cm), a shot camera (Artist G120), image processing equipment (microprocessor for rapid information processing), a personal computer and a printer. A program for monitoring the pattern of animal movement and the time spent getting from the maze entrance to exit was adopted. This program allowed direct recording of the X-Y coordinates of mouse movement on a computer disk file. The conditioning of all mice was the training of mice to drink water and was perfected using a simple maze of three partition walls 4 mo after the start of the feeding trial. The first maze trials were done after 24 h of water deprivation so that the thirsty

TABLE 1

Diet composition[1]

Ingredient	Control (palm oil)	DHA-EE	Egg-PC	DHA-EE+ Egg-PC
		g/kg		
Corn starch	488	488	488	488
Casein	200	200	200	200
Sucrose	150	150	150	150
Cellulose	50	50	50	50
Mineral mixture[2]	40	40	40	40
Vitamin mixture[3]	20	20	20	20
L-methionine	2	2	2	2
Palm oil	50	30	—	15
DHA-EE	—	20	—	10
Egg-PC	—	—	50	25

[1] DHA-EE, docosahexaenoic acid ethyl ester; Egg-PC, egg-phosphatidylcholine; DHA-EE+egg-PC, docosahexaenoic acid ethyl ester+egg-phosphatidylcholine.

[2] The mineral and vitamin mixtures were purchased from Oriental Yeast (Tokyo, Japan), and the compositions have previously been described by Kohashi et al. (1990). The mineral mixtures contained per kg: $CaHPO_4 \cdot 2H_2O$, 14.56 g; KH_2PO_4, 25.72 g; NaH_2PO_4, 9.35 g; NaCl, 4.66 g; Ca-lactate, 35.09 g; Fe-citrate, 3.18 g; $MgSO_4$, 7.17 g; $ZnCO_3$, 0.11 g; $MnSO_4 \cdot 4H_2O$, 0.12 g; $CuSO_4 \cdot 5H_2O$, 0.03 g; KI, 0.01 g.

[3] The vitamin mixtures contained per kg: retinyl acetate, 0.1 g; cholecalciferol, 0.00025 g; α-tocopheryl acetate, 0.5 g; menadione, 0.52 g; thiamin · HCl, 0.12 g; riboflavin, 0.4 g; pyridoxine · HCl, 0.08 g; cyanocobalamine, 0.00005 g; ascorbic acid, 3 g; biotin, 0.002 g; folic acid, 0.02 g; calcium pantothenate, 0.5 g; p-aminobenzoic acid, 0.5 g; niacin, 0.6 g; inositol, 0.6 g; choline chloride, 20 g; cellulose powder, 73.1 g.

TABLE 2

Fatty acid composition of lipids in different diets[1,2]

Fatty acids	Diet: Control (palm oil)	DHA-EE	Egg-PC	DHA-EE+Egg-PC
		g/100 g total fatty acids		
16:0	46.4	38.6	37.4	37.3
18:0	5.3	2.9	11.9	6.6
18:1(n-9)	38.3	26.5	29.1	27.5
18:1(n-7)	0.7	0.5	—	0.8
18:2(n-6)	9.0	6.2	15.9	11.3
18:3(n-3)	—	—	0.1	—
20:0	0.2	0.2	—	0.1
20:1(n-9)	0.1	—	0.1	0.1
20:3(n-6)	—	—	0.3	0.1
20:4(n-6)	—	—	3.3	1.3
20:5(n-3)	—	1.4	—	0.8
22:4(n-6)	—	—	0.2	—
22:5(n-6)	—	—	0.8	0.3
22:6(n-3)	—	23.7	0.9	13.8
ΣSFA[3]	51.9	41.7	49.3	44.0
ΣMUFA[4]	39.1	27.0	29.2	28.4
Σ(n-6) PUFA[5]	9.0	6.2	20.5	13.0
Σ(n-3) PUFA	—	25.1	1.0	14.6

[1] See Table 1 for diet abbreviations.

[2] Fatty acids in each diet were methylated with 14% boron trifluoride in methanol (AOCS 1998). The analysis of fatty acid methyl esters was done by a gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a 30 m × 0.25 mm i.d. capillary column (Supelcowax 10, Supelco, Bellefonte, PA). The fatty acid methyl esters were identified by comparison of their retention times with authentic standards.

[3] SFA, saturated fatty acid.

[4] MUFA, monounsaturated fatty acid.

[5] PUFA, polyunsaturated fatty acid.

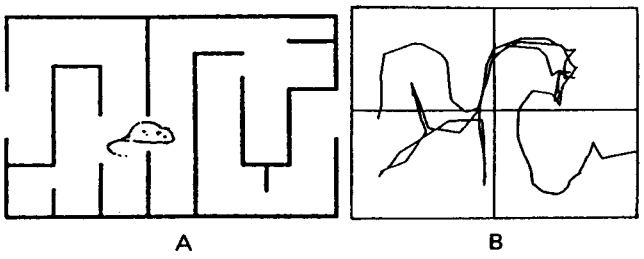

FIGURE 1 (*A*) Maze, which contains many blind alleys, used for measuring maze-learning ability and (*B*) typical maze-behavior pattern of mice.

animals sought water, which was placed outside the maze exit. The second trial was performed under the same condition on d 4 after the first trial; the third trial was conducted on d 4 after the second trial. The time required to reach the exit, the number of times that a mouse strayed into blind alleys and the behavior of a mouse in the maze were measured.

Statistics. All results were expressed as means ± SD, and significance was determined by two-way (age × diet) ANOVA using the SigmaStat statistical program package (Jandel, Erkrath, Germany). When the F-test was significant, comparisons between control and each dietary group were done using Dunnett's test at $\alpha = 0.05$.

RESULTS

Body weight and food intake. Body weight did not differ among young (44.8 ± 0.4g) or old (47.5 ± 0.5g) mice. The food consumption was 4.3 ± 0.2 g/d in both young and old

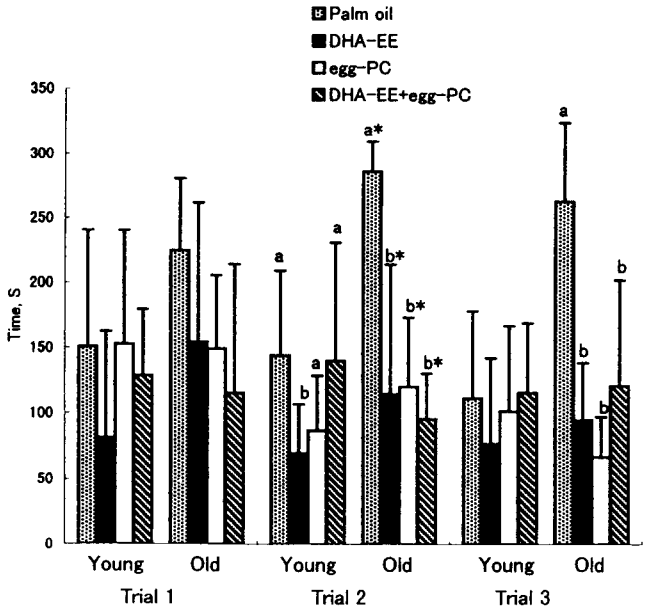

FIGURE 2 Differences in the time (s) required for young and old mice to reach the maze exit. Young mice aged 3 wk and old mice aged 14 mo were fed the control (palm oil) diet, docosahexaenoic acid ethyl ester (DHA-EE) diet, egg-phosphatidylcholine (egg-PC) diet and DHA-EE + egg-PC diet for 4 mo. Results are expressed as means ± SD, $n = 7$ for young mice and $n = 6$ for old mice. Differences were analyzed by two-way ANOVA and comparisons between the control and each dietary group were done using Dunnett's test. Bars not sharing a common letter indicate significant difference between the control and each experimental diet group at $P < 0.05$. Asterisks (*) indicate significant differences between young and old mice, $P < 0.05$.

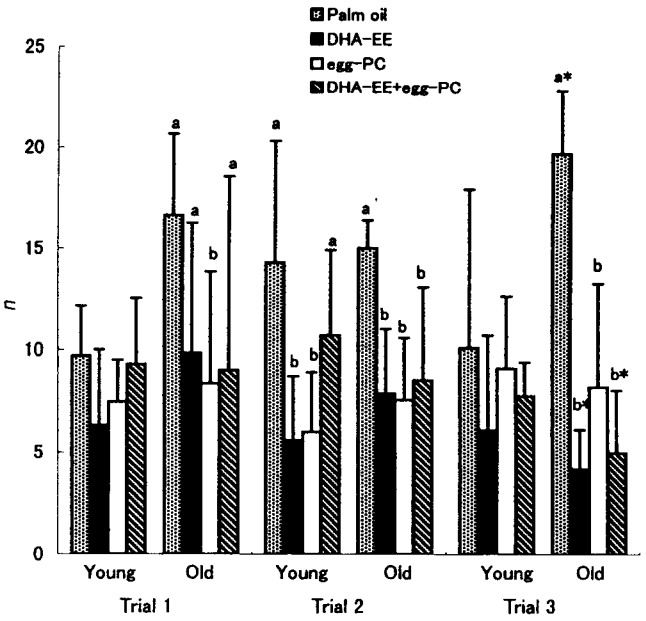

FIGURE 3 Differences in the number of times (*n*) that young and old mice strayed into blind alleys. Young mice aged 3 wk and old mice aged 14 mo were fed the control (palm oil) diet, docosahexaenoic acid ethyl ester (DHA-EE) diet, egg-phosphatidylcholine (egg-PC) diet and DHA-EE + egg-PC diet for 4 mo. Results are expressed as means ± SD, $n = 7$ for young mice and $n = 6$ for old mice. Differences were analyzed by two-way ANOVA and comparisons between the control and each dietary group were done using Dunnett's test. Bars not sharing a common letter indicate significant difference between the control and each experimental diet group at $P < 0.05$. Asterisks (*) indicate significant differences between young and old mice, $P < 0.05$.

mice, and the differences among the dietary groups were not significant.

Effect on maze-learning ability in young mice. During trial 2, the time required to reach the maze exit was significantly less in the DHA-EE diet group compared with the control group ($P < 0.05$) (**Fig. 2**). The number of times that a mouse strayed into blind alleys in the maze was significantly fewer in the DHA-EE or egg-PC groups than in the control group ($P < 0.05$) (**Fig. 3**). Groups did not differ significantly in time or number in trials 1 and 3 (Figs. 2 and 3).

Effect on maze-learning ability in old mice. During trial 2, the time required to find the exit was significantly less in the DHA-EE, egg-PC and DHA-EE + egg-PC diet groups compared with the control group ($P < 0.05$) (Fig. 2). When compared with young mice, the time required to reach the exit was longer in the palm oil, DHA-EE and egg-PC diet groups of old mice ($P < 0.05$), but the time was shorter in the DHA-EE + egg-PC diet group of old mice ($P < 0.05$). In trial 3, the mice fed DHA-EE, egg-PC and DHA-EE + egg-PC diets took less time to reach the exit ($P < 0.05$).

In trial 1, the egg-PC diet group strayed significantly into blind alleys of the maze fewer times than the control group ($P < 0.05$) (Fig. 3). During trials 2 and 3, the number of times the mice strayed into blind alleys was significantly fewer in all experimental groups compared with the control group ($P < 0.05$). In trial 3, the DHA-EE and DHA-EE + egg-PC groups of old mice strayed into blind alleys fewer times than the young mice, whereas the palm oil group of old mice strayed into blind alleys more frequently than young mice ($P < 0.05$).

DISCUSSION

The improved learning ability observed after the intake of DHA is in agreement with our previous study in which the learning ability of mice fed 5% sardine oil diet, which the lipid contains 9.3% DHA, was improved (Suzuki et al. 1998). We assume that modifications of HUFA in mouse brain after feeding of DHA, particularly an increase in DHA and a reciprocal decrease in arachidonic acid [20:4 (n-6)] levels, are associated with improved learning ability. Our previous report suggested that levels of DHA in brain lipids decrease with increasing age (Suzuki et al. 1989). Humans with senile dementia, treated for 6 mo with fish oil capsules (1400 mg DHA/d) in addition to the usual drugs, showed improvement in intellectual function (Miyanaga et al. 1995).

We reported that the majority of DHA incorporated into brain is found in microsomal, synaptosomal and mitochondrial fractions (Suzuki et al. 1997) . More recently, we showed that synaptic membrane fluidity in mice fed a DHA-rich diet was higher than that in control mice (Suzuki et al. 1998). This finding implied that DHA may influence the function of the synaptic membrane, which plays an important role in maintaining and improving learning ability and memory. Thus, the superior learning ability of mice fed the DHA-EE diet observed in this study may be associated with a higher level of synaptic membrane fluidity.

It has been shown that administration of PC containing 6.1% DHA improved the short-term memory of mice with dementia, but it did not alter the memory of normal mice (Chung et al. 1995). The improvement in memory associated with the intake of DHA-rich PC may be due to the influence of DHA on the phospholipids of neuronal membranes. However, the egg-PC diet used in this study contained very little (n-3) PUFA, including α-linolenic acid and DHA, suggesting that the increased learning ability of mice fed this PC diet might be due to a direct effect of PC. A possible explanation for the enhanced effect of PC on maze-learning ability is that an intake of PC provides choline, a precursor of the neurotransmitter acetylcholine, which will therefore be present in increased concentrations in the brain (Jope 1982). Thus, it may be that the mechanisms by which DHA-EE and egg-PC diets improved learning ability are different.

In old mice, the DHA-EE + egg-PC and egg-PC groups as well as the DHA-EE group took less time to reach the maze exit and strayed fewer times into blind alleys, indicating an increased learning ability. These results suggest that DHA and PC are necessary to maintain and improve learning ability and memory in elderly animals.

In conclusion, this study suggests that the intakes of DHA-EE and egg-PC considerably enhance maze-learning ability and brain functions in old mice.

LITERATURE CITED

Barnes, C. A. (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol. 93: 74–104.

Bartus, R. T., Fleming, D. & Johnson, H. R. (1978) Aging in the rhesus monkey: debilitating effects on short-term memory. J. Gerontol. 33: 858–871.

Bowen, D. M., Smith, C. B. & Davison, A. N. (1973) Molecular changes in senile dementia. Brain 96: 849–856.

Caird, W. K. (1966) Aging and short-term memory. J. Gerontol. 21: 295–299.

Chung, S. Y., Moriyama, T., Uezu, E., Uezu, K., Hirata, R., Yohena, N., Masuda, Y., Kokubu, T. & Yamamoto, S. (1995) Administration of phosphatidylcholine increases brain acetylcholine concentration and improves memory in mice with dementia. J. Nutr. 125: 1484–1489.

Decker, M. W. (1987) The effects of aging on hippocampal and cortical projections of the forebrain cholinergic system. Brain Res. Rev. 12: 423–438.

Drachman, D. A. & Leavitt, J. (1974) Human memory and the cholinergic system. Arch. Neurol. 30: 113–121.

Jope, R. S. (1982) Effects of phosphatidylcholine administration to rats on choline in blood and choline and acetylcholine in brain. J. Pharmacol. Exp. Ther. 220: 322–328.

Kohashi, M., Takahashi, A. & Iwai, K. (1990) Effect of a histidine-excess diet on a tetrahydrofolypolyglutamate pattern in rat liver. J. Nutr. Sci. Vitaminol. 36: 11–19.

Leathwood, P. D., Heck, E. & Mauron, J. (1982) Phosphatidyl choline and avoidance performance in 17 month-old SEC/1ReJ mice. Life Sci. 30: 1065–1071.

Miyanaga, K., Yonemura, K., Takagi, T., Kifune, R., Kishi, Y., Miyakawa, F., Yazawa, K. & Shirota, Y. (1995) Clinical effects of DHA in demented patients. J. Clin. Ther. Med. 11: 881–901.

Official Methods and Recommended Practices of the American Oil Chemists' Society (1998) AOCS Official Method Ce 1b-89, 5th ed., Champaign, IL.

Söderberg, M., Edlund, C., Kristensson, K. & Dallner, G. (1990) Lipid compositions of different regions of the human brain during aging. J. Neurochem. 54: 415–423.

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.

Suzuki, H., Hayakawa, S. & Wada, S. (1989) Effect of age on the modification of brain polyunsaturated fatty acids and enzyme activities by fish oil diet in rats. Mech. Ageing Dev. 50: 17–25.

Suzuki, H., Manabe, S., Wada, O. & Crawford, M. A. (1997) Rapid incorporation of docosahexaenoic acid from dietary sources into brain microsomal, synaptosomal and mitochondrial membranes in adult mice. Int. J. Vitam. Nutr. Res. 67: 272–278.

Suzuki, H., Park, S. J., Tamura, M. & Ando, S. (1998) Effect of the long-term feeding of dietary lipids on the learning ability, fatty acid composition of brain stem phospholipids and synaptic membrane fluidity in adult mice: a comparison of sardine oil diet with palm oil diet. Mech. Ageing Dev. 101: 119–128.

Umezawa, M., Ohta, A., Tojo, H., Yagi, H., Hosokawa, M. & Takeda, T. (1995) Dietary α-linolenate/linoleate balance influences learning and memory in the senescence-accelerated mouse (SAM). Brain Res. 669: 225–233.

Wurtman, R. J., Hirsch, M. J. & Growdon, J. H. (1977) Lecithin consumption raises serum-free-choline levels. Lancet ii: 68–69.

Yamamoto, N., Okaniwa, Y., Mori, S., Nomura, M. & Okuyama, H. (1991) Effects of a high-linoleate and a high-α-linolenate diet on the learning ability of aged rats. J. Gerontol. 46: B17–B22.

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