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Journal of Nutritional Biochemistry 18 (2007) 488–494
Journal of Nutritional Biochemistry

Effect of docosahexaenoic acid on brain 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in male ICR mice

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Received 7 June 2006; received in revised form 20 September 2006; accepted 2 October 2006

Abstract

We investigated the influence of docosahexaenoic acid ethyl ester (DHA-EE) on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in the brains of adult and aged mice. Male mice (Crlj:CD-1) were fed diets containing 3% lard plus 2% linoleic acid ethyl ester (LA-EE), or 2% DHA-EE, for 3 months. The brain HMG-CoA reductase activity of 8-month-old (adult) mice was not significantly influenced by dietary intake of DHA-EE. However, in 18-month-old (aged) mice, its activity was enhanced with dietary intake of DHA-EE. Brain HMG-CoA reductase activity and brain cholesterol content significantly increased with age. Hepatic HMG-CoA reductase activity and the cholesterol content of both adult and aged mice were reduced in DHA-EE diet groups, compared with LA-EE diet groups. The DHA percentages of brain and liver microsomal fractions increased with the intake of DHA-EE in adult and aged mice. These results suggest that DHA may enhance brain HMG-CoA reductase activity in aged mice.
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Keywords: Docosahexaenoic acid; 3-Hydroxy-3-methylglutaryl-coenzyme A reductase; Brain; Microsomal fraction; Age

1. Introduction

Studies have indicated that docosahexaenoic acid (DHA; 22:6$n-3$), an abundant component of marine fish oil, positively contributes to the maintenance of human health. Its effects have been demonstrated in the prevention and treatment of cardiovascular diseases [1], in improvement of visual function and in its use as an anticancer agent [2] and as an anti-inflammatory agent [3]. DHA helps prevent hypercholesterolemia by suppressing 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity [4].

There is high DHA content in the retinal and brain tissues of animals and humans, suggesting that this fatty acid plays an important role in neural function. This has been demonstrated in several animal studies where an increased intake of DHA in the diet influenced brain functions, such as learning ability and emotion [5–9]. In an early trial with mice, intake of docosahexaenoic acid ethyl ester (DHA-EE) or fish oil resulted in significant improvement in performance in a maze-learning ability test [5–9]. Human studies have indicated that the consumption of fish containing DHA may act as a defense against the onset of Alzheimer's disease (AD), suppress depression [10,11] and improve symptoms of dementia in the elderly [12]. Such intake also suppressed aggression in young adults [13].

Cholesterol is the principal component of certain membrane lipid domains and plays an important role in many physiological processes [14,15]. Studies indicate that cholesterol may be highly associated with AD and brain function [16–20]. In particular, HMG-CoA reductase inhibitors influence the pathogenesis of AD [19,20]. These studies suggest that appropriate regulation of cholesterol metabolism could be important in maintaining or improving brain function.

An increased intake of DHA causes reduction in both plasma and liver cholesterol levels and suppresses HMG-CoA reductase activity [4,21]. However, the influence of dietary DHA on HMG-CoA reductase activity in the brain is currently unknown. This study investigates the influence of DHA-EE-supplemented diets on HMG-CoA reductase activity in the brains of adult and aged mice.

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doi:10.1016/j.jnutbio.2006.10.001

2. Materials and methods

2.1. Diet

Lard was purchased from NOF Co., Ltd. (Tokyo, Japan). DHA-EE (95% purity) was purchased from Harima Chemicals, Inc. (Osaka, Japan). Linoleic acid ethyl ester (LA-EE; 90% purity) was purchased from Wako Pure Chemicals, Inc. (Osaka, Japan). The two experimental diet groups were: (a) 3% lard+2% LA-EE, and (b) 3% lard+2% DHA-EE. Experimental diets consisted of 48.8% corn starch, 20.0% casein, 15.0% granulated sugar, 5.0% cellulose powder, 4.0% salt mixture, 2.0% vitamin mixture and 0.2% L-methionine. Salt and vitamin mixtures were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). To prevent oxidative changes in fatty acids during storage, experimental diets were stored below −30°C.

Table 1 displays the main fatty acid composition of each experimental diet. The predominant fatty acids in all experimental diets were 16:0, 18:0, 18:1*n*−9 and 18:2*n*−6. In this study, the *n*−3 polyunsaturated fatty acid (PUFA) in the control diet was very low (DHA was not detected), as the only lipid component was lard. The main *n*−3 fatty acid contained in DHA-EE diets was 22:6*n*−3. We compared LA-EE to eliminate the influence of EE in the body.

Table 1
The main fatty acid composition of experimental diets (%)

	LA-EE	DHA-EE
14:0	1.2	1.2
16:0	17.6	15.5
18:0	9.0	8.4
16:1*n*−7	1.7	1.7
18:1*n*−9	30.2	26.3
18:1*n*−7	2.1	2.0
18:2*n*−6	35.9	5.4
18:3*n*−3	0.5	0.3
20:5*n*−3	–	1.6
22:6*n*−3	–	35.9
n−6/*n*−3	80.4	0.1

2.2. Animals

Male mice of Crlj:CD-1 (ICR) strain (4 weeks old) were obtained from Charles River Japan, Inc. (Atsugi, Kanagawa, Japan). All animals were switched from a laboratory chow (MF; Oriental Yeast Co., Ltd.) diet to an experimental diet at the age of 5 months for adult mice and at the age 15 months for aged mice. The components of the laboratory chow MF were as follows: 7.7% moisture, 23.6% protein, 5.3% fat, 2.9% dietary fiber and 6.1% ash. The predominant fatty acids of MF were 16:0, 19.4%; 18:1*n*−9, 24.2%; 18:2*n*−6, 50.1% and 0.9% DHA. Twenty adult mice (body weight, 40–50 g) were randomly divided into two groups of 10 animals. Each group was fed an experimental diet for 3 months. The same procedure was also used for aged mice. The animals were housed in suspended stainless steel cages with wire mesh bottoms. The animal room was kept at 24±0.5°C, and relative humidity was maintained at 65±5%. Room lighting consisted of 12-h periods of light and dark. The diets and water were given ad libitum. Food consumption (g/day) was monitored, and body weights were measured once a month. All mice were maintained according to the National Food Research Institute's (Tsukuba, Ibaraki, Japan) guidelines for the use of experimental animals.

2.3. Preparation of plasma samples and liver and brain homogenates

At the end of feeding trials, all mice fasted for 24 h before being anesthetized with diethyl ether. Blood from the inferior vena cava was then collected with a heparinized syringe and put into ice-cold tubes. Plasma was separated by centrifugation at 900×*g* for 20 min at 4°C. After blood collection, the whole liver and brain of each mouse were removed. A portion of the liver was homogenized with 0.05 mol/L ice-cold phosphate-buffered saline (pH 7.4) using a Teflon glass homogenizer. Brain homogenate samples were prepared from half of the brain (the right and left brain hemispheres were used at random) with 0.32 M ice-cold sucrose (9 ml/g tissue) using a Teflon glass homogenizer. Plasma samples and liver and brain homogenates were stored at −30°C until lipid and fatty acid analyses.

2.4. Preparation of liver and brain microsomal fractions

The liver (500 mg) and the remaining half of the brain were homogenized with 0.05 mol of ice-cold phosphate buffer (pH 7.4), 30 mM EDTA, 250 mM NaCl and 1 mM dithiothreitol using a Teflon glass homogenizer. The homogenate was centrifuged at 5000×*g* for 15 min. The resulting supernatant fraction was centrifuged at 15,000×*g* for 15 min. Microsomal fractions were obtained by centrifuging the 15,000×*g* supernatant fraction for 60 min at 105,000×*g*. The pellet containing microsomes was resuspended in 0.5 ml of the same buffer. Protein concentrations were determined by the method of Lowry et al. [22] using bovine serum albumin as standard.

2.5. Assay of HMG-CoA reductase

Hepatic and brain HMG-CoA reductase activities were measured according to the method of Shapiro et al. [23]. The liver (100 μg of protein) and brain microsomal (150 μg of protein) solutions were incubated with 0.1 M potassium phosphate buffer (pH 7.2), 20 mM EDTA, 40 μM NADPH, 10 mM dithiothreitol and 1 mM [^{14}C]HMG-CoA (4000–5000 dpm/nmol) for 30 min at 37°C. HCl (6 mol/L) was added to stop the reaction, and samples were further incubated for 30 min at 37°C. After centrifugation at 3000×*g* for 10 min, an aliquot of the supernatant was applied to a silica gel plate and developed in acetone–benzene (1:1, vol/vol). The area corresponding to mevalonolactone was removed, mixed with an ACSII scintillation cocktail (GE Healthcare Bio-Sciences Corp., New Jersey, USA) and counted for radioactivity.

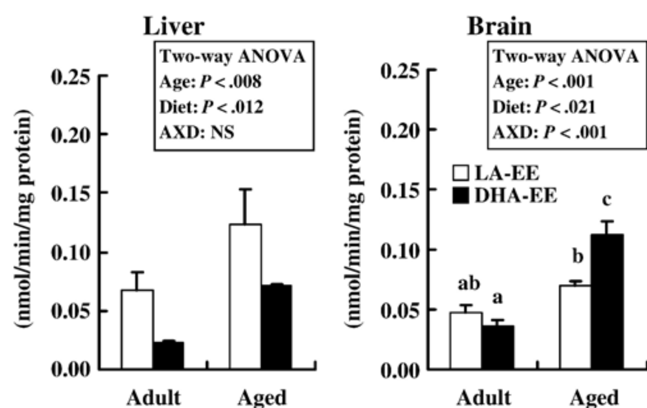

Fig. 1. HMG-CoA reductase activity of the liver and brain from adult and aged mice fed the LA-EE diet or the DHA-EE diet ($n=5$–6). Values for each dietary group with different letters are significantly different at $P<.05$ (Spjotroll–Stoline test).

2.6. Analysis of lipids and fatty acids

Total cholesterol (T-chol) concentrations in samples were determined with Wako's commercial analytical kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The fatty acids of total lipids from liver and brain homogenates were derivatized in a 0.5-mol/L NaOH methanol solution and in a 14% boron trifluoride methanol solution [24]. They were measured with a gas chromatograph (GC-18A; Shimadzu Co., Ltd., Kyoto, Japan) equipped with a fused silica capillary column Supelcowax 10 (30 m×0.25 mm i.d.; Supelco Co., Ltd., Bellefonte, USA) and manipulated with a Class 10 workstation (Shimadzu Co., Ltd.). The carrier gas was helium (flow, 1 ml/min), with a split injection of 40:1. The temperature profiles were as follows: initial temperature=175°C, heating rate=1°C/min, final temperature=225°C (final time=10 min), injector temperature=250°C and detector temperature=270°C. The fatty acids were identified by comparing their retention times with those of standard purified fatty acids.

2.7. Statistical analysis

All results were expressed as mean±S.E. The statistical significance of differences in lipid components, HMG-CoA reductase activity and fatty acid percentages between samples was determined by two-way analysis of variance (ANOVA; age and diet) using the STATISTICA statistical program package (StatSoft, Inc., Oklahoma, USA). For interactions between age and diet, one-way ANOVA was used to test for the significance of differences ($P<.05$, Spjotroll–Stoline test) between ages and/or between diets.

3. Results

There were no marked differences in average food consumption between adult groups (LA-EE diet group, 4.5 g/day/mouse; DHA-EE diet group, 4.5 g/day/mouse) or between aged groups (LA-EE diet group, 4.1 g/day/mouse; DHA-EE diet group, 3.9 g/day/mouse). In adult mice, the final body weights were as follows: LA-EE diet group, 45.2±1.0 g; DHA-EE diet group, 48.1±0.8 g. In aged mice, the final body weights were as follows: LA-EE diet group, 49.9±1.5 g; DHA-EE diet group, 47.8±0.7 g.

Fig. 1 displays the activities of HMG-CoA reductase in microsomal fractions from the livers and brains of adult and aged mice fed the LA-EE diet or the DHA-EE diet. HMG-CoA reductase activities from liver microsomal fractions in adult and aged mice were significantly lower in mice from the DHA-EE diet groups than from mice in the LA-EE diet groups. These activities were significantly higher in aged mice than in adult mice. Brain HMG-CoA reductase activity also accreted with aging. However, the activity of the enzyme in brain microsomal fractions was significantly greater in the DHA-EE diet group than in the LA-EE diet group in aged mice.

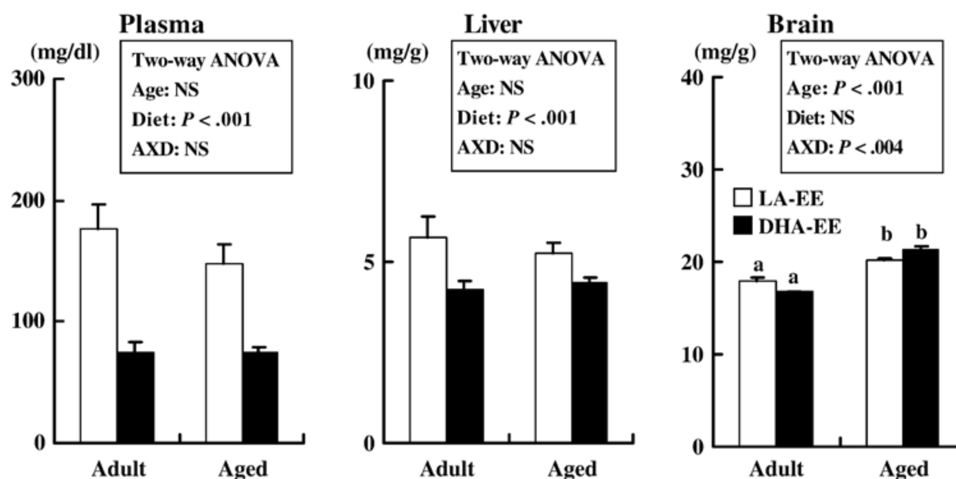

Fig. 2. Cholesterol content of plasma, liver and brain from adult and aged mice fed the LA-EE diet or the DHA-EE diet ($n=10$). Values for each dietary group with different letters are significantly different at $P<.05$ (Spjotroll–Stoline test).

Table 2
Fatty acid composition in liver microsomal fractions from adult and aged mice fed the LA-EE diet or the DHA-EE diet (n=5–6)

	Adult mice		Aged mice		Two-way ANOVA		
	LA-EE	DHA-EE	LA-EE	DHA-EE	Age	Diet	Age×Diet
Saturated fatty acid							
16:0	25.9±0.2	33.4±0.3	25.3±0.2	31.7±0.5	P<.005	P<.001	NS
18:0	14.1±0.3	13.3±0.2	16.3±0.8	15.2±0.2	P<.001	P<.014	NS
Others	0.6±0.0^{a,b}	0.4±0.0^{a,c}	0.9±0.0^{b}	0.4±0.1^{c}	NS	P<.001	P<.033
Total	40.5±0.2	47.1±0.3	42.5±0.7	47.2±0.6	P<.042	P<.001	NS
Monounsaturated fatty acid							
16:1	2.2±0.1	1.3±0.3	1.8±0.2	1.2±0.1	NS	P<.001	NS
18:1n−9	14.2±0.4	13.3±0.4	13.1±0.7	14.1±0.4	NS	NS	NS
18:1n−7	2.7±0.2	1.2±0.0	3.4±0.0	1.4±0.1	NS	P<.001	NS
Others	0.4±0.0	–	0.6±0.0	0.3±0.1	P<.006	P<.001	NS
Total	19.5±0.5	15.9±0.5	18.6±1.2	17.0±0.4	NS	P<.001	NS
Polyunsaturated fatty acid							
18:2n−6	14.4±0.6	11.5±0.7	13.0±0.1	12.4±0.5	NS	P<.001	NS
20:4n−6	18.3±0.2	1.5±0.1	18.3±0.4	2.2±0.3	NS	P<.001	NS
20:5n−3	–	2.9±0.1	–	2.9±0.2	NS	P<.001	NS
22:5n−6	1.7±0.1	–	2.4±0.1	–	P<.018	P<.001	P<.018
22:6n−3	3.7±0.3^{a}	19.8±0.4^{b}	3.2±0.1^{a}	16.7±0.3^{c}	P<.001	P<.001	P<.001
Others	1.7±0.0^{a}	1.2±0.1^{b}	1.5±0.0^{a,b}	1.6±0.1^{a,b}	NS	NS	P<.009
Total	39.5±0.3	37.0±0.5	38.3±0.5	35.7±0.5	P<.018	P<.001	NS
Unknown	0.4±0.1	–	0.7±0.0	0.1±0.1	P<.016	P<.001	NS
n−6/n−3	8.9±0.5^{a}	0.6±0.0^{b}	11.0±0.2^{c}	0.8±0.1^{b}	P<.002	P<.001	P<.008

Values for each dietary group with different letters are significantly different at P<.05 (Spjotroll–Stoline test).

Fig. 2 displays the plasma, liver and brain cholesterol contents from adult and aged mice in the LA-EE and DHA-EE diet groups. In both adult and aged mice, plasma and liver T-chol contents were significantly lower in the DHA-EE diet group than in the LA-EE diet group. There were no significant influences in plasma and liver cholesterol contents with aging. However, the cholesterol content of the brain in both LA-EE and DHA-EE diet groups was

Table 3
Fatty acid composition in brain microsomal fractions of adult and aged mice fed the LA-EE diet or the DHA-EE diet (n=5–6)

	Adult mice		Aged mice		Two-way ANOVA		
	LA-EE	DHA-EE	LA-EE	DHA-EE	Age	Diet	Age×Diet
Saturated fatty acid							
16:0	24.4±0.0	24.6±0.2	24.7±0.1	24.5±0.1	NS	NS	NS
18:0	21.0±0.2	20.4±0.1	20.7±0.2	20.4±0.1	NS	P<.006	NS
Others	–	0.3±0.1	0.1±0.1	0.2±0.1	NS	P<.047	NS
Total	45.4±0.2	45.3±0.1	45.6±0.2	45.2±0.2	NS	NS	NS
Monounsaturated fatty acid							
16:1n−7	0.3±0.1	0.5±0.0	0.6±0.2	0.5±0.0	NS	NS	NS
18:1n−9	12.2±0.1	13.8±0.1	12.9±0.1	14.2±0.1	P<.001	P<.001	NS
18:1n−7	4.6±0.0	4.1±0.0	4.8±0.0	4.2±0.0	P<.001	P<.001	NS
Others	0.8±0.0	0.9±0.0	0.9±0.0	0.9±0.0	P<.011	P<.019	NS
Total	17.9±0.2	19.2±0.1	19.1±0.1	19.9±0.1	P<.001	P<.001	NS
Polyunsaturated fatty acid							
20:4n−6	10.8±0.1	6.1±0.1	10.8±0.1	6.1±0.1	NS	P<.001	NS
20:5n−3	–	0.4±0.0	–	0.4±0.0	NS	P<.001	NS
22:4n−6	2.2±0.0^{a}	–^{b}	2.0±0.0^{c}	0.7±0.0^{d}	P<.001	P<.001	P<.001
22:5n−6	1.3±0.1^{a}	0.7±0.0^{b}	1.5±0.1^{a}	–^{c}	P<.001	P<.001	P<.001
22:6n−3	17.6±0.2^{a}	23.2±0.1^{b}	16.7±0.1^{c}	21.5±0.2^{d}	P<.001	P<.001	P<.018
Others	0.6±0.0	1.5±0.1	0.7±0.0	1.3±0.1	NS	P<.001	NS
Total	32.5±0.1^{a}	31.5±0.2^{b}	31.7±0.1^{b}	30.1±0.2^{c}	P<.001	P<.001	P<.046
Dimethyl acetal							
16:0	0.8±0.1	1.2±0.1	1.1±0.1	1.3±0.1	P<.021	P<.021	NS
18:0	1.8±0.0	1.9±0.1	1.8±0.1	2.3±0.1	NS	P<.006	NS
18:1n−7	–^{a}	–^{a}	0.1±0.1^{a}	0.5±0.0^{b}	P<.001	P<.002	P<.002
Total	2.7±0.0	3.1±0.2	3.0±0.2	4.1±0.1	P<.001	P<.001	NS
Unknown	1.5±0.1^{a}	0.8±0.1^{b}	0.7±0.0^{b}	0.8±0.1^{b}	P<.001	NS	P<.001
n−6/n−3	0.8±0.0	0.3±0.0	0.9±0.0	0.4±0.0	P<.001	P<.001	NS

Values for each dietary group with different letters are significantly different at P<.05 (Spjotroll–Stoline test).

significantly higher in aged mice than in adult mice. The intake of DHA-EE did not significantly influence T-chol content in the brains of adult and aged mice.

Table 2 lists the fatty acid compositions of liver microsomal fractions from adult and aged mice fed the LA-EE and DHA-EE diets. Percentages of 16:0 and saturated fatty acid (SFA) in both adult and aged mice were significantly higher in the DHA-EE diet groups than in the LA-EE diet groups. The 18:0 percentages were significantly higher in aged mice than in adult mice. A lower percentage of 18:2$n-6$ in adult and aged mice in the DHA-EE diet group, compared with that in the LA-EE diet group, was significant in adult mice but not in aged mice. The percentages of 20:4$n-6$ and 22:5$n-6$ were significantly lower — and the percentages of 20:5$n-3$ and 22:6$n-3$ were significantly higher — in both adult and aged mice in the DHA-EE diet groups than in the corresponding LA-EE diet groups. Therefore, the $n-6/n-3$ ratio was significantly lower in DHA-EE diet groups than in LA-EE diet groups. In aged mice, the percentage of 22:6$n-3$ was significantly lower and the percentage of 22:5$n-6$ was significantly higher than those in adult mice. With those changes, the $n-6/n-3$ ratio was significantly higher in aged mice than in adult mice.

Table 3 presents the fatty acid composition of brain microsomal fractions from adult and aged mice fed the DHA-EE or the LA-EE diet. The percentages of 20:4$n-6$, 22:4$n-6$ and 22:5$n-6$ were significantly lower — and the percentages of 18:1$n-9$ and 22:6$n-3$ were significantly higher — in both adult and aged mice in the DHA-EE diet groups than in the corresponding LA-EE diet groups. The total dimethylacetal (DMA) percentages were significantly higher in the DHA-EE diet groups than in the LA-EE diet groups. In aged mice, the percentages of 22:6$n-3$ and PUFA were significantly lower — and the total DMA percentage and $n-6/n-3$ ratio were significantly higher — than those in adult mice.

4. Discussion

The suppression of HMG-CoA reductase activity in liver microsomal fractions, following intake of DHA by adult and aged mice, was consistent with results obtained in previous studies [4,21]. Furthermore, these changes were mirrored by those seen in liver cholesterol levels. However, although brain HMG-CoA reductase activity was not significantly changed with the intake of DHA-EE in adult mice, it was enhanced in the brain of aged mice on the same diet. Other studies have demonstrated that HMG-CoA reductase is rapidly degraded in mammalian cells supplemented with sterols or mevalonic acid [25,26]. However, administration of these compounds to aged rats did not have this effect, suggesting that degradation mechanisms may be less effective in older animals [26]. This may have contributed to enhanced levels of the enzyme in brains of aged mice in this study.

In general, cholesterol levels in the brain rise as age increases [27,28]. In this study, the significant increase of brain cholesterol content was observed with age. However, cholesterol content did not show significant differences in experimental diets. Shahdat et al. [29] and Hashimoto et al. [30] indicate that DHA decreases amyloid β (Aβ) and cholesterol levels in detergent-insoluble membrane fractions, but there were no significant differences in synapse fractions. Furthermore, the infusion of Aβ increases cholesterol content in detergent-insoluble membrane fractions [30]. The effect of DHA on brain cholesterol content may be different in brain fractions. However, enhanced membrane cholesterol reduces the membrane-disordering effect of Aβ [31,32]. Moreover, low cellular cholesterol levels induce pathophysiological features of mood disorders [33]. Furthermore, recent studies revealed that low levels of brain cholesterol induced the suppression of dendrite outgrowth and enhanced τ protein phosphorylation, which is a feature of AD [34,35]. Thus, the enhancement of HMG-CoA reductase activity with DHA-EE intake may contribute to the maintenance of brain function in aging.

The mechanisms by which the transition in HMG-CoA reductase activity from adult to aging mice occurred, in response to the DHA-EE diet, are currently unknown. Recent studies indicate that $n-3$ PUFAs reduce the expression of the hepatic HMG-CoA reductase gene and the sterol-regulatory element-binding protein-1 gene that codes for a transcription factor that mediates insulin effects on hepatic gene expression [36–38]. The effect of aging on the regulation of this expression remains unclear. Oligomeric Aβ, which is likely to be present in older brains, suppressed cholesterol synthesis in cultured neurons [39]. Such a mechanism could have partially counteracted an increased production of cholesterol by the elevated HMG-CoA reductase activity in older animals fed a DHA-EE diet. Determination of the extent of any oligomeric Aβ present in the brains of these aged mice was beyond the scope of the present study.

Pallottini et al. [40] also demonstrated that hepatic HMG-CoA reductase activity increased with age, and this correlated with an increased production of reactive oxygen species. Hossain et al. [41] suggested that DHA intake might suppress oxidative stress in the brain. Reactive oxygen species may not be associated with the difference in DHA-EE intake in brain HMG-CoA reductase activities between adult and aged mice. Eckert et al. [32] indicated that the disordering effect of Aβ on the brain membrane is modified by brain aging. This finding suggested that aging might modify the function of the brain membrane. However, the marked increases in the percentage of DHA in brain microsomal fractions were similar in aged and young mice. Therefore, the differences in HMG-CoA reductase activities between the two age groups may be independent of membrane fatty acid composition. Further detailed studies are necessary to clarify the mechanisms associated with different outcomes in brain HMG-CoA reductase activity following an intake of DHA-EE during aging. Our present findings suggest that DHA intake could enhance brain HMG-CoA reductase activity in aged mice.

Acknowledgment

This research was supported by a research fellowship from the Japan Society for the Promotion of Science. Special thanks is extended to Dr. Takashi Ide (National Food Research Institute) for his advice on the measurement of HMG-CoA reductase activity.

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