Long-term n-3 Fatty Acid Deficiency Induces No Substantial Change in the Rate of Protein Synthesis in Rat Brain and Liver

Akira SATO,¹⁾ Tomomi OSAKABE, Atsushi IKEMOTO, Shiro WATANABE,²⁾ Tetsuyuki KOBAYASHI,³⁾ and Harumi OKUYAMA*

Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan. Received January 5, 1999; accepted May 12, 1999

The influence of long-term n-3 fatty acid deficiency on the rate of protein synthesis in rat brain and liver was investigated in relation to learning behavior or a presumed survival time-shortening factor (SSF) in rapeseed oil, using a large-dose [³H]phenylalanine (Phe) injection method. When Wistar rats were made n-3 fatty acid-deficient by feeding a safflower oil (α -linolenate-deficient) diet for 2 generations, conditions under which the safflower oil group had been shown to exhibit altered learning behaviors, compared with the perilla oil group, no significant changes in the rate of protein synthesis were observed compared with the perilla oil (α -linolenate-sufficient) or rapeseed oil (α -linolenate-sufficient but SSF-containing) groups. However, the rapeseed oil group had a reduced specific radioactivity of free Phe in the cerebral cortex, compared with the safflower oil group. In contrast to the reported observation of very long-term n-3 fatty acid deficiency inducing an almost 2-fold increase in the rate of protein synthesis in the brain, our results indicate that altered learning behavior resulting from n-3 fatty acid deficiency in rats is not associated with any substantial changes in the rate of protein synthesis in the brain.

Key words brain protein synthesis; n-3 fatty acid deficiency; learning behavior; perilla oil; rapeseed oil; survival time-shortening factor

Tissue polyunsaturated fatty acids (PUFA) are classified mainly into the linoleic acid (n-6) and α -linolenic acid (n-3) series depending on their metabolism, and these two fatty acid series are not interconvertible in the animal body. They cannot be synthesized by the mammals and, therefore, have to be obtained from the diet. The dietary n-6/n-3 fatty acid balance has been shown to influence the development of chronic diseases in the elderly as reviewed elsewhere.⁴⁾

Long-term deprivation of n-3 fatty acids has been shown to reduce the docosahexaenoic acid (DHA, 22:6n-3) content in the brain and cause impairment and/or modification of central nervous system (CNS) function.^{5–9)} In particular, it is well demonstrated that rats fed diets supplemented with vegetable oils, containing limited amounts of α -linolenic acid (n-3) and sufficient amounts of linoleic acid (n-6), exhibit altered learning behavior.^{10–14)} We have found that these alterations in learning behavior are related to reduced synaptic vesicle density in the hippocampus CA1 region and some functional changes in the brain microsomal membrane surfaces in n-3 fatty acid-deficient rats (safflower oil diet), compared with n-3 fatty acid-sufficient rats (perilla oil diet).^{15–17)} It has also been reported that n-3 fatty acid deficiency alters the content and/or receptor density of monoamines, such as dopamine and serotonin, in the frontal cortex of rats.¹⁸⁾ However, the biochemical basis for the link between n-3 fatty acid deficiency and altered CNS function has not yet been fully elucidated.

Protein metabolism is one of the crucial processes in the nervous system, because most neurotransmitters are amino acids, their derivatives or peptides. Furthermore, the receptors, enzymes and ion channels are also proteins. Therefore, anomalous protein turnover in the nervous system will affect these proteins and neurotransmitters, leading to altered learning behavior. Recently, Giaume *et al.*¹⁹⁾ reported that n-3 fatty acid deficiency in peanut oil-fed rats induced a 2-fold increase in the rate of protein synthesis in the brain, com-

pared with that of rats fed peanut oil plus rapeseed oil containing a significant amount of α -linolenic acid. However, increased protein synthesis resulting from n-3 fatty acid deficiency was not easy to explain. We speculated that n-3 fatty acid deficiency itself did not increase the rate of protein synthesis in the brain, but a presumed survival time-shortening factor (SSF) in rapeseed oil, which is used as a dietary oil containing n-3 fatty acid, suppressed protein synthesis as observed by Giaume *et al.*¹⁹⁾ The presence of this factor has been confirmed in our previous experiments on stroke-prone spontaneously hypertensive (SHRSP) rats.^{20–23)} In addition, we have also found that feeding rapeseed oil resulted in anomalous behavioral patterns and drug responses in mice, compared with feeding other vegetable oils.²⁴⁾ Therefore, we cannot exclude the additional possibility that this SSF in rapeseed oil might reach the nervous system, alter protein metabolism associated with neurotransmission, and induce an altered behavioral pattern.

In the present study, we examined the aforementioned possibilities by measuring the rate of protein synthesis in the brain and liver of rats fed a diet supplemented with safflower oil (α -linolenate-deficient), perilla oil (α -linolenate-sufficient) or rapeseed oil (α -linolenate-sufficient but containing SSF) for 2 generations, conditions under which it has been repeatedly shown that there is a significant difference in learning behavior between the safflower oil- and perilla oil-fed groups.^{11,25–28)}

MATERIALS AND METHODS

Materials L-[4-³H]Phenylalanine (Phe) (25 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO, U.S.A.). L(-)-Phe was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and β -phenylethylamine was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents and solvents were analytical grade.

* To whom correspondence should be addressed.

Table 1. Fatty Acid Composition of the Experimental Oils

Fatty acid ^{a)}	Safflower oil	Perilla oil	Rapeseed oil
% (w/w) of total fatty acids			
14:0	tr. ^{b)}	tr.	0.5
16:0	6.6	6.8	4.4
16:1	tr.	tr.	0.2
18:0	2.5	2.3	2.6
18:1	13.4	21.9	61.8
18:2n-6	76.5	13.2	22.3
18:3n-3	0.2	54.7	8.2
n-6/n-3	383	0.24	2.7

The fatty acid composition of each oil was analyzed by GLC. *a)* Fatty acids are designated by the number of carbon atoms; the number of double bonds, and the position of the first double bond numbered from the methyl terminus as n-6 or n-3. *b)* tr., trace.

Animals and Diets Female Wistar rats (Japan SLC, Shizuoka, Japan), from 3 weeks of age, were fed a semipurified diet supplemented with safflower oil, perilla oil or rapeseed oil at 5% (w/w). They were mated at 11 weeks of age, the same diet was continued through gestation, parturition, and subsequent lactation, and the male progeny were also fed diets identical to those of the dams. At 11 weeks of age, the progenies were used to estimate the rate of protein synthesis. The test diets were replaced every other day, and water was provided *ad libitum*. The test diets were prepared as follows: the fat-free basal diet (Clea Japan, Inc., Tokyo, Japan) contained 47% corn starch, 24.6% milk casein, 8% cellulose, 6% mineral mixture, 5% sucrose, 2% α -starch, 1.4% vitamin mixture, 0.6% choline chloride, and 0.4% DL-methionine (by weight % of the test diet). This basal diet was mixed with 5% dietary vegetable oil, and pelleted with water. The fatty acid compositions of the dietary oils are shown in Table 1.

Injection of [³H]Phe and Dissection The fractional rates of protein synthesis in the brain and liver were measured by the large-dose [³H]Phe injection method described by Garlick *et al.*²⁹⁾ L-[4-³H]Phe was combined with unlabeled Phe to give 150 μ mol (50 μ Ci)/ml phosphate-buffered saline (PBS; pH 7.4). Rats were injected with 1.0 ml/100 g body weight of the [³H]Phe solution *via* a lateral tail vein with no anesthesia. At 3 or 10 min after injection, the rats were decapitated, blood was collected in a tube containing 0.5–1.0 ml 3.8% (w/v) sodium citrate, and centrifuged at 3000 rpm for 5 min at 4 °C. The brain and liver were rapidly excised and the brain regions were dissected. The tissues were quickly frozen in dry ice-ethanol. Plasma and tissue samples were stored at –80 °C until analysis.

Measurement of the Specific Radioactivity of [³H]Phe in Free Amino Acid and Protein Pools in the Tissues Tissue samples were homogenized with 10 volumes ice-cold 2% (w/v) perchloric acid and then centrifuged at 2150 $\times g$ for 15 min at 4 °C. The pH of the supernatant was adjusted to between 6.0 and 7.0 with 1.5 ml saturated potassium citrate solution, and the mixture was again centrifuged at 2150 $\times g$ for 15 min at 4 °C. The supernatant was used for measurement of the specific radioactivity of free Phe in tissues. The precipitate containing protein was washed three times with 5 ml 2% perchloric acid, suspended in 5 ml 0.3 M NaOH, and incubated at 37 °C for 1 h. Protein-bound Phe in the tissues was obtained by reprecipitating the protein with 1 ml 20% (w/v) perchloric acid, washing the pellet twice with 5 ml 2% per-

chloric acid and hydrolyzing the protein in 5 ml 6 M HCl at 110 °C for 23–24 h. After the HCl was evaporated to dryness, the free amino acids obtained by protein hydrolysis were dissolved in 3 ml 0.5 M sodium citrate buffer (pH 6.3). The specific radioactivity of [³H]Phe involved its enzymatic conversion into phenylethylamine was determined, followed by measurement of radioactivity and fluorometric determination.³⁰⁾

Calculation of the Rate of Protein Synthesis in Tissues

The fractional rate of protein synthesis (k_s) was calculated from the mean specific radioactivity of free Phe in the tissues between 0 and 10 min (S_A) and the specific radioactivity of protein-bound Phe in the tissues 10 min (S_B) after the injection of [³H]Phe. The value of S_A was obtained after sacrificing the animals 3 and 10 min after the injection of [³H]Phe, and k_s was calculated using the formula given by Garlick *et al.*²⁹⁾,

$$k_s = (S_B \times 100) / (S_A \times t) \quad (1)$$

where t is the incorporation time in days and k_s is expressed as % per day.

Lipid Analysis To determine the fatty acid composition of the tissues, total lipids were extracted with chloroform-methanol according to the method of Bligh and Dyer.³¹⁾ Heptadecanoic acid was added to the sample prior to lipid extraction as the internal standard. Fatty acid methyl esters were prepared by treating the extracted lipids with 5% (w/v) HCl in methanol for 1 h in a boiling water bath. They were then extracted with petroleum ether, and quantitated by gas-liquid chromatography (GLC) on a capillary column (DB-225; J&W Scientific, Folsom, CA, U.S.A.).

Statistics The statistical significance of difference among the dietary groups was analyzed by a one-way ANOVA using Bonferroni's post-hoc test for multiple comparisons. In all cases, a value of $p < 0.05$ was considered significant.

RESULTS

Fatty Acid Composition of Tissues in Rats Fed Safflower, Perilla or Rapeseed Oil There were no statistically significant differences in the body weight, and weight of the cerebral cortex, cerebellum and liver among the safflower, perilla and rapeseed oil groups (data not shown).

The effect of the dietary vegetable oils on the fatty acid composition of the total lipids of the cerebral cortex was different from that on those of the liver (Table 2). Long-term feeding of the n-3 fatty acid-deficient safflower oil resulted in a more significant reduction in DHA (22:6n-3) content with a concomitant increase in 22:4n-6 and 22:5n-6 content in the cerebral cortex compared with the other two dietary oils. The DHA content in the cerebral cortex in the perilla oil group was significantly higher than that in the safflower oil group but was similar to that in the rapeseed oil group. The arachidonate (20:4n-6) content in the cerebral cortex in the perilla oil group was significantly lower than that in the other two dietary groups. The fatty acid composition of the total lipids of the cerebellum was relatively similar to that of the cerebral cortex (data not shown).

Feeding vegetable oils with different fatty acid compositions resulted in significant differences in the fatty acid composition of the total lipids of the liver (Table 2). The octade-

Table 2. Fatty Acid Composition of the Total Lipids of the Cerebral Cortex and Liver in Rats Fed Safflower Oil, Perilla Oil, or Rapeseed Oil Diet

Fatty acid ^{a)}	Cerebral cortex			Liver		
	Safflower	Perilla	Rapeseed	Safflower	Perilla	Rapeseed
	% (w/w) of total fatty acids					
14:0	0.4±0.2	0.3±0.2	0.3±0.2	N.D. ^{b)}	N.D.	N.D.
14:1	0.3±0.3	0.3±0.2	0.2±0.2	N.D.	N.D.	N.D.
16:0DMA	1.6±0.5	1.8±0.7	1.9±0.6	N.D.	N.D.	N.D.
16:0	20.8±1.0	20.4±1.2	20.0±1.0	20.1±1.1	25.5±3.8	24.3±2.7
18:0DMA	2.7±0.9	3.0±1.0	3.2±0.8	N.D.	N.D.	N.D.
18:1DMA	0.9±0.3	1.0±0.3	1.2±0.2	N.D.	N.D.	N.D.
18:0	22.8±0.7	22.8±0.4	22.8±0.4	16.5±2.9	15.4±0.4	18.7±1.4
18:1	17.5±0.9 ^a	21.3±0.4 ^b	19.9±0.7 ^b	12.2±1.7 ^a	16.2±0.9 ^{ab}	21.3±2.7 ^b
18:2n-6	0.7±0.1	0.7±0.1	0.5±0.0	19.4±2.8 ^b	10.2±0.5 ^a	10.1±1.6 ^a
18:3n-3	N.D.	N.D.	N.D.	N.D. ^a	7.4±1.8 ^b	0.5±0.3 ^a
20:0	1.2±1.3	0.4±0.0	0.4±0.1	0.1±0.1	N.D.	N.D.
20:1	1.0±0.1	1.0±0.0	1.2±0.1	0.1±0.1	N.D.	N.D.
20:3n-6	0.3±0.0 ^a	0.6±0.1 ^b	0.3±0.1 ^a	0.5±0.1 ^a	0.8±0.1 ^{ab}	1.2±0.3 ^b
20:4n-6	11.0±0.8 ^c	8.0±0.1 ^a	9.6±0.2 ^b	25.7±1.0 ^c	4.5±0.5 ^a	14.6±2.2 ^b
20:5n-3	N.D.	N.D.	N.D.	N.D. ^a	10.7±0.4 ^c	1.7±0.4 ^b
22:0	0.7±0.6	0.4±0.0	0.4±0.1	N.D.	N.D.	N.D.
22:4n-6	3.7±0.2 ^c	1.5±0.1 ^a	2.7±0.1 ^b	1.2±0.2 ^b	N.D. ^a	N.D. ^a
22:5n-6	7.8±0.5 ^b	N.D. ^a	0.2±0.0 ^a	2.5±0.6 ^b	N.D. ^a	N.D. ^a
22:5n-3	N.D. ^a	0.7±0.0 ^b	N.D. ^a	N.D. ^a	2.8±0.2 ^b	0.5±0.3 ^a
22:6n-3	3.7±0.0 ^a	13.6±0.5 ^b	12.9±1.1 ^b	1.6±0.2 ^a	6.3±0.8 ^b	6.9±0.3 ^b
24:0	1.7±1.0	1.1±0.1	1.1±0.2	0.3±0.3	0.3±0.2	0.3±0.2
24:1	1.1±0.2	1.2±0.0	1.2±0.1	N.D.	N.D.	N.D.
Total n-6	23.5±1.4 ^c	10.8±0.2 ^a	13.4±0.3 ^b	49.2±1.6 ^c	15.5±0.9 ^a	25.9±3.5 ^b
Total n-3	3.7±0.0 ^a	14.3±0.5 ^b	12.9±1.1 ^b	1.6±0.2 ^a	27.2±2.6 ^c	9.6±1.0 ^b
n-6/n-3	6.4±0.3 ^b	0.8±0.0 ^a	1.0±0.1 ^a	31.5±4.9 ^b	0.6±0.0 ^a	2.7±0.2 ^a
Total fatty acids (μg/mg of protein)	331±17	326±33	355±13	149±19	173±24	155±20

a) Values (means±S.D.) with different superscripts (a, b or c) are significantly different among the dietary groups for each tissue ($p<0.05$ by one-way ANOVA using Bonferroni's post-hoc test) ($n=3$). DMA denotes dimethylacetal derived from plasmalogen. b) N.D., not determined.

Fig. 1. Time-Dependent Changes in the Specific Radioactivity of Free Phe in the Cerebral Cortex (A), Cerebellum (B), Plasma (C) and Liver (D) of Rats Fed Safflower Oil (■—), Perilla Oil (△---) and Rapeseed Oil (○---

Rats were injected with 150 μmol (50 μCi) [³H]Phe/100 g body weight via a lateral tail vein, and tissues were taken from 4 rats sacrificed after 3 min and 6 rats sacrificed after 10 min. The specific radioactivity of free Phe in tissues was measured as described in Materials and Methods.

cenoate (18:1) content of the liver in the rapeseed oil group tended to be higher than that in the other two dietary groups, reflecting the high 18:1 content of rapeseed oil (Table 1). Feeding linoleate (18:2n-6)-rich safflower oil resulted in significant elevation of 18:2n-6, arachidonate (20:4n-6), 22:4n-6 and 22:5n-6 in the liver, compared with the other two dietary groups. Similarly, feeding α -linolenate (18:3n-3)-rich perilla oil resulted in a significant increase in 18:3n-3, 20:5n-3 and 22:5n-3 in the liver, compared with the other two dietary groups. There was a significantly greater difference in arachidonate (20:4n-6) in the liver than in the brain of the three dietary groups. The DHA (22:6n-3) content of the liver in the perilla oil group was significantly higher than that in the safflower oil group but was similar to that in the rapeseed oil group; this was also true in the brain.

Protein Synthesis in Rats Fed Safflower, Perilla or Rapeseed Oil The specific radioactivity of free Phe in the cerebral cortex, cerebellum, plasma and liver of rats 3 and 10 min after the injection of 150 μ mol (50 μ Ci) [3 H]Phe/100 g body weight is shown in Fig. 1. The specific radioactivity at 10 min decreased in the plasma and liver, but increased in the cerebral cortex and cerebellum, compared with the values at 3 min, indicating that the free Phe pool of the liver equilibrated more rapidly with the plasma pool than that of the cerebral cortex and cerebellum, and that the transfer of free Phe from the plasma to the cerebral cortex and cerebellum is slower than that to the liver. The specific radioactivity during the 10 min period in the plasma and brain of the rapeseed oil group tended to be lower, compared with that of the other groups.

The mean specific radioactivity of free Phe between 0 and 10 min (S_A), the specific radioactivity of protein-bound Phe at 10 min (S_B) and the rate of protein synthesis (k_S) in the cerebral cortex, cerebellum and liver are summarized in Table 3. The S_A value of the cerebral cortex in the rapeseed oil group was significantly lower than that in the safflower oil group and tended to be lower than that in the perilla oil group. The S_A value of the cerebellum in the rapeseed oil group tended to be lower than that in the other dietary groups. However, the k_S in each tissue did not differ significantly among the safflower oil, perilla oil and rapeseed oil groups. The RNA and protein concentrations in the cerebral cortex, cerebellum and liver did not differ significantly between the dietary groups (data not shown).

DISCUSSION

In this study, we investigated how dietary fatty acid balance affects protein metabolism in the brain; we also looked at the liver as a non-neuronal control tissue. Most methods for measuring the rate of protein synthesis *in vivo* are based on administration of trace-amounts of labeled amino acids to the animal with subsequent measurement of the labeled proteins. The main problem with these methods is the accurate assessment of the specific radioactivity of the precursor amino acid for protein synthesis. When a trace-dose of labeled amino acid is administered, the specific radioactivity of the precursor amino acids often differs widely between species and between different compartments (*e.g.* plasma and tissues), and may vary due to dilution of the precursor pool by unlabeled amino acids which are present in the tissues and

Table 3. Effect of Dietary Oils on the Specific Radioactivity of Phe and the Rate of Protein Synthesis in the Cerebral Cortex, Cerebellum and Liver of Rats^{a)}

	Dietary group		
	Safflower	Perilla	Rapeseed
S_A (dpm/nmol) ^{b)}			
Cerebral cortex	447 $\pm$ 35	427 $\pm$ 17	403 $\pm$ 13*
Cerebellum	437 $\pm$ 25	437 $\pm$ 17	409 $\pm$ 21
Liver	559 $\pm$ 31	561 $\pm$ 15	544 $\pm$ 50
S_B (dpm/nmol) ^{c)}			
Cerebral cortex	0.50 $\pm$ 0.03	0.48 $\pm$ 0.02	0.47 $\pm$ 0.04
Cerebellum	0.45 $\pm$ 0.04	0.45 $\pm$ 0.03	0.47 $\pm$ 0.05
Liver	2.70 $\pm$ 0.29	2.86 $\pm$ 0.56	2.79 $\pm$ 0.23
Protein synthesis: k_S (%/d) ^{d)}			
Cerebral cortex	16.2 $\pm$ 1.0	16.1 $\pm$ 1.2	16.9 $\pm$ 1.8
Cerebellum	15.0 $\pm$ 1.9	14.8 $\pm$ 1.3	16.5 $\pm$ 2.0
Liver	69.7 $\pm$ 8.6	73.3 $\pm$ 14.4	74.1 $\pm$ 4.8

Values are means $\pm$ S.D. for 6 rats. *, Significantly different from the safflower oil group at $p < 0.05$ by one-way ANOVA using Bonferroni's post-hoc test. a) Rats were injected with 150 μ mol (50 μ Ci) [3 H]Phe/100 g body weight via a lateral tail vein, and tissues were taken from 4 rats sacrificed after 3 min and 6 rats sacrificed after 10 min. b) S_A is the mean specific radioactivity of free Phe in tissue between 0 and 10 min. c) S_B is the specific radioactivity of protein-bound Phe in tissue at 10 min. d) The protein synthesis rate (k_S) was calculated from Eq. 1 as described in Materials and Methods.

due to recycling of the labeled amino acids, derived from degradation of the labeled proteins, into the precursor pool.^{32,33)} Using this method, Giaume *et al.*¹⁹⁾ reported that n-3 fatty acid deficiency in rats increased protein synthesis in the brain approximately 2-fold, following administration of a trace-dose of [35 S]methionine; they did this by autoradiography and densitometry without measuring the specific radioactivity of free and protein-bound methionine in the brain regions. Furthermore, the amount of 35 S in the acid-insoluble (protein-bound) fraction of the frontal cortex in n-3 fatty acid-deficient rats showed an approximately 1.6-fold increase, compared with control rats, although the protein content did not differ between the two groups. Therefore, Giaume *et al.*¹⁹⁾ concluded that the overall protein turnover in the brain is increased by n-3 fatty acid deficiency. In the present study, we injected a large-dose of [3 H]Phe to determine the rate of protein synthesis in the brain and liver to minimize any difference in the specific radioactivity of free Phe in the precursor pools, a method which has been used successfully to measure the rate of protein synthesis in the brain^{34,35)} and liver²⁹⁾ of rats *in vivo*. Our results showed that, unlike the results of Giaume *et al.*,¹⁹⁾ n-3 fatty acid deficiency caused by safflower oil-feeding for 2 generations did not affect the rate of protein synthesis in the brain or liver (Table 3).

This discrepancy in the results may be partly due to a methodological difference in measuring the rate of protein synthesis. Another methodological difference involves the dietary conditions: African peanut oil, as n-3 fatty acid-deficient oil, and peanut oil plus rapeseed oil, as a control, were fed to 3 generations in the study by Giaume *et al.*¹⁹⁾ whereas safflower oil, as n-3 fatty acid-deficient oil, and perilla oil, as a control, were fed to 2 generations in this work. Although the DHA levels in the brain fell by a similar extent in the peanut oil group³⁶⁾ and safflower oil group (Table 2), compared with control levels, the arachidonate content was similar in the peanut oil and control groups.³⁶⁾ However, a signifi-

cant difference in arachidonate content was observed between the safflower oil and perilla oil groups (Table 2). Despite the difference in arachidonate content, we observed no significant difference in the rate of protein synthesis among the three dietary groups (Table 3).

We have previously demonstrated that feeding rapeseed oil shortens the survival time of SHRSP rats by 40%, compared with soybean, perilla, and fish oils,^{20–23)} and that mice fed rapeseed oil exhibit unusual behavioral patterns, compared with mice fed other vegetable oils,²⁴⁾ which cannot be accounted for simply by the fatty acid compositions. Our present results indicate that the presumed SSF or any other component does not affect the rate of protein synthesis (Table 3).

Although we could not find a rational explanation for the apparent discrepancy in the n-3 fatty acid deficiency-induced rate of brain protein synthesis, our results suggest that chronic n-3 fatty acid deficiency does not affect the rate of protein synthesis in the brain or liver, and that the altered learning behavior resulting from n-3 fatty acid deficiency observed in rats is not associated with substantial changes in the rate of protein synthesis in the brain.

Acknowledgements We thank Prof. H. Yokogoshi, School of Food and Nutritional Sciences, University of Shizuoka, for his generous technical advice. This work was supported in part by the Special Coordination Funds for Promoting Science and Technology of the Science and Technology Agency of the Japan Government and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES AND NOTES

- 1) Present address: Laboratory for Molecular Neurogenesis, Brain Science Institute (BSI), The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan.
- 2) Present address: Department of Clinical Application, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan.
- 3) Present address: Department of Biology, Faculty of Science, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan.
- 4) Okuyama H., Kobayashi T., Watanabe S., *Prog. Lipid Res.*, **35**, 409–457 (1997).
- 5) Salem N., Jr., Kim H. Y., Yergey J. A., "Docosahexaenoic Acid: Membrane Function and Metabolism, The Health Effect of Polyunsaturated Fatty Acids in Seafoods," ed. by Simopoulos A. P., Kifer R. R., Martin R., Academic Press, New York, 1986, pp. 263–317.
- 6) Neuringer M., Anderson G. J., Connor W. E., *Annu. Rev. Nutr.*, **8**, 517–541 (1988).
- 7) Bazan N. G., *Nutr. Brain*, **8**, 1–24 (1990).
- 8) Bourre J. M., Bonneil M., Clement M., Dumont O., Durand G., Lafont H., Nalbone G., Piciotti M., *Prostaglandins Leukot. Essent. Fatty Acids*, **48**, 5–15 (1993).
- 9) Yoshida S., Sato A., Okuyama H., *Jpn. J. Pharmacol.*, **77**, 11–22 (1998).
- 10) Lamprey M. S., Walker B. L., *J. Nutr.*, **106**, 86–93 (1976).
- 11) Yamamoto N., Saitoh M., Moriuchi A., Nomura M., Okuyama H., *J. Lipid Res.*, **28**, 144–151 (1987).
- 12) Bourre J. M., Francois M., Youyou A., Dumont O., Piciotti M., Pascal G., Durand G., *J. Nutr.*, **119**, 1880–1892 (1989).
- 13) Enslen M., Milon H., Malnoë A., *Lipids*, **26**, 203–208 (1991).
- 14) Umezawa M., Ohta A., Tojo H., Yagi H., Hosokawa M., Takeda T., *Brain Res.*, **669**, 225–233 (1995).
- 15) Yoshida S., Yasuda A., Kawazato H., Sakai K., Shimada T., Takeshita M., Yuasa S., Kobayashi T., Watanabe S., Okuyama H., *J. Neurochem.*, **68**, 1261–1268 (1997).
- 16) Yoshida S., Miyazaki M., Takeshita M., Yuasa S., Kobayashi T., Watanabe S., Okuyama H., *J. Neurochem.*, **68**, 1269–1277 (1997).
- 17) Yoshida S., Miyazaki M., Sakai K., Takeshita M., Yuasa S., Sato A., Kobayashi T., Watanabe S., Okuyama H., *Biospectroscopy*, **3**, 281–290 (1997).
- 18) Delion S., Chalon S., Hérault J., Guilleateau D., Besnard J. C., Durand G., *J. Nutr.*, **124**, 2466–2476 (1994).
- 19) Giaume M., Gay N., Baubet V., Gharib A., Durand G., Bobillier P., Sarda N., *J. Neurochem.*, **63**, 1995–1998 (1994).
- 20) Huang M. Z., Naito Y., Watanabe S., Kobayashi T., Kanai H., Nagai H., Okuyama H., *Biol. Pharm. Bull.*, **19**, 554–557 (1996).
- 21) Huang M. Z., Watanabe S., Kobayashi T., Nagatsu A., Sakakibara J., Okuyama H., *Lipids*, **32**, 745–751 (1997).
- 22) Miyazaki M., Huang M. Z., Watanabe S., Kobayashi T., Okuyama H., *Nutr. Res.*, **18**, 1049–1056 (1998).
- 23) Miyazaki M., Huang M. Z., Takemura N., Watanabe S., Okuyama H., *Lipids*, **33**, 655–661 (1998).
- 24) Kameyama T., Ohhara T., Nakashima Y., Naito Y., Huang M. Z., Watanabe S., Kobayashi T., Okuyama H., Yamada K., Nabeshima T., *Biol. Pharm. Bull.*, **19**, 400–404 (1996).
- 25) Yamamoto N., Hashimoto A., Takemoto Y., Okuyama H., Nomura M., Kitajima R., Togashi T., Tamai Y., *J. Lipid Res.*, **29**, 1013–1021 (1988).
- 26) Yamamoto N., Okaniwa Y., Mori S., Nomura M., Okuyama H., *J. Gerontol.*, **46**, B17–22 (1991).
- 27) Nakashima Y., Yuasa S., Hukamizu Y., Okuyama H., Ohhara T., Kameyama T., Nabeshima T., *J. Lipid Res.*, **34**, 239–247 (1993).
- 28) Okaniwa Y., Yuasa S., Yamamoto N., Watanabe S., Kobayashi T., Okuyama H., Nomura M., Nagata Y., *Biol. Pharm. Bull.*, **19**, 536–540 (1996).
- 29) Garlick P. J., McNurlan M. A., Preedy V. R., *Biochem. J.*, **192**, 719–723 (1980).
- 30) Suzuki O., Yagi K., *Anal. Biochem.*, **75**, 192–200 (1976).
- 31) Bligh E. G., Dyer W. J., *Can. J. Biochem. Physiol.*, **37**, 911–917 (1959).
- 32) Lestage P., Gonon M., Lepetit P., Vitte P. A., Debilly G., Rossatto C., Lecestre D., Bobillier P., *J. Neurochem.*, **48**, 352–363 (1987).
- 33) Smith C. B., Deibler G. E., Eng N., Schmidt K., Sokoloff L., *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9341–9345 (1988).
- 34) Yokogoshi H., Hayase K., Yoshida A., *J. Nutr.*, **122**, 2210–2217 (1992).
- 35) Hayase K., Yokogoshi H., *J. Nutr.*, **124**, 683–688 (1994).
- 36) Gazzah N., Gharib A., Croset M., Bobillier P., Lagarde M., Sarda N., *J. Neurochem.*, **64**, 908–918 (1995).