Effects of α-Linolenic, Eicosapentaenoic and Docosahexaenoic Acids on the Content and Fatty Acid Composition of Brain Phospholipid in Rats

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Received April 19, 1999

The effects of dietary n-3 fatty acids, α-linolenic acid (18:3), eicosapentaenoic acid (EPA, 20:5), and docosahexaenoic acid (DHA, 22:6), on brain phospholipid content and fatty acid composition were compared in rats fed with a diet containing constant ratios of saturated fatty acid/monounsaturated fatty acid/polyunsaturated fatty acid (PUFA) and n-3/n-6. The dietary fat in each diet was added at the level of 10%. In each diet, n-3 PUFA comprised two-thirds of the PUFA and the remaining one-third was linoleic acid (18:2). Dietary fat containing linoleic acid as the sole source of PUFA was also given to the control group. The content of brain phospholipid in the three n-3 PUFA groups was significantly lower than that of the linoleic acid group. This reduction was greater in the EPA and DHA groups than in the α-linolenic acid group. The decrease in phospholipid content in rats fed n-3 fatty acid-rich diets was largely due to the decrease in the phosphatidylethanolamine fraction. Each dietary n-3 PUFA was found to affect the fatty acid composition of brain phospholipids; the most pronounced alteration was observed in phosphatidylethanolamine fraction. Furthermore, the proportion of DHA in the phosphatidylethanolamine fraction tended to be higher in the DHA group than in other PUFA groups. In conclusion, dietary α-linolenic acid, EPA and DHA can influence the phospholipid content, phospholipid subclass, and fatty acid composition in rat brain.

Key words : *brain, phospholipid, α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid.*

Polyunsaturated fatty acids are the major components in the membrane lipids of most tissues, particularly those of the brain and retina.[1,2] The n-3 PUFAs are essential in brain development, learning ability and nervous system function in humans and animals.[3-5] Dietary manipulation of PUFA intake results in substantial alteration of membrane fatty acid composition in many tissues[6,7] and may bring about changes in cell membranes. This indicates the importance of dietary fat balance to the structure and function of cells. DHA is found in the phospholipids of brain synaptosomes, retina and sperm at relatively high concentrations, while it is a minor constituent of blood and most tissues.[6,8] The fatty acid composition in most tissues is altered by dietary fat manipulation,[6,7] while the lipid composition of the brain is remarkably constant.[9,10]

The marine n-3 fatty acids, EPA and DHA, were in the past referred to as "fish oil" without any further distinction. However, recent studies demonstrated that some of the functions of EPA and DHA were different *in vivo*.[11-13] It is reported that restriction of n-3 PUFAs during development significantly decreased exploratory behavior in animals.[6,14,15] As n-3 PUFAs are important for brain functions,[11,13] it is of interest to know the effects of individual n-3 fatty acid, α-linolenic acid, EPA and DHA, on the lipid profiles of brain. The present study, therefore, examined the influence of ethyl EPA, ethyl DHA, α-linolenic acid and the n-6 PUFA, linoleic acid, on the contents, subdistributions, and fatty acid compositions of brain phospholipid in rats.

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Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; LPC, lysophosphatidylcholine; SPM, sphingomyelin; PC, phosphatidylcholine; PS+PI, phosphatidylserine +phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid.

Materials and Methods

Materials. EPA (purity>97%) and DHA (purity>97%) were isolated from sardine oil and the orbital fat of tuna, respectively, and the ethyl esters were prepared using ethanolic hydrogen chloride solution. These lipids were gifts from Dr. K. Yazawa of Sagami Central Chemical Institute (Tokyo, Japan). Safflower oil, palm oil, and perilla oil were obtained from Linoru Oil Mills Industrial (Nagoya, Japan) and Ohta Oil (Okazaki, Japan), respectively. Vitamin and mineral mixtures (AIN-93) were purchased from Nihon Nosan Kogyo (Tokyo, Japan). All other chemicals and reagents were of the best commercial grade available.

Diet and animal experiments. Male Sprague-Dawley rats aged four weeks were purchased from Nippon SLC Co. (Hamamatsu, Japan). Rats were housed individually in suspended wire-mesh stainless steel cages in a temperature-controlled room (21~23°C) with a 12-hr light/dark cycle

(07:00~ 19:00). The semipurified basal diet used in this study contained vitamin-free casein 20%, corn starch 15%, cellu-lose 5%, mineral mixture (AIN-93)[16] 4%, vitamin mixture (AIN-93)[16] 1%, choline bitartrate 0.2% and sucrose to make a total content of 100%. Dietary fat was added at the level of 10%. Dietary fatty acid compositions were compared in rats fed a diet containing constant ratios of saturated fatty acid/monounsaturated fatty acid/ polyunsaturated fatty acid and n-3/n-6. A diet containing linoleic acid as the sole PUFA was prepared by mixing high oleic safflower oil, high linoleic safflower oil and palm oil (10:33:57, by %). Fat in the α-linolenic acid diet was prepared by mixing high oleic safflower oil, palm oil and perilla oil (7:57:37, by %). Dietary fat rich in EPA and DHA was prepared by mixing high oleic safflower oil, high linoleic safflower oil and palm oil (13:3:62, by %), and either EPA or DHA. The fatty acid composition of each dietary fat is shown in Table 1. EPA or DHA was mixed with the fat mixture daily before the administration of diets to rats in order to prevent the spoilage of highly unsaturated fatty acids. These rats were given only a freshly preared diet daily from 19:00 for 2 weeks. Water was provided *ad libitum*. Body weight was recorded every other day. Food intake was determined daily.

Analysis of lipids. At the end of the treatment period, the animals were killed by decapitation between 8:00 and 9:00. The brain was removed and kept frozen at -80°C until needed for analysis. The brain lipids were extracted and purified by the method reported previously.[17] The contents of brain triglyceride and cholesterol were measured by the methods of Fletcher *et al.*[18] and Sperry and Webb,[19] respectively. The brain phospholipid was quantified by phos-phorus content according to the method reported previously.[20] The phospholipid classes were separated by thin-layer chro-matography on Silica Gel H-plates using a solvent system composed of chloroform/ methanol/acetic acid/water=25/15/4/2, by volume.[21] The fatty acid composition of total phospho-lipids and the phosphatidylethanolamine fraction was deter-mined by gas-liquid chromatography (GC-14, Shimadzu, Kyoto, Japan) using a fused Omega Wax Capillary Column (30×0.25 μm, Supelco, U.S.A.) after separation by thin-layer chromatography and transmethylation. The column, injector, and detector temperatures were 180°C, 250°C, and 260°C, respectively.

Statistical analyses. Each value is presented as the mean±SE. Data were analyzed by one-way ANOVA, and all differences were evaluated by Duncan's new multiple-range test.[22] A difference was considered significant at $p<0.05$.

Results and Discussion

It is well known that brain has an unusually high content of lipids, two-thirds of which are phospholipids. Phospholipids are structurally and functionally important constituents of cell membranes.[1,2] They play roles in modifying the structure, fluidity, and function of brain membranes. Table 2 shows the concentrations of brain lipids after administration of experimental diets. The content of brain phospholipid in the n-3 PUFA groups significantly decreased by 10~12% as compared to the linoleic acid group. The reduction was greater in the EPA and the DHA groups than in the α-linolenic acid group. In contrast, cholesterol content in the EPA and the DHA groups was significantly higher (13~16%) than in the linoleic acid group. It has been suggested that the patterns of phospholipid in the brain are influenced by many factors, including alterations in diet.[6,7] Foot and Cladinine examined the influence of dietary fat on the lipid composition of synaptosomal and microsomal membranes in rat brain.[23] They found that the contents of cholesterol and phosphatidylcholine in these membranes varied depending on the diet. Moreover, increase in the cholesterol content of the membrane was strongly correlated with increase in the membrane phosphatidyl-choline content. As shown in Table 3, the increase in brain cholesterol content likely was compensating for the

Table 1. Fatty acid composition of dietary fat.

Fatty acid	Linoleic acid (n-6)	α-Linolenic acid (n-3)	Eicosapentaenoic acid (n-3)	Docosahexaenoic acid (n-3)
		(weight %)		
14 : 0	0.9	0.9	0.8	0.8
16 : 0	31.1	31.1	31.5	31.5
16 : 1	0.1	0.1	0.1	0.1
18 : 0	3.0	3.0	2.7	2.7
18 : 1	32.3	32.3	32.6	32.6
18 : 2 n-6	31.9	31.9	10.0	10.0
18 : 3 n-3	0.0	22.2	0.1	0.1
20 : 5 n-3	0.0	0.0	22.2	0.0
22 : 6 n-3	0.0	0.0	0.0	22.2
SFA	35.0	34.9	34.9	34.9
MUFA	32.0	32.4	32.7	32.7
PUFA (n-6)	31.9	10.0	10.0	10.0
(n-3)	0.0	22.2	22.2	22.2

Table 2. Effects of dietary fatty acids on the concentration of brain lipids in rats.

Fatty acid	Linoleic acid	α-Linolenic acid	Eicosapentaenoic acid	Docosahexaenoic acid
		(mg/g brain)		
Triglyceride	9.4±0.5	9.5±0.8[a]	8.7±0.2[a]	7.7±0.7[a]
Phospholipid	49.2±0.8[a]	44.0±0.5[b]	43.7±0.3[b]	43.4±0.9[b]
Cholesterol	14.8±0.5[a]	16.6±0.3[ab]	17.1±0.8[b]	17.7±0.6[b]
PC	16.8±10.3[a]	16.2±0.7[ab]	15.7±0.4[ab]	15.3±0.3[b]
PE	21.9±0.4[a]	19.4±0.8[b]	18.3±0.4[b]	18.2±0.4[b]

Rats were fed semipurified diets containing linoleic acid, α-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid for 2 weeks. Values are given as the means±SE of 6 rats in each experimental group. Between the groups, values with different letters are significantly different at $p< 0.05$.

Table 3. Effects of dietary fatty acids on the compositions of brain phospholipid in rats.

Fatty acid	Linoleic acid	α-Linolenic acid	Eicosapentaenoic acid	Docosahexaenoic acid
		(% of total phospholipid)		
LPC	0.72±0.07	0.87±0.57	0.90±0.20	0.52±0.06
SPM	2.45±0.09[a]	5.10±0.13[b]	4.75±0.11[b]	5.13±0.04[b]
PC	34.2±0.24[a]	36.1±0.01[b]	36.1±0.60[b]	35.3±0.49[ab]
PS+PI	16.0±0.53	15.0±1.09	14.8±0.32	15.5±1.11
PE	44.5±0.60[a]	41.7±0.48[b]	42.2±0.34[ab]	41.9±1.35[ab]
PA	2.12±0.24	1.27±0.05	1.25±0.03	1.63±0.27

Rats were fed semipurified diets containing linoleic acid, α-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid for 2 weeks. Values are given as the means±SE of 6 rats in each experimental group. Between the groups, values with different letters are significantly different at $p< 0.05$.

fluidizing effect of increased membrane phosphatidylcholine composition.

Phosphatidylcholine and phosphatidylethanolamine are the major phospholipid components in brain of rats. The proportion of phosphatidylcholine was higher, whereas that of phosphatidylethanolamine was lower in the n-3 fatty acid groups (Table 3). A significant increase in the proportion of sphingomyelin was also noted in the n-3 PUFA groups. As sphingomyelin appears to be important for the regulation of cell growth and differentiation, and possibly for interactions with cell receptors and signaling systems,[24] an increase in its level may alter cell functions. It has been reported that abnormal quantities of phospholipids and/or sphingolipids are associated with the nervous system disorders such as the sphingolipidosis and demyelinating diseases.[24]

Alterations of the phospholipid acyl moieties might also induce changes in the physical properties of the membranes and in the activities of membrane-bound enzymes.[12] It is reported that n-3 fatty acids are important in brain development and learning ability in rats[6,15] and essential for nervous system functions in humans.[6] Furthermore, DHA is the most prominent fatty acid in the membranes of the brain and the retina.[2] Recent studies demonstrated that a lowered concentration of DHA in the nervous system is associated with a loss of nervous system function.[5] However, it has been reported that brain lipids are relatively resistant to diet-induced alterations compared to other organs.[9,10] In agreement with previous reports,[9,10] the fatty acid moieties of brain phospholipids were not modified markedly by dietary manipulation (Table 4). However, as shown in Tables 4, the DHA supplemented diet resulted in increased EPA levels in phospholipid, suggesting that retroconversion of DHA to EPA may have occurred, as has been suggested by others.[6,25] In contrast, EPA supplementation did not increase the DHA level. This suggests that the elongation and desaturation of EPA to DHA was not affected by dietary EPA in rats.

Arachidonic acid (20:4, n-6) is one of the most abundant PUFAs in membrane phospholipids and is the precusor substrate of eicosanoid synthesis.[26] The level of arachidonic acid was not altered significantly by dietary restriction, indicating that the brain content of this fatty acid is relatively resistant to large changes in the ratio of n-3 to n-6 fatty acids in the diet. However, Bourre et al. reported[7] that feeding large amounts of fish oil reduced the arachidonic acid level of brain, indicating that the type and amount of n-3 fatty acids in the diet, in addition to the n-6 PUFA/n-3 PUFA ratio, may be important factors in determining the interactions of these fatty acids with arachidonic acid metabolism. Our results showed that the proportion of arachidonic acid in both total phospholipids and phosphatidylethanolamine fractions in the brain was unaffected by dietary manipulation (Tables 4 and 5). The level of arachidonic acid in the phosphatidylcholine fraction tended to be higher in the linoleic acid group than in the n-3 PUFA groups (data not shown), in agreement with the other reports.[7,27] These results also suggest that dietary n-3 fatty acids inhibit the delta 5 desaturase-catalyzed conversion of dihomo-r-linolenic acid (20:3 n-6) to arachidonic acid.

The brain is known to possess the necessary pathway

Table 4. Effects of dietary fatty acids on the fatty acid compositions and concentrations of brain phospholipid in rats.

Fatty acid	Linoleic acid	α-Linolenic acid	Eicosapentaenoic acid	Docosahexaenoic acid
		(% of total fatty acids)		
14 : 0	0.43±0.13	0.37±0.18	0.22±0.05	0.19±0.01
16 : 0	27.33±0.51[a]	25.10±0.17[ab]	23.56±1.80[bc]	24.37±0.53[bc]
16 : 1	0.33±0.03	0.28±0.03	0.35±0.04	0.29±0.00
18 : 0	29.15±0.89	28.71±1.77	29.02±2.04	29.84±0.00
18 : 1	22.60±1.11	24.31±0.82	25.98±0.93	24.40±0.89
18 : 2 n-6	0.68±0.02[ab]	0.71±0.04[ab]	0.54±0.01[b]	0.76±0.15[a]
20 : 4 n-6	8.46±0.29	8.82±0.46	8.62±0.40	7.66±0.14
20 : 5 n-3	n.d.	0.03±0.03	0.14±0.06	0.25±0.15
22 : 6 n-3	11.01±0.27	11.68±0.49	11.59±0.76	12.24±0.53
SFA	56.91	54.18	52.86	54.40
MFA	22.93	24.59	26.33	24.69
PUFA n-6	9.14	9.53	9.16	8.42
n-3	11.01	11.71	11.73	12.49
		(mg fatty acid/g brain)		
20 : 4 n-6	4.16	3.88	3.76	3.32
22 : 6 n-3	5.42	5.14	5.06	5.31

Rats were fed semipurified diets containing linoleic acid, α-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid for 2 weeks. Values are given as the means±SE of 6 rats in each experimental group. Between the groups, values with different letters are significantly different at $p< 0.05$. n.d. : not detected.

Table 5. Effects of dietary fatty acids on the fatty acid compositions of brain phosphatidylethanolamine in rats.

Fatty acid	Linoleic acid	α-Linolenic acid	Eicosapentaenoic acid	Docosahexaenoic acid
		(% of total fatty acids)		
14 : 0	0.33±0.11[a]	0.38±0.08[a]	2.95±0.54[b]	2.97±0.56[b]
16 : 0	13.50±0.64	13.42±0.94	12.91±0.63	17.76±7.84
18 : 0	37.62±2.87[a]	39.37±1.44[a]	34.55±1.07[ab]	29.88±1.54[b]
18 : 1	25.72±2.21	25.22±0.93	23.12±2.68	21.87±1.73
18 : 2 n-6	0.21±0.05	0.29±0.00	0.24±0.04	0.53±0.30
18 : 3 n-3	0.89±0.05	0.78±0.07	0.43±0.43	0.34±0.34
20 : 1	5.00±1.22	4.74±0.13	3.16±1.53	4.84±0.30
20 : 4 n-6	6.12±0.86	5.77±0.32	6.20±1.65	7.51±0.12
20 : 5 n-3	n.d.	0.49±0.15	n.d.	n.d.
22 : 4 n-6	3.60±0.41	2.98±0.01	6.10±2.19	3.64±0.13
22 : 6 n-3	4.05±0.65[a]	4.65±0.29[a]	8.89±2.25[ab]	10.41±0.60[b]
SFA	51.54	53.17	50.41	50.61
MFA	30.82	30.18	26.44	26.96
PUFA n-6	11.44	9.04	12.54	11.68
n-3	6.20	7.61	10.61	10.75

Rats were fed semipurified diets containing linoleic acid, α-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid for 2 weeks. Values are given as the means±SE of 6 rats in each experimental group. Between the groups, values with different letters are significantly different at $p< 0.05$. n.d. : not detected.

to convert α-linolenic acid to DHA.[28] When the diet was supplemented with α-linolenic acid, the level of DHA increased significantly only in the ethanolamine- and inositol-phosphoglycerides.[29] However, when DHA is supplied directly in the diet, the proportion and the mass of DHA may increase, because the direct incorporation of dietary long chain PUFAs into the developing brain has also been demonstrated.[30] The present study showed that the proportion of α-linolenic acid in the phosphatidylethanolamine fraction was decreased, whereas that of DHA was increased in the n-3 fatty acid groups, in agreement with the finding of others.[5,30] The lack of α-linolenic acid in the brain lipids suggests that dietary α-linolenic acid taken up by the brain is rapidly desaturated and elongated to docosapentaenoic acid (22:5 n-3) and DHA, or that α-linolenic acid is desaturated and elongated to docosapentaenoic acid at the blood-brain barrier and then taken up by the brain.[31] It was reported that after administration of [1-^{14}C]linolenic acid the major incorporation of radioactivity was into DHA of brain lipids within 48 hours.[32]

When male rats had been maintained for 2 weeks on the semipurified diets differing in fatty acid composition, the final body weight (225~232 g) and daily intake of food (15.8~16.4 g/day) were nearly the same in all four groups.

In conclusion, the present study demonstrated that the

brain phospholipid mass was lower in the n-3 PUFA groups than in the linoleic acid group. The alteration of the proportion of fatty acids in brain phospholipids by dietary fats was relatively small. However, the proportion of DHA tended to increase in the group fed DHA. Additional experimentation will be required to determine whether and how the alteration of the phospholipid profile influences the structural and functional parameters of the brain.

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