Modulation of fatty acid composition in murine brain by dietary unsaturated fats

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The response of individual phospholipid class fatty acid composition to dietary fats was studied in murine brain. Purified diets containing 10% (by weight) safflower oil, olive oil, linseed oil, or a mix of 9:1 (wt/wt) fish oil/safflower oil were fed to female mice. Offspring continued on the diets and were sacrificed on 3, 18, and 42 days after parturition. Docosahexaenoic acid (DHA;22:6n-3), abundant in the fish oil fed group, was particularly enriched in phosphatidyl ethanolamine and phosphatidyl serine. Fish oil-fed mice accumulated more DHA than the linseed oil-fed mice. While olive oil contained just 0.6% n-3 PUFA, DHA levels in linseed oil-fed animals overall were only slightly higher than olive oil-fed mice and were only elevated in the phospholipids classes phosphatidyl ethanolamine and phosphatidyl inositol. By contrast, DHA levels in the safflower oil-fed group were dramatically lower, containing instead elevated levels of 22:4n-6 and 22:5n-6. Eicosapentaenoic acid (EPA; 20:5n-3) levels were significantly lower in the brain relative to the other tissues we examined, implying that this fatty acid was selectively excluded from the brain or immediately metabolized to DHA in the brain. The amount of EPA in the brain was independent of the form of n-3 fatty acids in the diet, whereas DHA levels were strongly dependent. The type of n-3 PUFA was more important to 22:6n-3 accumulation in brain than the absolute quantity of 18:3n-3 and the vast majority of n-3 accumulation in all lipid classes examined occurred prior to weaning.

Keywords: eicosapentaenoic acid (EPA; 20:5n-3); docosahexaenoic acid (DHA; 22:6n-3); phospholipid; n-3 and n-6 polyunsaturated fatty acids; brain; diet

Introduction

Considerable research on the roles of n-3 polyunsaturated fatty acid (PUFA) in essential cell and tissue functions[1-3] and in modulating n-6 PUFA metabolism[2,4] have led to recommendations to increase the n-3 PUFA content of human diets.[5-8] It is apparent that both n-6 and n-3 fatty acids are essential for normal development in mammals, and that each has specific functions in the body. n-6 fatty acids are required primarily for growth, reproduction,[9-11] and the maintenance of skin integrity, whereas n-3 fatty acids are involved in the development and function of the retina and cerebral cortex and perhaps other organs.[2,12] Fetal life and infancy are particularly critical for the nervous tissue development.[13] Docosahexaenoic acid (DHA, 22:6n-3) is the prominent polyunsaturate in the brain and occurs mainly in the phosphatidylserine (PS), phosphatidylethanolamine (PE), and aminophospholipids of neuronal cells.[12,14] This distribution in phospholipids (PL) suggests that they are substantial in the metabolically active and excitable subcellular membranes of the retina and nervous system. Therefore, with respect to human nutrition, adequate amounts of n-3 fatty acids should be provided not only during pregnancy, lactation, and infancy, but probably throughout life.[13]

It is still quite controversial as to the precise form in which these PUFA should be fed. Both the short- and long-chain n-6 and n-3 PUFA in the diet are rapidly absorbed and incorporated into plasma lipids over several hours.[15] In contrast, the incorporation of these

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PUFA into cellular membrane PL requires more time and appears to occur during cell formation.[15] However, most of the putative therapeutic effects of n-3 PUFA have been obtained using fish oil feeding,[16–18] which contains eicosapentaenoic acid (EPA; 20:5n-3) and DHA, but not by the precursor form, α-linolenic acid (18:3n-3). To further evaluate uptake and incorporation of n-3 PUFA into brain PL, we conducted a long-term feeding study to determine the effects of varying types of n-3 PUFA and their concentration on brain PL.

Offspring, continued on the diets containing n-6 PUFA (safflower oil) without significant n-3, accumulated primarily n-6 and less n-3 PUFA in the brain PL. Although the diets contained n-3 PUFA, as either 18:3n-3 or 20:5n-3 plus 22:6n-3, the form of the n-3 PUFA fed had a greater effect than the absolute quantity. DHA was the major n-3 PUFA accumulated in brain PL. Although fish oil contained 20% total n-3 PUFA and linseed oil 60% n-3 PUFA, fish oil-fed mice had significantly greater levels of DHA overall and in each individual PL class. In contrast, olive oil contained just 0.6% n-3 PUFA. DHA levels in linseed oil-fed animals overall were slightly higher than olive oil-fed mice and were only elevated in the PL classes PE and phosphatidyl inositol (PI). This confirms that 22:6n-3 and its accumulation is highly regulated and presumably largely at the metabolism of 18:3n-3 to 22:6n-3, because when excess 22:6n-3 was fed as fish oil, accumulation was higher.

One intriguing observation was the apparent exclusion of EPA from brain PL in contrast to its abundance in the PL of most other tissues. This was independent of the form of n-3 fatty acids in the diets. Even when EPA was fed intact, it did not accumulate in the brain. This suggested two possible mechanisms: EPA is quantitatively metabolized in the brain tissue to longer chain products, i.e., DHA; or the uptake of fatty acid in PL by the brain is acyl chain specific.

Methods and materials

Animals and diets

Thirty-six 4-week-old Swiss female Webster mice (Bantin & Kingman, Fremont, CA USA), were randomized into four groups and received laboratory chow (Rodent Laboratory Chow #5001, Purina Mills Inc., St. Louis, MO USA) for 2 weeks before being switched to experimental diets. The mice were fed fat-free AIN 76A diets (Dyets Inc., Bethlehem, PA USA) modified with either 10 wt % safflower oil (Dyets Inc.); olive oil (G. Sensat, extra virgin #5, Specialty Food and Beverage Sales, West Milford, NJ USA); linseed oil (Spectrum Marketing, Petaluma, CA USA); or fish oil (National Institute of Health Biomedical Test Material L89195BB, Bethesda, MD USA). Safflower oil (a 9:1 fish/safflower oil ratio, wt/wt) was added to the latter to ensure linoleic acid requirements and to achieve a 3:1 ratio of n3 to n6 in both the linseed and fish groups. The fatty acid composition of the diets is shown in *Table 1*.

Mice were allowed free access to feed and water in a room with a 12-hour dark cycle. Dams were fed experimental diets for 5 months before the study and mated with male mice fed chow. The pregnant mice were kept in the plastic cages individually. Litters were normalized from 10–15 pups to 10 pups on day 3. On day 18 (weaning), the mothers were removed from the pups and four litters were analyzed. The remaining pups continued on the same diets and were analyzed on day 42. The whole brain samples (excluding the optic nerves) were obtained on days 3, 18, and 42 after parturition. They were weighed and stored at −70° C for lipid analyses.

Table 1 Fatty acid composition of the diets

Fatty acids[a]	SAF	OLV	LIN	FSH
14:0	0.2	0.0	0.0	5.6
16:0	7.2	10.7	5.0	14.1
16:1n-7	0.0	0.8	0.0	7.6
18:0	2.2	3.6	4.1	2.5
18:1n-9	12.0	78.3	16.3	8.7
18:2n-6	77.7	5.3	16.3	8.9
18:3n-3	0.0	0.6	58.2	0.9
20:0	0.3	0.4	0.0	0.2
20:1n-9	0.1	0.2	0.0	1.4
20:5n-3	0.0	0.0	0.0	11.2
22:6n-3	0.0	0.0	0.0	9.8
c22+c24[b]	0.3	0.1	0.0	13.4
others	0.0	0.0	0.0	15.7
SUM of n-3	0.1	0.7	58.2	28.6
SUM of n-6	77.7	5.3	19.4	10.0
n-3/n-6	0.0013	0.1	3.0	2.9

Values are means of two determinations per group and expressed as mol %. 0.0 indicates that levels were below those detectable, in most cases less than 0.01 %.
Diets contained either 10 wt% safflower oil (SAF), olive oil (OLV), linseed oil (LIN), or a 9/1 (wt/wt) fish oil/safflower oil mix (FSH).
[a]Fatty acids are designated by the (n-x) numbering system.
[b]Total amount of fatty acids included 22 and 24 carbons.
[c]Others include 15:0, 16:1n-5, 16:2n-7, 16:2n-4, 16:3n-4, 16:4n-1, 18:1n-7, 18:4n-3, 18:4n-3, 20:2n-6, 20:3n-3, 20:4n-3.

Lipid analyses

The brain samples were homogenized and extracted.[19] PL were separated by high-performance thin layer chromatography (HPTLC)[20] by using HPTLC pre-coated Silica Gel 60 plates (E. Merck, Darmstadt, Germany) and a solvent system of chloroform: methanol:acetic acid:water (50/37.5/3.5/2,vol/vol/vol/vol). Samples were visualized by spraying with 8-hydroxyl-1,3,6-pyrenetrisulfonic acid trisodium salt (20 mg/100 mL methanol) and viewing under UV light. Lipid classes, i.e., phosphatidyl choline, serine, inositol, and ethanolamine (PC, PS, PI, and PE respectively) were identified and scraped by comparison against PL standards (Sigma Chemical Co., St. Louis, MO USA). Phospholipids isolated by all the procedures described above may include alkylacyl- and alkenylacyl-forms of the glycerophosphatide in addition to the more common diacyl derivatives. Acyl groups were quantitatively converted to fatty acid methyl esters (FAME) by heating to 100° C for 1 hour with acetyl chloride as an acid catalyst.[21]

The FAME were separated and quantified by capillary gas liquid chromatography (GLC) (Hewlett Packard Gas Chromatograph Model 5890A equipped with a DB-23 capillary column; 50% cyanopropyl phase, 25 m × 0.25 mm i.d., 0.25 mm film thickness; J & W Scientific, Folsom, CA USA); a flame-ionization detector, and a 3392 A integrator. The GLC conditions were as follows: oven initial and final temperature: 170° C and 210° C; rate: 5° C/min.; initial and final column hold time: 1 and 5 min.; injector temperature: 250° C; detector temperature: 280° C. FAME, with heptadecanoic acid methyl

ester as an internal standard, were identified by comparison of retention times to authentic standards (NuChek Prep, Elysian, MN USA). Burdick and Jackson (Muskegon, MI USA) High Grade and Capillary GC/MS Grade solvents were used for all analyses.

Statistics

Analysis of variance[22] (ANOVA) with Fischer's protected least significant difference was used to compare the difference of fatty acid composition between dietary treatments and within each treatment. The data were expressed on a molar percentage basis.

Results

To distinguish the influence of various dietary fats on the modification of the brain PL membrane composition during the life time of the mice, three time points (days 3, 18, and 42) were selected for the age of offspring. Maternal plasma was the major nutrient source for the pups at day 3 after parturition. Moreover, the mice were nursed with breast milk till weaning, and the nutrients were supplied from the diet thereafter. It is important to understand what would be the adequate form of n-3 fatty acids and whether they should be provided during pregnancy, lactation, infancy, and throughout life.

Body weights ranged from 8.2–10.6 g on day 18. Safflower- and linseed oil-fed groups were heavier than the other groups, and the fish oil-fed group was the lightest. On day 42 the average weight was 33.5 g for the linseed- and olive oil-fed groups and 30.1 g for the fish oil-fed group. The body weights were significantly different for the various treatments ($P < 0.05$; ANOVA), but these differences were of small magnitude.

Effects of diet on the acyl modification of weaning murine brain phospholipids (day 18)

The fatty acid composition in weaning murine brain phospholipids was altered while the food source was from the breast milk of the dams. Weaning mice in the fish oil group accumulated significantly ($P < 0.05$ by ANOVA) more DHA in the brain PE and PS than the other groups (safflower, linseed, and olive oil groups) (*Figure 1*). Also, importantly, the olive oil group accumulated more DHA than the safflower oil group in all PL measured ($P < 0.05$). Interestingly, the DHA level in the olive oil group also showed no significant ($P > 0.05$) difference from the linseed oil-fed group in PC and PS (*Figure 1*). The amount of EPA was low among all the groups, even in the fish oil group, which had been fed 11.2 wt % fat of EPA in the diet.

Safflower oil, rich in n-6 PUFA and low in n-3 PUFA, resulted in low n-3 fatty acid levels (22:5n-3 and DHA) in murine brain PL and compensatorily high n-6 PUFA–arachidonic acid (AA; 20:4n-6) (PE), 22:4n-6 (PC, PS, PE), and especially 22:5n-6 (all PL classes) (*Figure 1*). Mice in the linseed oil-fed group had more AA and 22:4n-6 and less DHA in PE than the fish oil group ($P < 0.05$). With respect to n-6 PUFA, there was more ($P < 0.05$) AA, 22:4n-6, and 22:5n-6 in the olive oil-fed group than the linseed and fish oil groups in PC, PE, and PS (*Figure 1*).

Effects of diet on the acyl modification of brain phospholipids on day 42

PL PUFA composition at 42 days (*Table 2*) basically paralleled the changes at weaning. The n-6 PUFA were not generally changed by various dietary fats for the additional 2 weeks feeding after weaning. However, the level of 22:5n-6 was still rising in PS from safflower oil feeding. Again, only trace amounts of EPA were found.

At the three time points examined, the amounts of n-6 PUFA in PS and PE (*Table 3, 4*) of the fish oil-fed group were the lowest compared with the other dietary groups on day 3 and remained low through day 42. The quantities of the n-3 PUFA in the fish oil-fed group were the highest compared with the other treatments on day 3 and remained high through day 42. Contrarily, the fatty acid composition was highest in n-6 PUFA and lowest in n-3 PUFA of the safflower oil-fed group compared with the other groups through day 42.

Influence of maturity on the acyl modification of brain phospholipids

Development did not strongly affect the accumulation of n-3 PUFA in the brain via dietary means. The amounts of n-3 PUFA in PS, PE, and PC (*Table 3,4,5*) did not change after day 18. There was an indication that mice fed n-3–rich oils (fish and linseed) changed less over time than did the safflower oil- and olive oil-fed animals. EPA was low in all phospholipid fractions on any one of the time points (*Table 3,4,5,6*), even in fish oil-fed mice where EPA itself was abundant in the diets of the dams before and during the pregnancy.

The data on the brain PC (*Table 5*) of the linseed and fish oil groups showed that 20:3n-6, AA, 22:4n-6 and 22:5n-6 were raised on day 18 relative to day 3. The evidence for the reduction of 20:3n-6 and AA, as well as the increment of 22:4n-6 and 22:5n-6 from day 18 to day 42, suggested further elongation and desaturation processes (*Table 5*). Despite the modification of PUFA in PS, PE, and PC by dietary means, the amounts of AA in PI were not affected at any time point in all treatments (*Table 6*).

Some fatty acids were affected during the life span of the mice by various fats supplied. The amount of 22:4n-6 and 22:5n-6 in PC (*Table 4,5*) was elevated in the safflower oil treatment from day 3 to day 18 but not changed afterwards. However, differences between 3 and 18 days in PE and PS (two PL having high 22:4n-6 and 22:5n-6) were not significant statistically, possibly due to sample size. The amounts of DHA in the murine brain PC (*Table 5*) of offspring from the dams fed linseed oil or fish oil were different between 3 and 18 days. The DHA content in PE and PS (two PL having high DHA) was not different between days 3 and 18. Therefore, the change of brain DHA between days 3 and 18 was small.

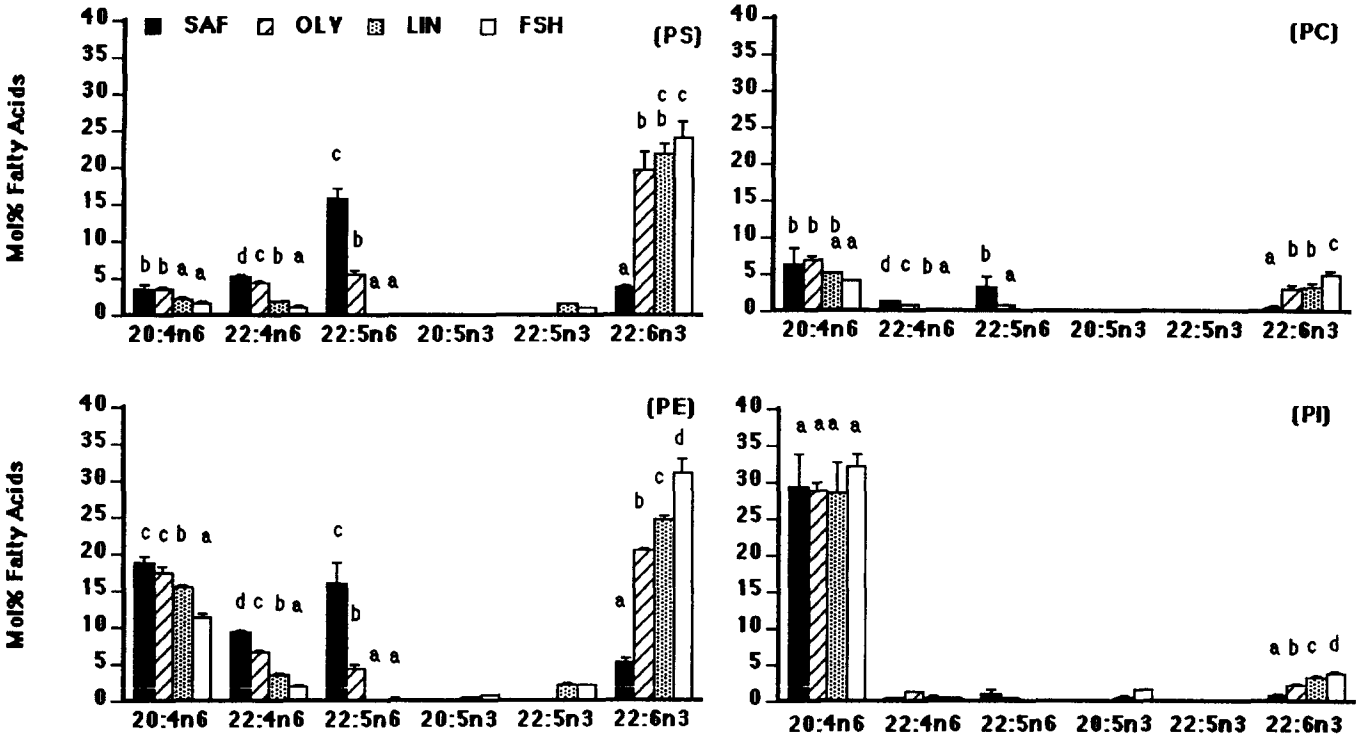

Figure 1 Major n-6 and n-3 polyunsaturated fatty acid (PUFA) composition of murine brain phospholipids from the offspring on day 18 after parturition. Values are expressed as mol % and the means ± SD for three determinations each group. Values with a different letter differ significantly ($P < 0.05$, Fisher's protected least significant difference) between groups. Abbreviations are as follows: SAF, safflower oil-fed group; OLV, olive oil-fed group; LIN, linseed oil-fed group; FSH, fish oil-fed group. PS, phosphatidyl serine; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol.

Table 2 Major n-6 and n-3 PUFA composition of the murine brain phospholipids from the offspring on day 42 after parturition

Fatty acid	SAF	OLV	LIN	FSH	SAF	OLV	LIN	FSH
	PS				PC			
20:4n-6	2.5^{b}	2.6^{b}	1.8^{ab}	1.4^{a}	7.6^{b}	4.6^{a}	3.7^{a}	3.0^{a}
22:4n-6	4.3^{c}	3.0^{b}	2.0^{a}	1.2^{a}	1.0^{c}	0.6^{b}	0.6^{ab}	0.4^{a}
22:5n-6	21.9^{c}	3.8^{b}	0.3^{a}	0.4^{a}	3.2^{c}	0.4^{b}	0.3^{b}	0.1^{a}
20:5n-3	0.0	0.0	0.1	0.1	0.0^{a}	0.0^{a}	0.2^{b}	0.3^{b}
22:5n-3	0.0^{a}	0.0^{a}	0.9^{b}	0.8^{b}	0.0^{a}	0.0^{a}	0.2^{b}	0.3^{b}
22:6n-3	4.7^{a}	19.4^{b}	24.2^{c}	25.7^{c}	0.9^{a}	2.9^{b}	3.5^{b}	4.5^{b}
	PE				PI			
20:4n-6	17.8^{c}	13.9^{b}	10.8^{a}	8.7^{a}	26.8	32.0	30.4	24.3
22:4n-6	8.8^{d}	6.0^{c}	3.9^{b}	2.6^{a}	0.7^{b}	0.7^{b}	1.7^{c}	0.0^{a}
22:5n-6	20.4^{c}	2.7^{b}	0.8^{a}	0.4^{a}	1.1^{b}	0.1^{a}	0.0^{a}	0.0^{a}
20:5n-3	0.0^{a}	0.0^{a}	0.6^{b}	0.9^{b}	0.0^{a}	0.1^{a}	1.0^{b}	1.1^{b}
22:5n-3	0.1^{a}	0.2^{a}	1.8^{b}	2.2^{c}	0.0^{a}	0.0^{a}	0.7^{b}	0.0^{a}
22:6n-3	6.0^{a}	21.2^{b}	25.0^{c}	34.6^{d}	0.6^{a}	2.2^{b}	1.9^{b}	2.8^{c}

Values are expressed as mol % and represent the means for three determinations each group. 0.0 indicates that levels were below those detectable, in most cases less than 0.01 %. Values sharing a same letter or without a letter did not differ significantly ($P < 0.05$, Fisher's protected least significant difference).
Abbreviations are the same as in Fig. 1.

Discussion

The results demonstrate that considerable adjustment of the long-chain PUFA in the brain lipids occurs in response to maternal diets. This study found that fish oil providing the highly unsaturated fatty acids, EPA and DHA, in contrast to 18:3n-3, resulted in elevated accumulations of DHA in the membrane PL of the brain. At this point we are reluctant to conclude that by passing an obvious regulatory point, i.e., metabolism of 18:3n-3 to 22:6n-3, is a net beneficial direction. This may lead to excessive accumulation of 22:6n-3 in brain. Second, there were no significant differences between developmental stages (3, 18, and 42 days). This long-

Table 3 Fatty acid composition of the murine brain phosphatidylserines from the offspring on days 3, 18, and 42 after parturition

	SAF			OLV			LIN			FSH		
	D3*	D18	D42	D3*	D18	D42	D3	D18	D42	D3	D18	D42
16:0	6.7	4.1	2.4	5.2	2.5	1.8	4.8	3.5	2.6	5.3^{b}	3.1^{a}	2.4^{a}
18:0	61.2	54.3^{b}	49.5^{a}	49.8	44.8	45.1	53.2	48.4	59.4	57.3	46.6	46.1
18:1n-9+7	4.1	15.4	10.8	8.1	13.8	18.9	6.6^{a}	16.2^{b}	14.2^{ab}	6.3^{a}	15.8^{b}	15.7^{b}
18:2n-6	0.0	0.3^{b}	0.1^{a}	0.0	0.2^{b}	0.1^{a}	0.4	0.3	0.2	0.0^{a}	0.2^{c}	0.1^{b}
20:3n-6	0.0	0.7	0.4	0.6	0.8^{b}	0.5^{a}	0.9	1.3	0.9	0.0^{a}	1.2^{c}	0.7^{b}
20:4n-6	5.5	3.6	2.5	6.4	3.6^{b}	2.6^{a}	3.8^{b}	2.1^{a}	1.8^{a}	1.1	1.8	1.4
22:4n-6	5.4	5.5	4.3	5.3	4.4^{b}	3.0^{a}	1.7	2.0	2.0	0.0^{a}	1.1^{b}	1.2^{b}
22:5n-6	11.5	15.9^{a}	21.9^{b}	5.3	5.6^{b}	3.8^{a}	0.0^{a}	0.3^{b}	0.3^{b}	0.0^{a}	0.3^{b}	0.4^{b}
20:5n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0^{a}	0.1^{b}	0.1^{b}
22:5n-3	0.0	0.0	0.0	0.0	0.2^{b}	0.0^{a}	2.9^{b}	1.6^{a}	0.9^{a}	1.3	1.2	0.8
22:6n-3	3.5	3.9	4.7	16.3	19.7	19.4	22.9	22.1	24.2	23.0	24.3	25.7
others†	1.9	3.6	2.2	1.8	3.2	3.6	3.0	2.8	3.5	5.8^{b}	3.2^{a}	3.8^{a}

Values are expressed as mol % and represent the means for three determinations each group. "18:1n-9+7" denotes the sum of 18:1n-9 and 18:1n-7. Values sharing a same letter or without a letter did not differ significantly ($P < 0.05$, Fisher's protected least significant difference) between time points in each diet group.
*One sample was determined on day 3 data due to limited tissue availability. Day 3 data were not included in statistics analysis in this situation.
†Others include 14:0, 14:1n-5, 16:1n-7, 20:0, 22:0, 22:1, 24:0, and 24:1n-9.

Table 4 Fatty acid composition of the murine brain phosphatidylethanolamines from the offspring on days 3, 18, and 42 after parturition

	SAF			OLV			LIN			FSH		
	D3*	D18	D42	D3*	D18	D42	D3	D18	D42	D3	D18	D42
16:0	12.5	7.3	6.1	10.8	7.4^{a}	7.6^{a}	11.8^{b}	7.8^{a}	6.9^{a}	12.2^{c}	7.7^{b}	5.2^{a}
18:0	27.7	25.9	26.1	24.9	25.3^{a}	25.3^{a}	26.5^{a}	25.9^{a}	23.8^{a}	23.3	24.8	24.1
18:1n-9+7	12.2	10.5	10.3	11.8	12.1	16.3	11.0^{a}	12.7^{a}	17.7^{b}	10.8^{a}	13.2^{b}	14.9^{b}
18:2n-6	2.6	0.7^{b}	0.4^{a}	0.3	0.3^{b}	0.1^{a}	0.7^{b}	0.6^{b}	0.4^{a}	0.6^{b}	0.4^{b}	0.2^{a}
20:3n-6	0.5	0.8^{b}	0.4^{a}	0.4	0.7	0.0	1.0^{ab}	1.2^{b}	0.8^{a}	1.0	1.3	1.0
20:4n-6	20.6	18.9	17.8	21.8	17.4^{b}	13.9^{a}	15.8^{b}	15.5^{b}	10.8^{a}	9.8^{ab}	11.4^{b}	8.7^{a}
22:4n-6	6.6	9.5	8.8	5.8	6.6^{b}	6.0^{a}	2.0^{a}	3.8^{b}	3.9	1.0^{a}	2.0^{b}	2.6^{b}
22:5n-6	11.4	16.2	20.4	4.3	4.6^{b}	2.7^{a}	0.2	0.2	0.8	0.5	0.4	0.4
20:5n-3	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.6	0.6	1.1	0.8	0.9
22:5n-3	0.0	0.0	0.1	0.0	0.2^{b}	0.2^{a}	3.5	2.4	1.8	3.3^{b}	2.1^{a}	2.2
22:6n-3	3.1	5.2	6.0	14.5	20.6	21.2	23.4	24.7	25.0	32.8	31.2	34.6
others	1.0	3.4	2.7	3.6	3.8^{a}	5.1^{a}	2.8	3.2	5.0	2.4^{a}	3.6^{b}	5.3^{c}

See footnotes in *Table 3*.

Table 5 Fatty acid composition of the murine brain phosphatidylcholines from the offspring on days 3, 18, and 42 after parturition

	SAF			OLV			LIN			FSH		
	D3	D18	D42	D3*	D18	D42	D3	D18	D42	D3	D18	D42
16:0	51.3	42.6	40.7	48.0	44.7	45.7	52.1^{b}	45.4^{a}	42.3^{a}	52.9^{b}	43.8^{a}	39.1^{a}
18:0	6.9^{a}	11.2^{b}	14.3^{b}	7.1	10.0	13.0	7.1^{a}	11.3^{ab}	15.7^{b}	8.9^{a}	10.5^{ab}	15.7^{b}
18:1n-9+7	21.8^{a}	24.8^{ab}	26.8^{b}	25.5	26.3	27.7	24.0	26.2	29.0	24.6^{a}	27.0^{b}	32.3^{c}
18:2n-6	1.6	1.5	1.2	0.7	0.7^{b}	0.3^{a}	1.3^{ab}	1.2^{b}	0.6^{a}	1.0^{b}	1.0^{b}	0.4^{a}
20:3n-6	0.5	0.5	0.3	0.4	0.4^{b}	0.2^{a}	0.4^{a}	0.7^{b}	0.4^{a}	0.3^{a}	0.8^{b}	0.4^{ab}
20:4n-6	5.6	6.5	7.6	4.8	6.9^{b}	4.6^{a}	2.4^{a}	5.2^{c}	3.7^{b}	1.7^{a}	4.3^{c}	3.0^{b}
22:4n-6	0.7^{a}	1.4^{b}	1.0^{a}	0.7	0.8	0.6	0.1^{a}	0.4^{b}	0.6^{c}	0.0^{a}	0.2^{b}	0.4^{c}
22:5n-6	1.2^{a}	3.5^{b}	3.2^{ab}	0.4	0.8^{b}	0.4^{a}	0.0	0.0^{a}	0.3^{b}	0.0^{a}	0.1^{b}	0.1^{c}
20:5n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.1	0.2	0.3	0.3	0.3
22:5n-3	0.0	0.0	0.0	0.0	0.1	0.0	0.4	0.3	0.2	0.2	0.3	0.3
22:6n-3	0.3	0.7	0.9	1.7	3.2	2.9	1.6^{a}	3.4^{b}	3.5^{b}	1.6^{a}	5.2^{b}	4.5^{b}
others	3.7	3.8	2.3	3.8	2.8	2.4	3.8^{b}	3.0^{a}	2.5^{a}	3.5	3.0	2.5

See footnotes in *Table 3*.

Table 6 Fatty acid composition of the murine brain phosphatidylinositols from the offspring on days 3, 18, and 42 after parturition

	SAF			OLV			LIN			FSH		
	D3*	D18	D42	D3*	D18	D42	D3	D18	D42	D3*	D18	D42
16:0	5.6	6.9^{ab}	9.6^{b}	11.1	6.0	6.9	5.2^{a}	7.5^{a}	12.2^{b}	4.6	6.9	9.1
18:0	58.5	54.1	55.8	48.8	41.7^{a}	50.3^{b}	56.0	49.8	57.2	44.0	47.5	52.8
18:1n-9+7	4.3	4.8	5.3	5.1	5.5	5.8	5.0	5.6	5.4	5.2	5.2	6.7
18:2n-6	0.0	0.8^{b}	0.4^{a}	0.0	0.2^{b}	0.0^{a}	0.4	0.4	0.0	0.0	0.2^{b}	0.0^{a}
20:3n-6	0.0	0.3^{b}	0.0^{a}	0.0	0.5^{b}	0.3^{a}	0.0	0.8^{b}	0.0^{a}	1.0	0.0	0.0
20:4n-6	19.2	29.4	26.8	29.3	29.0	32.0	29.4	28.6	30.4	39.6	32.2^{b}	24.3^{a}
22:4n-6	0.0	0.7	0.7	0.0	1.3^{b}	0.7^{a}	0.0	0.7^{a}	1.7^{b}	0.0	0.6^{b}	0.0
22:5n-6	0.0	1.3	1.1	0.0	0.7^{b}	0.1^{a}	0.0	0.0	0.0	0.0	0.0	0.0
20:5n-3	0.0	0.0	0.0	0.0	0.0	0.1	0.8	0.7	1.0	0.0	1.7	1.1
22:5n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0^{a}	0.7^{b}	0.0	0.4^{b}	0.0^{a}
22:6n-3	0.0	1.0^{b}	0.6^{a}	0.0	2.2	2.2	1.3^{a}	3.4^{b}	1.9^{ab}	2.5	3.8	2.8
others	1.4	1.0	0.0	2.8	1.0	1.6	2.5	1.6	2.0	3.2	1.3	0.8

See footnotes in *Table 3*.

term study confirms and adds strength to the theory that dietary effects on brain PL fatty acid composition are early events. We also suggest that the maternal diet is important and will affect the incorporation of n-3 fatty acids in the brain of the fetus. n-3 Fatty acids should be provided in the maternal diet until weaning to supply the needs of n-3 fatty acids in the brain of the young. Also for the young low in n-3 PUFA in the brain, providing an n-3–rich diet to the dams would help us to supply the needs of n-3 fatty acids. n-3 Fatty acids enriched in the diet had no further effects on mice after they were nursed by dams fed an n-3–rich diet.

The fatty acid profile in weaning brain reflected that of the maternal diets

The data resemble previous studies in suckling rats,[23,24] mouse,[25,26] monkey,[27] and chick.[28] The maternal diet directly affected the type of nutrient absorption of the neonates that take up essential components through the placenta. The dams received experimental diets for 5 months and continued through the nursing period. Safflower oil, richest in n-6 PUFA among the groups, led to the highest n-6 PUFA—mainly 22:5n-6. In contrast, fish oil rich in EPA plus DHA resulted in significant accumulation of n-3 PUFA—especially DHA in day 18-mouse brain PE and PS (*Figure 1*). The greatest depletion of the longer chain n-3 fatty acids such as DHA was probably due to the high ratio of dietary linoleic acid (18:2n-6) to α-linolenic acid (18:3n-3)[3] in safflower oil. It has been suggested that n-3 and n-6 fatty acids compete for the desaturase enzymes, especially the rate-limiting Δ6 desaturase, so that the conversion of linolenic acid in the n-3 series to 18:4n-3,[13] is inhibited by high levels of linoleic acid. The lower oxidation rate of the post-Δ6 desaturation products ensure the incorporation of the long-chain PUFA into PL, part of the stable structural components.[29] The low DHA in the brain of the safflower oil-fed group is not likely caused by its oxidation.

DHA, a 22-carbon chain-length fatty acid, may be essential for the development of the brain nervous system.[6,7] How then is it preferentially deposited in brain? The lipid transport protein in the brain of the neonates may affect the fatty acid composition.[30,31] Such lipid transport proteins may specifically take up 22-carbon chain-length fatty acids, i.e., DHA[32] in the fish oil-fed group and 22:5n-6 in the safflower oil fed group. Our intriguing observation that EPA was extremely low from the brain, even in the fish oil-fed group (*Figure 1, Table 3,4*), may also support the preferential selectivity of DHA incorporation into the brain.[33] EPA was present in the plasma of the neonates who have the enzymes necessary for further elongation and desaturation.[34] Yet DHA instead of EPA is the form being preferentially taken up.[32,35]

Linseed oil contained the same calorie percentage in the diet and n-3 to n-6 ratio as fish oil. However, the n-3 PUFA was in the form of 18:3n-3 rather than EPA or DHA. The data again showed extensive DHA incorporation relative to EPA and 18:3n-3 in PL of the brain (*Figure 1*). The fact that less DHA was incorporated into the brain PE in the weaning linseed oil-fed group (*Figure 1*) suggested that DHA rather than its precursor (18:3n-3) was more efficient at incorporation into the brain PL. Low desaturase activities may prolong the accumulation of DHA from 18:3n-3.[13,36–38] The limited transport of 18:3n-3 through the blood brain barrier[36,39] could be another explanation. However more studies are needed to establish this mechanism.[40,41]

Regarding the essentiality of DHA for the development of the brain, it may also be important to consider maintaining membrane integrity by keeping the appropriate quantity of n-6 PUFA.[6,26,42] Research[43,44] has demonstrated that increasing quantities of dietary 18:3n-3 resulted in an overall increase of DHA and inversely a decrease of 22:5n-6 in rat brain. In whole brain the level of DHA was shown to increase with 18:3n-3 intake varying from 1 to 200–250 mg/100 g of diet, then reaching a plateau (the inverse was observed for 22:5n-6). The level of arachidonic acid in membranes did not change by differing the 18:3n-3 in the diet.[44] The largest

absolute changes in 22:5n-6 and DHA levels occurred as the 18:2n-6 to 18:3n-3 ratio increased from 36 to 165.[45] It was suggested that the 18:2n-6 to 18:3n-3 ratio for the weaning rat should be <36, and possibly even as low as 9, to reach the adequate amount of DHA. This study suggests that long-chain n-3 PUFA differ from 18:3n-3.

Olive oil, with 78.3% of oleic acid (18:1n-9) and 0.6% 18:3n-3 in the diet, accumulated less (average of 5% less in PE) DHA than the linseed oil group (*Figure 1*). However, olive oil contained just 0.6% n-3 PUFA. DHA levels in linseed oil-fed animals overall were just slightly higher than olive oil-fed mice and were only elevated in the PL classes PE and PI.

High oleic-low linoleic acid diet feeding[46] showed 18:1 and 20:3n9 accumulation in fetal rat tissues, suggesting an essential fatty acid (EFA) deficiency state. Nevertheless, the mice did not exhibit functional deficiency symptoms in this study. Fish oil provided 40 times more dietary n-3 fatty acids than olive oil, but the amount of DHA was only about 30% more than that in the olive oil group.

The selective incorporation of fatty acid into the brain

We fed the mice 11.2% of EPA in the present study, but the amount of this fatty acid in brain PL was hardly detectable. Even after 42 days of fish-oil feeding to the offspring, the highest amount was 1.1 mol% in PC. EPA is either quickly metabolized into DHA after entering the brain,[47] or it is selectively excluded from the brain. EPA was formed in cultured murine cerebromicrovascular endothelia[48] and astrocytes but not neurons[49] and large vessel endothelial cells[50,51] incubated with 1-^{14}C-18:3n3. The endothelial cells at the blood-brain barrier may take up [1-^{14}C]-18:3n3 from the circulation, convert them into EPA that can be more effectively utilized in neural tissues, and release EPA into the brain.[48] The blood-brain barrier may play a role in controlling the elongation and desaturation of n-3 fatty acids. However, experiments on hepatectomized rats given intracarotid injection of [1-^{14}C]-18:3n-3, which was converted to DHA and became an active component in brain, suggested a higher metabolism to DHA rather than EPA in adult animals.[39,52–53] The blood-brain barrier did not influence the uptake of DHA.[53] Intraperitoneal injection of [1-^{14}C]-18:3n-3 into 3-day-old mouse pups resulted in a rapid decline in liver and serum lipid labeling.[35] In contrast, labeling of the brain and retinal lipids were initially low and increased with time. It was hypothesized that the requirements for DHA are met by a signal that was sent by the appropriate tissues to the liver to induce the secretion of DHA-containing lipoproteins.

The effects of development on fatty acid modification

The consequence of age on fatty acid modification via dietary means is controversial. Dyer and Greenwood[45] showed that in the well-developed brain of the weaning rat, the neural 22-carbon fatty acids could be altered within 2 weeks by changing the dietary 18:2n-6 to 18:3n-3 ratio, and that the magnitude of the effect increases with time. The brain fatty acid composition could also be altered by dietary fats in short-term studies after the animals are weaned.[3,7,54] In contrast, others using shorter and longer periods of experimental studies reported that the brain was less responsive[25,33] or recovered remarkably slowly[3,23] to dietary modulation after weaning. The length of time that the dams were on the experimental diets,[8] the dosage of the fats in the diets,[26,33,54–56] the species of animal, the age of the animals,[57–59] and the time point selected to be examined[33,57] could affect the results. The day 42 data (*Table 2*) revealed that longer dietary feeding did not change the fatty acid composition compared with that in day 18 mice nursed by the mothers. n-3 PUFA were not changed by development when the maternal diets were already rich in n-3 PUFA, that is, the day-3 data remained constant throughout. It might be that the turnover of neurons and oligodendrocytes and the renewal of brain membranes are slow. When the brain maturation is complete, the modification of fatty acid becomes limited.[3,29] Milk from the dams is the major food source for the pups before weaning, as synthetic diets are after weaning. It is possible to alter the pups' brain PL composition by changing the food for the dams, that is, changing the fatty acid composition in the milk, or changing the diets for the pups after weaning. Although we did not design the changes in the diets for the dams or diet for the pups, we suggest that weaning is the time point at which to alter the fatty acid composition in the brain by dietary means. For future work the appropriate amount of n-3 fatty acids in the diet, the age of the animals, time point for the diet and the regulating mechanism of the incorporation of EPA and DHA in the brain should be investigated.

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