Differential effects of n-3 fatty acid deficiency on phospholipid molecular species composition in the rat hippocampus

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Abstract **In this study, we have examined the effects of n-3 fatty acid deficient diets on the phospholipids (PL) molecular species composition in the hippocampus. Female rats were raised for two generations on diets containing linoleic acid (18:2n-6), with or without supplementation of α-linolenic acid (18:3n-3) or 18:3n-3 plus docosahexaenoic acid (22:6n-3). At 84 days of age, the hippocampal phospholipids were analyzed by reversed phase HPLC-electrospray ionization mass spectrometry. Depleting n-3 fatty acids from the diet led to a reduction of 22:6n-3 molecular species in phosphatidylcholine (PC), phosphatidylethanolamine (PE), PE-plasmalogens (PLE), and phosphatidylserine (PS) by 70–80%. In general, 22:6n-3 was replaced with 22:5n-6 but the replacement at the molecular species level did not always occur in a reciprocal manner, especially in PC and PLE. In PC, the 16:0,22:6n-3 species was replaced by 16:0,22:5n-6 and 18:0,22:5n-6. In PLE, substantial increases of both 22:5n-6 and 22:4n-6 species compensated for the decreases in 22:6n-3 species in n-3 fatty acid deficient groups. While the total PL content was not affected by n-3 deficiency, the relative distribution of PS decreased by 28% with a concomitant increase in PC. The observed decrease of 22:6n-3 species along with PS reduction may represent key biochemical changes underlying losses in brain-hippocampal function associated with n-3 deficiency.**—Murthy, M., J. Hamilton, R. S. Greiner, T. Moriguchi, N. Salem, Jr., and H-Y. Kim. **Differential effects of n-3 fatty acid deficiency on phospholipid molecular species composition in the rat hippocampus.** ***J. Lipid Res.*** **2002.** 43: **611–617.**

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Neural membranes accumulate and conserve high levels of 22:6n-3, which may be derived from its dietary precursor 18:3n-3 and from preformed 22:6n-3 during neurodevelopment (1–3). It is becoming increasingly apparent that rigorous maintenance of 22:6n-3 is necessary for optimal neural development and function (4–8).

In animal studies, it has been shown that a reduction in brain 22:6n-3 leads to a variety of behavioral and visual deficits (7–15). The evidence in human infant studies suggests that feeding formulas containing low n-3 PUFA leads to suboptimal cognitive and visual function (5, 6, 15–18). The reduction of 22:6n-3 resulting from an n-3 fatty acid deficiency is generally associated with concomitant increases in 22:5n-6 (8, 19–24). It has been shown that DHA-deficient animals recover from their 22:6n-3 losses in the brain when switched to an n-3 fatty acid adequate diet (9, 23) and they also recover from deficits in spatial task performance (24); however, changes in electroretinograms (9) and mean arterial blood pressure do not appear to be reversible (25).

The glycerophospholipids in neural membranes are the main reservoirs of PUFAs, including 22:6n-3. However, each phospholipid class in these membranes has a distinct and unique molecular species profile. Both de novo biosynthetic and remodeling pathways appear to contribute to the unique molecular species assembly in these phospholipids (PL) classes. Studies in the rat pineal gland (26) and in both monkey (27) and guinea pig brains (28) have shown that n-3 fatty acid deficiency markedly alters the composition of PL molecular species. Furthermore, it has been demonstrated that loss of docosahexaenoic acid (DHA) due to n-3 fatty acid deficiency decreases the phosphatidylserine (PS) concentration in neuronal membranes (29–31). These diet-induced changes in PL molecular species may significantly alter membrane properties (32, 33), subsequently leading to adverse functional consequences (1, 9, 34).

Abbreviations: 20:4n-6, arachidonic acid; DHA (22:6n-3), docosahexaenoic acid; 22:5n-6, docosapentaenoic acid; HPLC-MS, HPLC-mass spectrometry; LA (18:2n-6), linoleic acid; LNA (18:3n-3), α-linolenic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipids; PLE, PE-plasmalogens; PS, phosphatidylserine.

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It has been previously shown that olfactory-based (14, 22) and spatial task performance (8, 11, 13) in rats responds to the brain n-3 fatty acid status. It is well established that the hippocampus plays a crucial role in learning and memory-related tasks, particularly in configural discriminations (35). In an attempt to understand the potential importance of diet-induced modification of membrane PL molecular species in the observed functional deficits, we have examined in detail the effects of n-3 fatty acid deficiency on PL molecular species composition of the rat hippocampus.

MATERIALS AND METHODS

Boron trifluoride (BF_3) was obtained from Sigma or Alltech (Deerfield, IL). Deuterium labeled or non-labeled phospholipid standards were purchased from or custom synthesized by Avanti Polar Lipids (Alabaster, AL). All solvents were analytical or HPLC grade and purchased from Fisher (Pittsburgh, PA).

Animals and diets

All experimental procedures were carried out in accordance with the policies of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Animal Care and Use Committee. All animals were bred and housed in the NIAAA animal facility. The animals were kept on a 12 h light-dark cycle with light onset at 0600 h. The level of fluorescent luminescence averaged 65 lux in the colony. Room temperature was maintained at 21°C, with average relative humidity at 50%. Food and water were provided ad libitum.

Long Evans female rats (first generation, F1) received from a commercial source (Charles River Portage, MI) at 3 weeks of age, were randomly divided into four groups and raised on four semisynthetic diets based on the AIN-93 diet as previously described (7, 13, 23). The n-3 deficient diets contained coconut oil and safflower oil nearly devoid of 18:3n-3. The two low n-3 diets were labeled as linoleic acid (LA) (containing 1.9% safflower oil and 8.1% coconut oil) or high-LA (8.5% safflower oil and 1.5% coconut oil). The two n-3 fatty acid diets were supplemented with 0.48% flaxseed oil with or without 0.63% DHASCO oil (contained DHA, Martek Biosciences Corporation, Columbia, MD). These two diets were labeled as +LNA (α-linolenic acid) (≈0.3 g 18:3n-3 per 100 g diet) and +LNA/DHA (≈0.3 g 18:3n-3 and 0.128 g DHA per 100 g diet). The fatty acid composition of each diet is shown in **Table 1**. At 8 weeks of age, female rats were allowed to mate with chow-fed (NIH-31 chow) male rats. The dams continued with their experimental diets during the suckling period. Each experimental group was composed of pups taken from each of 10 litters at weaning for an n = 10/group. These pups (F2) were then given the same diets as their dams. At 12 weeks of age, 24 female rats were euthanized by decapitation. Both right and left hippocampi were rapidly dissected from the brain and immediately stored at −80°C until use for lipid analysis. The hippocampal layer and neuron sections were stained in cresyl violet, and examined microscopically for morphology prior to using them for subsequent lipid molecular species analysis as described previously (36).

Lipid and fatty acid analysis

The hippocampi were homogenized and the lipids extracted by the method of Folch (37). About half of the lipid extract from each hippocampus sample (lipid extracts from about 30–50 mg wet weight of hippocampal tissue) was used for fatty acid analysis. To determine total fatty acids, small aliquots of the total lipid extracts (about 5% of total) were transmethylated with 14% BF_3 in methanol following the method of Morrison and Smith (38). Fatty acid methyl esters were analyzed on a Hewlett-Packard gas chromatograph (Series 5890 II) equipped with a flame ionization detector. Unknown fatty acid peaks were identified and quantified using 23:0 as an internal standard, as previously described (39).

Table 1. Fatty acid composition of diets

Fatty Acid	LA	High-LA	+LNA	+LNA/DHA
	% total fatty acids			
8:0	0.8	0.3	1.86	2.1
10:0	3.8	0.8	3.8	3.9
12:0	39.7	7.0	36.5	35.3
14:0	16.7	3.0	15.8	15.5
16:0	9.8	7.0	9.6	9.5
18:0	9.8	3.9	9.5	9.0
20:0	0.2	0.3	0.2	0.2
22:0	0.1	0.2	0.1	0.1
24:0	0.1	0.1	0.1	0.1
Total saturates	80.9	22.7	77.2	75.6
16:1n-7	0.03	0.1	0.03	0.1
18:1n-9	3.6	12.3	3.9	4.4
18:1n-7	0.3	0.7	0.3	0.3
20:1	0.1	0.2	0.1	0.1
22:1	0.01	0.02	0.02	0.02
Total mono unsaturates	3.9	13.0	4.3	4.8
18:2n-6	15.1	63.9	15.3	15.7
20:2n-6	0.1	0.1	0.0	0.1
20:3n-6	nd	nd	nd	nd
20:4n-6	nd	0.1	nd	nd
Total n-6 fatty acids	15.1	64.0	15.3	15.8
18:3n-3	0.1	0.1	3.1	2.6
20:5n-3	nd	nd	nd	nd
22:5n-3	nd	nd	nd	nd
22:6n-3	nd	nd	nd	1.3
Total n-3 fatty acids	0.1	0.1	3.1	3.9
18:2n-6/18:3n-3	345	529	5	6
n-6/n-3	346	530	5	4
Total PUFA	15	64	18	20

Phospholipid molecular species analysis by HPLC-mass spectrometry

The other half of the lipid extracts were used for the separation and quantitation of phospholipid molecular species by reversed-phase HPLC-electrospray ionization mass spectrometry with a C_{18} column (150 × 20 mm, 5 μm, Phenomenex, Torrance, CA) as described previously (40). Lipid extracts of rat hippocampi containing a mixture of d_{35}-labeled phospholipids as internal standards were injected directly onto a C_{18} column. The separation of phospholipid molecular species from n-3 fatty acid supplemented or deficient groups was accomplished with a mobile phase containing water: 0.5% ammonium hydroxide in methanol-hexane. The gradient increased linearly from 12:88:0 to 0:88:12 in 17 min after holding the initial composition for 3 min at a flow rate of 0.4 ml/min (41). A Hewlett-Packard HPLC-MS series 1100 MSD instrument was employed to detect the phospholipid molecular species. For electrospray ionization, the drying gas temperature was 350°C while the drying gas flow rate and nebulizing gas pressure were 13 l/min and 32 psi, respectively. The capillary voltage was set at 4,000 V, and the exit voltage was set at 200 V. Under these conditions, PS, phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA) fragmented into diglyceride (DG^+) ions. However, phosphatidylcholine (PC) species were detected mainly as pro-

tonated $(M+H)^+$ and natriated $(M+Na)^+$ ions. Individual molecular species from n-3 deficient or supplemented hippocampal lipids were quantified using deuterated phospholipid standards representing each of the phospholipid classes (**Fig. 1**). As shown in Fig. 1, the 22:5n-3 species were chromatographically separated from the 22:5n-6 species. The concentration of 22:5n-3 in samples from both the deficient and supplemented groups was small and therefore not included in our tables.

Statistical analysis

One-way ANOVA was used to analyze the effect of diets on the percentages of the phospholipid molecular species. Significant differences between diets were determined by post-hoc Tukey honestly significant differences test.

RESULTS

We observed that n-3 fatty acid deficient diets (LA and high-LA) led to a dramatic decline in the total 22:6n-3 content (from 13% to 1.5%) and this decrease was primarily compensated for by 22:5n-6 (from 0.4% to 11%) in all four hippocampal PLs. This was an expected result based on previous investigations (19–24). However, the extent of compensatory replacement of 22:6n-3 with 22:5n-6 was not always reciprocal and varied among PL classes. This difference is apparent in PC as illustrated in Fig. 1 where the 18:0,22:5n-6 replaced the 18:0,22:6n-3 species to a significantly greater extent when compared with PS or PE. We consistently observed little or no difference in species composition between the two n-3 fatty acid deficient groups (LA and high-LA groups) or between the two n-3 fatty acid supplemented groups (+LNA and +LNA/DHA).

Molecular species composition of PC

In **Table 2**, we have summarized the effects of the n-3 fatty acid deficiency upon the molecular species composition of PC. Phospholipid species containing 22:5n-6 represented only 3.6% and 4.2% of the total PC species in the +LNA and +LNA/DHA diet groups, respectively, whereas the 22:6n-3 species ranged from 15.5–16.8% in these diet groups. In contrast, in the LA and high-LA groups, 22:5n-6 species comprised approximately 19% with only small percentages of 22:6n-3 species (4.5–4.7%). The 16:0,22:6n-3-PC was the most abundant polyunsaturated PC species in both n-3 supplemented groups. After a low n-3 fatty acid diet, however, both 16:0,22:5n-6 and 18:0,22:5n-6 were prominent polyun-

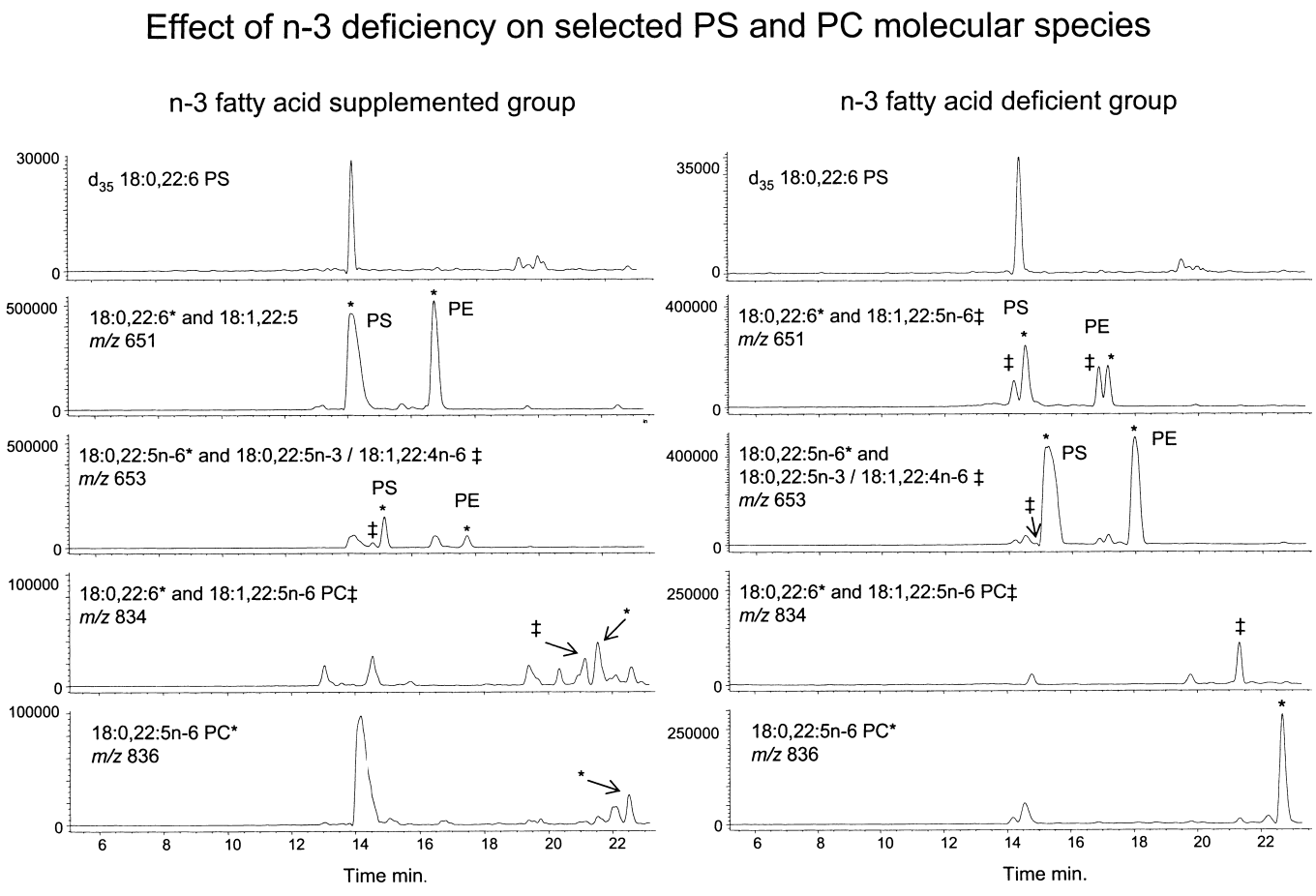

Fig. 1. Mass ion chromatograms of selected phospholipid molecular species in the rat hippocampus obtained after n-3 fatty acid supplementation or depletion. Phospholipid molecular species were separated by reversed-phase HPLC and subsequently detected by electrospray ionization mass spectrometry as diglyceride (DG^+) ions for phosphatidylserine (PS) and phosphatidylethanolamine (PE) or $(M+H)^+$ ions for phosphatidylcholine (PC) molecular species. Note that the 18:0,22:5n-6 and 18:0,22:5n-3 species were chromatographically separated. The level of 18:0,22:5n-3 was very low even in the n-3 fatty acid supplemented groups.

Table 2. Phosphatidylcholine molecular species composition of the hippocampus

Species	Diet			
	LA ($n = 6$)	High-LA ($n = 11$)	+LNA ($n = 6$)	+LNA/DHA ($n = 10$)
16:0,18:1	16.8 ± 0.5*	16.3 ± 0.3	16.0 ± 0.4	15.7 ± 0.3
18:0,18:1	18.4 ± 0.7^{c,f,i}	20.6 ± 0.4^{c}	21.0 ± 0.5^{f}	21.4 ± 0.5^{i}
18:1,18:1	2.5 ± 0.2^{a,c}	3.4 ± 0.2^{a}	2.7 ± 0.2^{b}	4.0 ± 0.2^{b,c}
16:0,20:4	11.1 ± 0.2	11.1 ± 0.3	11.8 ± 0.5	11.4 ± 0.4
18:0,20:4	10.1 ± 0.3	11.5 ± 0.3	10.6 ± 0.6	10.3 ± 0.3
18:1,20:4	4.2 ± 0.2^{a}	5.1 ± 0.4	5.1 ± 0.3	4.4 ± 0.4^{a}
16:0,22:4	0.5 ± 0.3	0.9 ± 0.2	0.7 ± 0.1	0.2 ± 0.1
18:0,22:4	2.1 ± 0.1^{a,d}	0.9 ± 0.3^{a}	1.0 ± 0.2^{d}	1.2 ± 0.2
16:0,22:5	9.8 ± 0.5^{c,f}	10.5 ± 0.9^{i,l}	2.0 ± 0.4^{c,i}	2.4 ± 0.2^{f,l}
18:0,22:5	7.5 ± 0.5^{c,f}	6.5 ± 0.5^{i,l}	0.8 ± 0.1^{c,i}	0.8 ± 0.1^{f,l}
18:1,22:5	2.0 ± 0.2^{b,f}	1.7 ± 0.2^{h,l}	0.8 ± 0.1^{b,h}	1.0 ± 0.2^{f,l}
16:0,22:6	4.1 ± 0.2^{c,f}	3.9 ± 0.2^{i,l}	12.9 ± 0.7^{c,i}	13.7 ± 0.5^{f,l}
18:0,22:6	nd	nd	1.0 ± 0.1^{a,c,i}	1.4 ± 0.1^{a,f,l}
18:1,22:6	0.6 ± 0.1^{c,f}	0.6 ± 0.1^{i,l}	1.6 ± 0.0^{c,i}	1.7 ± 0.2^{f,l}

* Numbers represent the percent of total (mean ± SEM) for six or more rats; some minor species were omitted from the table. Comparisons were made with one-way ANOVA. The same superscripts indicate that statistical significance was reached at the indicated level as measured by the posthoc Tukey HSD test.

^{a,d} $P < 0.05$.

^{b,h} $P < 0.005$.

^{c,f,i,l} $P < 0.001$.

nd, not detected.

saturated PC species. It is worth noting that 18:0,22:5n-6-PC (7.5%) increased substantially while the 18:0,22:6n-3 species was only a minor component (1%) even after 22:6n-3 supplementation. The monoenoic species 18:0,18:1 and 16:0,18:1 were the major components in PC and together represented 35–37% of the total across all four dietary groups. Phospholipid species containing 20:4n-6 ranged from 25–28% in PC.

Table 3. Phosphatidylethanolamine molecular species composition of the hippocampus

Species	Diet			
	LA ($n = 6$)	High-LA ($n = 11$)	+LNA ($n = 6$)	+LNA/DHA ($n = 10$)
16:0,18:1	2.3 ± 0.2*	2.4 ± 0.2	1.8 ± 0.1	2.1 ± 0.1
18:0,18:1	6.8 ± 0.6^{c}	7.8 ± 0.6^{b}	9.2 ± 0.6	11.5 ± 0.8^{b,c}
18:1,18:1	2.9 ± 0.2	1.6 ± 0.2	2.4 ± 0.2	2.8 ± 0.3
16:0,20:4	4.9 ± 0.3	5.3 ± 0.5	4.6 ± 0.3	4.1 ± 0.3
18:0,20:4	24.2 ± 0.4	23.6 ± 1.0	22.3 ± 1.1	20.2 ± 1.0
18:1,20:4	4.8 ± 0.1	5.0 ± 1.7	7.1 ± 0.3	7.0 ± 0.3
16:0,22:4	3.3 ± 0.3	4.0 ± 0.7	3.9 ± 0.9	2.4 ± 0.2
18:0,22:4	4.4 ± 0.3	4.5 ± 0.2	4.0 ± 0.3	4.1 ± 0.2
16:0,22:5	11.7 ± 0.2^{c,f}	11.1 ± 0.4	0.5 ± 0.1^{c}	1.0 ± 0.2^{f}
18:0,22:5	20.3 ± 0.6^{c,f}	22.1 ± 0.7^{i,l}	1.0 ± 0.1^{c,i}	1.0 ± 0.2^{f,l}
18:1,22:5	5.8 ± 0.3^{c}	4.5 ± 0.3^{c}	nd	nd
16:0,22:6	3.5 ± 0.3^{c,f}	3.6 ± 0.2^{i,l}	14.7 ± 1.3^{c,i}	15.4 ± 1.1^{f,l}
18:0,22:6	3.7 ± 0.3^{c,f}	3.6 ± 0.2^{i,l}	21.9 ± 1.1^{c,i}	21.1 ± 1.2^{f,l}
18:1,22:6	0.7 ± 0.3^{c,f}	0.4 ± 0.2^{i,l}	5.3 ± 0.4^{c,i}	6.1 ± 0.1^{f,l}

* Numbers represent the percent of total (mean ± SEM) for six or more rats; some minor species were omitted from the table. Comparisons were made with one-way ANOVA. The same superscripts indicate that statistical significance was reached at the indicated level as measured by the posthoc Tukey HSD test.

^{b} $P < 0.005$.

^{c,f,i,l} $P < 0.001$.

nd, not detected.

Table 4. PE-plasmalogens (PLE) molecular species composition of the hippocampus

Species	Diet			
	LA ($n = 6$)	High-LA ($n = 11$)	+LNA ($n = 6$)	+LNA/DHA ($n = 10$)
16:0,18:1	7.5 ± 0.3*^{,a,c,f}	6.1 ± 0.2^{a,d,g}	4.4 ± 0.5^{c,d}	4.7 ± 0.4^{f,g}
18:0,18:1	5.3 ± 1.5	6.4 ± 1.0	4.8 ± 0.9	6.3 ± 0.7
18:1,18:1	4.8 ± 0.4	6.1 ±0.4^{a}	5.9 ± 0.5^{a,d}	6.8 ± 1.1^{d}
16:0,20:4	11.0 ± 0.5^{c}	10.0 ± 0.4^{b}	9.3 ± 0.5	7.5 ± 0.6^{b,c}
18:0,20:4	8.8 ± 0.5	8.2 ± 0.6	8.5 ± 1.1	8.2 ± 0.6
18:1,20:4	10.9 ± 0.6	11.3 ± 0.9	12.3 ± 0.5	11.3 ± 0.5
16:0,22:4	11.2 ± 0.9^{a,g}	9.2 ± 0.9^{d,j}	6.0 ± 0.6^{a,d}	5.2 ± 1.0^{g,j}
18:0,22:4	8.4 ± 1.1^{a,d}	8.6 ± 0.5^{b,g}	4.8 ± 0.7^{a,b}	5.7 ± 0.5^{d,g}
16:0,22:5	9.6 ±0.5^{a,c,f}	8.3 ± 0.3^{a,i,l}	0.8 ± 0.0^{c,i}	1.0 ± 0.0^{f,l}
18:0,22:5	7.1 ± 0.5^{a,c}	8.9 ± 0.8^{f,i}	1.6 ± 0.1^{c,f}	3.7 ± 0.7^{a,i}
18:1,22:5	3.6 ± 0.2	4.0 ± 1.3	nd	nd
16:0,22:6	2.9 ± 0.2^{c,f}	4.5 ± 0.7^{i,l}	16.5 ± 0.5^{c,i}	16.2 ± 0.5^{f,l}
18:0,22:6	4.7 ± 0.1^{c,f}	3.4 ± 0.2^{i,l}	16.7 ± 1.1^{c,i}	16.0 ±0.3^{f,l}
18:1,22:6	1.0 ± 0.6^{c,f}	1.4 ± 0.2^{i,l}	7.0 ± 0.4^{c,i}	6.6 ± 0.7^{f,l}

* Numbers represent the percent of total (mean ± SEM) for six or more rats; some minor species were omitted from the table. Comparisons were made with one-way ANOVA. The same superscripts indicate that statistical significance was reached at the indicated level as measured by the posthoc Tukey HSD test.

^{a,d,g,j} $P < 0.05$.

^{b} $P < 0.005$.

^{c,f,i,l} $P < 0.001$.

nd, not detected.

Neither 18:1- nor 20:4-containing PC species were affected significantly by the n-3 fatty acid deficient diets.

Molecular species composition of PE and PE-plasmalogens

The molecular species compositions of PE and PE-plasmalogens (PLE) are summarized in **Tables 3** and **4**, respectively. The 18:1 species ranged from 12–16% and 15–19% in PE and PLE fractions, respectively, in all four dietary groups. The percentages of 20:4n-6 species found in PE and PLE fractions (27–34% across the four dietary groups) were slightly higher than the levels found in PC. An unusually high concentration of the 18:1,20:4 species was observed only in PLE, however, its content was not altered by n-3 fatty acid deficiency. The percentages of 22:4n-6 species found in the PE or PLE fraction (6.5–8.5% and >10% across the four dietary groups, respectively) were significantly higher than those found in PC where it only ranged from 1.4–2.6%. The 22:6n-3 species in PE ranged from 42–43% in both n-3 fatty acid supplemented groups with 18:0,22:6n-3 (~21%) and 16:0,22:6n-3 (~15%) as the major species. N-3 fatty acid deficient diets decreased 22:6n-3 species to about 8% and increased 22:5n-6 species dramatically from about 2% to 38% of the total PE species. Although the levels of 22:6n-3 species were comparable in PE and PLE (about 38%) in both n-3 fatty acid supplemented groups, 22:5n-6 levels in the PLE fraction were significantly lower (about 20%) than the levels found in PE (about 38%) in both n-3 fatty acid deficient groups. A substantial increase of 22:4n-6 species (from about 11% to 19%) occurred and the 22:5n-6 and 22:4n-6 species in combination accounted for the compensation for the loss of 22:6n-3 in PLE. There was also a small but significant increase in the 16:0,20:4-PLE species in deficient groups relative to that of the +LNA/DHA diet group.

Table 5. Phosphatidylserine molecular species composition of the hippocampus

Species	Diet			
	LA ($n = 6$)	High-LA ($n = 11$)	+LNA ($n = 6$)	+LNA/DHA ($n = 10$)
16:0,18:1	$1.0 \pm 0.2^{*,c,i}$	$0.6 \pm 0.2^{f,l}$	$2.0 \pm 0.3^{c,f}$	$1.6 \pm 0.2^{i,l}$
18:0,18:1	16.3 ± 0.6	15.2 ± 0.9	16.1 ± 0.7^{a}	16.5 ± 0.7^{a}
18:1,18:1	3.6 ± 0.6^{a}	$5.1 \pm 0.3^{a,c,f}$	2.1 ± 0.1^{c}	2.4 ± 0.3^{f}
16:0,20:4	0.6 ± 0.1^{a}	0.4 ± 0.1^{c}	$1.3 \pm 0.2^{a,c,e}$	0.4 ± 0.1^{e}
18:0,20:4	12.4 ± 0.9	12.9 ± 0.7^{a}	10.0 ± 0.8	10.2 ± 0.7^{a}
18:1,20:4	1.8 ± 0.2	2.0 ± 0.2	1.5 ± 0.2	2.1 ± 0.2
16:0,22:4	nd	nd	nd	nd
18:0,22:4	$5.3 \pm 0.4^{b,c}$	$5.3 \pm 0.2^{e,f}$	$2.9 \pm 0.4^{c,f,h}$	$3.5 \pm 0.4^{b,e,h}$
16:0,22:5	1.4 ± 0.2^{a}	0.9 ± 0.3^{a}	nd	nd
18:0,22:5	$41.8 \pm 0.6^{c,f}$	$43.4 \pm 0.8^{i,l}$	$3.1 \pm 0.3^{c,i}$	$3.3 \pm 0.3^{f,l}$
18:1,22:5	nd	nd	nd	nd
16:0,22:6	nd	nd	$3.5 \pm 0.4^{c,f}$	2.8 ± 0.6^{a}
18:0,22:6	$13.1 \pm 0.4^{c,f}$	$12.5 \pm 0.6^{i,l}$	$54.0 \pm 0.7^{c,i}$	$52.9 \pm 0.8^{f,l}$
18:1,22:6	$0.7 \pm 0.4^{b,f}$	$0.7 \pm 0.2^{i,l}$	$3.5 \pm 0.7^{b,i}$	$5.3 \pm 0.7^{f,l}$

* Numbers represent the percent of total (mean ± SEM) for six or more rats; some minor species were omitted from the table. Comparisons were made with one-way ANOVA. The same superscripts indicate that statistical significance was reached at the indicated level as measured by the posthoc Tukey HSD test.

[a] $P < 0.05$.

[b,e,h] $P < 0.005$.

[c,f,i,l] $P < 0.001$.

nd, not detected.

Molecular species composition of PS

In **Table 5**, we have summarized the effects of our experimental diets on the molecular species compositions of PS. In general, the percentage of total 18:1 species in PS was lower than the level of 18:1 species in PC. Similarly, the 20:4n-6 and 22:4n-6 species were also lower in PS when compared with their levels in PC, PE, and PLE. In the +LNA and +LNA/DHA groups, 18:0,22:6n-3 was the major species found in PS (>50%), which was to a large extent replaced by 18:0,22:5n-6-PS in both of the n-3 fatty acid deficient dietary groups. The 18:0,22:5n-6 species increased markedly from 3% to 43%, while 18:0,22:6n-3-PS was reduced from 54% to 13% by n-3 fatty acid deficiency.

Overall effect of n-3 deficiency on phospholipid molecular species composition

It is apparent from our results that the replacement of 22:6n-3 with 22:5n-6 in groups fed with n-3 fatty acid deficient diets does not always occur in a reciprocal manner. For example, 16:0,22:6n-3 species were replaced with both 16:0,22:5n-6 and 18:0,22:5n-6 in PC. In PE, 16:0,22:6n-3 and 18:0,22:6n-3 species were proportionately replaced with 16:0,22:5n-6 and 18:0,22:5n-6. In PLE, 16:0,22:6n-3 and 18:0,22:6n-3 species were replaced not only with 16:0,22:5n-6 or 18:0,22:5n-6 but also with 16:0,22:4n-6 and 18:0,22:4n-6 species and, to a lesser extent, with 16:0,20:4n-6. In PS, 18:0,22:6n-3 species were replaced mainly with 18:0,22:5n-6. In PI, where the predominant species was 18:0,20:4, little change was observed in 22:6n-3 or 22:5n-6 species (data not shown). The combined PI and PA represented less than 5% of the total glycerophospholipids.

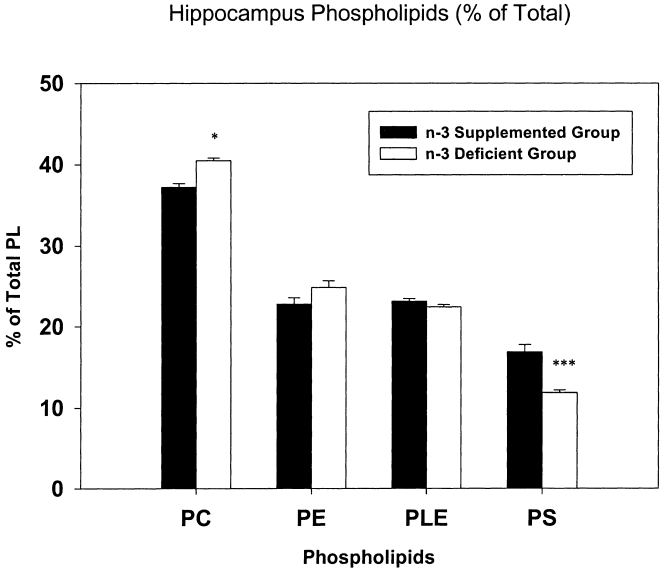

Fig. 2. The numbers in this figure are expressed as mean ± SEM, and represent percent of total glycerophospholipids (including only PC, PE, plasmalogens (PLE), and PS). The data from two n-3 fatty acid deficient groups or two n-3 fatty acid supplemented groups were combined and referred to as "deficient" (n = 10) or "supplemented" groups (n = 10), respectively. The comparison was made by Student's *t*-test. The percentage of PC in the deficient group was significantly higher than that of the supplemented group (*$P < 0.05$). The PS in the deficient group was significantly lower (***$P < 0.001$) in comparison to the supplemented group.

Effect of n-3 deficiency on the PS content

In addition to the replacement of 22:6n-3 with 22:5n-6 species by the depletion of n-3 fatty acids, there were also significant alterations in the phospholipid class profile. When the distribution of each phospholipids class was calculated as the percent of the total glycerophospholipid, it was shown that the PS proportion was significantly lower (by 28%, $P < 0.001$) in the n-3 fatty acid deficient groups (**Fig. 2**). It is also interesting to note that this PS class decrease was associated with a slight but significant increase in PC ($P < 0.05$) in these deficient groups. The PE content also showed a tendency to increase although it was not statistically significant. The total phospholipid concentration as determined by phosphorous assay did not show significant differences among the four diet groups (data not shown).

DISCUSSION

In this report, we present the effects of n-3 fatty acid supplementation or depletion on the glycerophospholipid molecular species profile in the rat hippocampus. Our findings indicate that 18:3n-3 supplementation at 0.3 g/100 g diet may be sufficient to support the maintenance of 22:6n-3 concentration in hippocampal phospholipid classes, since adding 22:6n-3 in the diet did not significantly influence the 22:6n-3 levels. A marked loss of hippocampal 22:6n-3 phospholipid species was apparent in

both n-3 fatty acid deficient diets and it was generally compensated for by the corresponding 22:5n-6 species and, to a lesser extent, with the 22:4n-6 species in all of the major glycerophospholipids (PC, PE, PLE, and PS) examined in this study. However, the extent of reciprocal replacement of 22:6n-3 with 22:5n-6 varied across different PL classes. It was also apparent in this study that the calculated fatty acid percentages from the molecular species data were in general agreement with the individual PL fatty acid compositions (36). The changes observed in individual PL molecular species in n-3 fatty acid deficient groups are also in substantial agreement with those reported for monkey brain (27), guinea pig brain (28), and rat cortex (31).

Our results support the concept that the 22:6n-3 losses in PC, PE, and PS were to a large extent reciprocally compensated with 22:5n-6 (19). In PLE, however, the increase of 22:5n-6 was not sufficient to account fully for the loss of 22:6n-3, suggesting that the turnover of PLE may be differentially affected by n-3 fatty acid deficiency as opposed to diacyl-PLs. The increases that we observed in the 16:0,22:4n-6 and 18:0,22:4n-6 species of PLE during n-3 deficiency are similar to those shown by Lin et al. (27) in the rhesus monkey frontal cortex. Kurvinen et al. (28) also reported increases of 18:0,22:4n-6 species in both PLE and alkylacyl-PE but not in the diacyl form of PE during n-3 fatty acid deficiency in the guinea pig cerebrum. These findings together with our present data support the notion that polyunsaturated species in various forms of PE may have different metabolic fates. It is possible that 16:0,22:5n-6 and 18:0,22:5n-6 alkylacyl species may not be as efficiently converted to PLE as the corresponding 22:6n-3 or 22:4n-6 species. Alternatively, acyltransferase may not favor 22:5n-6 esterification to form alkylacyl or alkenylacyl PLE. The small decrease (from about 25% to 20–22%) in total *sn*-1 18:1 PLE species observed in n-3 deficient rats (Table 4) is consistent with an earlier report indicating that fish oil feeding increases the proportion of PLE species containing *sn*-1 18:1 in monkey brain (27).

In general, 20:4n-6 species in PC, PE, PLE, and PS were not significantly affected by n-3 fatty acid deficiency and these findings corroborate those reported for the guinea pig brain (28), rat brain microsomes (30), and rat pineal (26). The previous report by Garcia et al. (30) indicated that in rat brain microsomes the absolute concentration of the major arachidonoyl species in PS (18:0,20:4) decreased significantly. However, when the data from Garcia et al. (30) were recalculated as a percentage, the 18:0,20:4-PS species remained unchanged, in agreement with our current data.

Garcia et al. (30) and Hamilton et al. (31) have reported reductions in the PS content of rat brain cortex microsomal and mitochondrial subcellular fractions and in the olfactory bulb. A similar decrease in total PS has also been reported in the retina of herring when fed an n-3 deficient diet (42). It has also been shown that an intraamniotic injection of 22:6n-3 ethyl esters increases the PS content in rat pup brains (29). Furthermore, it has been reported that the cerebral cortex PLs obtained from infants fed breast milk and who had died of sudden infant death syndrome contained 39% more PS relative to that of infants who had received vegetable oil-based formula containing no 22:6n-3 (43). In agreement with these earlier reports, the present study demonstrates that the PS content in the rat hippocampus is considerably (28%) lower than that of the deficient groups (Fig. 2) despite the marked replacement of 22:6n-3 with 22:5n-6. The reduction of the hippocampal PS level induced by n-3 fatty acid deficiency has been previously observed in aged rats (44). The data from this and other studies (30, 31) indicate that the PS decrease is predominantly associated with the decrease of 18:0,22:6n-3-PS. It is also noteworthy that 18:0,22:5n-6-PC increased during n-3 deficiency in the hippocampus as has been reported in rat brain microsomes and the pineal gland (26, 30), suggesting that the 18:0,22:5n-6-PC may not be as good a substrate for hippocampal PS synthesis as is 18:0,22:6n-3-PC, thus contributing to the decrease in total PS. Alternatively, microsomal PS synthase activity may have been compromised by a change in membrane properties secondary to altered lipid composition after n-3 fatty acid deficiency.

It has been shown that the depletion of n-3 fatty acids in the diet leads to a reduction of PS, concomitantly increasing monoamine oxidase activity in hippocampus (44). Conversely, Kim et al. (45) have demonstrated that supplementation of a neuronal cell culture with 22:6n-3 increases the PS content, subsequently inhibiting neuronal apoptosis via the signaling pathways of cell survival. Furthermore, both 22:6n-3 and PS have been shown to influence hippocampal neurotransmission and synaptic efficacy (46, 47). Therefore, it is evident that both phospholipid and 22:6n-3 status altered by n-3 fatty acid deficiency can affect various components involved in hippocampal function, such as neurotransmitter levels, signaling pathways as well as synaptic efficacy.

In conclusion, the present data demonstrate that hippocampal 22:6n-3 species decreased by n-3 fatty acid deficient diets was predominantly replaced with 22:5n-3 species in a highly reciprocal manner in PE but not in PC, PS, and PLE. The differential effects of n-3 fatty acid deficiency on hippocampal PL molecular species, particularly in 22:6n-3, 22:5n-6, and 22:4n-6 species, observed in this study together with the PS decrease, may be important in neuronal signaling and hippocampus-mediated tasks such as spatial task-related memory and learning.

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