Effects of Maternal Dietary n-3 and n-6 Fatty Acids (Pre- and Post-Δ6 Desaturation) on Tissue Glycerophospholipid Fatty Acid Compositions in Dams and Suckling Mice

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Abstract. **The present study examined the effects of supplementation of either 18:3n-3 or a mixture of its post-Δ6-desaturation metabolites, 20:5n-3/22:6n-3, in combination with either 18:2n-6 or its immediate Δ6-desaturation product, 18:3n-6, in the maternal diet (n-3 to n-6 ratio at 0.25) on brain, liver, heart, and kidney glycerophospholipid fatty acid composition in dams (B6D2F$_1$ mice) and their 12-day-old suckling pups. As expected, n-3 and n-6 fatty acids competed for incorporation into tissue glycerophospholipids in both dams and their suckling pups. Feeding a 20:5n-3/22:6n-3 as compared with an 18:3n-3 rich diet increased the tissue levels of 20:5n-3 and 22:5n-3, whereas it decreased those of 20:3n-6 and 20:4n-6. Replacing 18:2n-6 with 18:3n-6 in the maternal diet increased significantly the levels of 18:3n-6, 20:3n-6, and 20:4n-6, whereas it reduced those of 20:5n-3. However, the effects of maternal dietary fats on tissue fatty acid compositions in pups were qualitatively similar to but quantitatively smaller than those in dams. The discrepancy might be due to differences in the composition of fatty acids taken up and synthesized by the dams and that transferred to the pups.**

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It is generally agreed that 18:3n-3 and 18:2n-6 are metabolized by the same enzyme systems in humans and animals (1). Thus, the metabolism and incorporation of n-3 and n-6 fatty acids in animal tissues is regulated, at least in part, by the ratio of n-3 and n-6 fatty acids in the diet (2, 3). We have previously analyzed the liver, heart, and kidney fatty acid composition in adult animals and shown that competition between n-3 and n-6 fatty acids tends to favor the latter (4, 5). On the other hand, the tissue n-3 to n-6 ratios in pups from dams fed a diet containing various ratios of long-chain (C_{20}+C_{22}) n-3 fatty acids to 18:2n-6 were consistently greater than the n-3 to n-6 ratio in the milk fat, suggesting that long-chain (C_{20}+C_{22}) n-3 fatty acids are more favorably taken up and incorporated into tissue lipids in suckling pups (6, 7).

There are numerous factors that may contribute to the differences between adults and neonates in responding to different dietary n-3 to n-6 ratios. Brenner (8) has demonstrated previously that the activities of Δ6 and Δ5 desaturases are lower in neonates than in adult animals. Thus, age differences affect the ability of animals to metabolize n-3 and n-6 fatty acids. Crawford and his colleagues (9, 10) have suggested that the selective increasing concentrations of long-chain C_{20} and C_{22} polyunsaturated fatty acids from the maternal system to the placental tissue and then to the fetal liver and brain is a process of compartmentalization on the fetal side of the placenta: the long-chain fatty acids are incorporated preferentially into glycerophospholipids, whereas the parent C_{18} fatty acids are more evenly distributed between neutral lipids and glycerophospholipids. The neutral lipids are either stored or oxidized

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for energy utilization, whereas the glycerophospholipids are incorporated into the stable cell membranes.

In line with these views, the greater incorporation of n-3 fatty acids in neonatal tissues reported in our previous studies (6, 7) might be explained as follows: First, the supplemented n-3 fatty acids (20:5n-3 and 22:6n-3) used in those studies had already undergone Δ6 desaturation, whereas the n-6 fatty acids (18:2n-6) had not. Since long-chain n-3 fatty acids suppress the desaturation of 18:2n-6 (11, 12), feeding these acids would reduce the accumulation of long-chain n-6 fatty acids in dams, and consequently, the transfer of long-chain n-6 fatty acids through the placenta to the fetus or through the milk to the neonates. Second, since acyltransferase, which is responsible for the acylation of fatty acids to glycerophospholipids, favors the long-chain (C_{20}+C_{22}) over the 18:2n-6 as substrate (13), a reduced supply of long-chain n-6 fatty acids (e.g., 20:4n-6) from dams would reduce the ability of n-6 fatty acids to compete with preformed long-chain n-3 fatty acids for incorporation into tissue glycerophospholipids in pups.

It is evident that both factors discussed above stressed the amounts of long-chain n-6 fatty acids synthesized by the dams that are transferred to the pups. It is, therefore, of interest to examine in more detail whether the type of n-3 fatty acids (18:3n-3 or a mixture of its post-Δ6-desaturation products, 20:5n-3/22:6n-3) supplemented to the dams affects differently the tissue composition of n-6 fatty acids in dams and their pups. We also examine whether the inhibitory effect of long-chain n-3 fatty acids on accumulation of long-chain n-6 fatty acids in dams can be alleviated if 18:3n-6, a post-Δ6-desaturation product of 18:2n-6, is supplemented in place of 18:2n-6 in the maternal diet.

Materials and Methods

Animals. Both male and female hybrid mice ($B6D2F_1$, 16–20 weeks old) were purchased from Charles River Breeding Laboratories (St. Constant, Quebec). Animals were maintained in a room with constant humidity and temperature (22°C) under a reversed light:dark (12:12 hr) cycle as described previously (6, 7, 14). Females were group housed (a male was only housed with females when mated) in standard plastic cages containing Beta-Chip hardwood bedding (Northeastern Products Corp., Warrensburg, NY). Animals were mated, and conception (Day 0) was confirmed by the presence of vaginal plugs.

At conception, the pregnant dams were assigned randomly to one of four different liquid-diet regimens. The detailed composition of liquid diet (F2187; Bio-Serv, Frenchtown, NJ) has been described previously (14). In general, the formulated diet contains (by calorie) 20% fat-free casein, 20% fat supplement and 60% carbohydrate (maltose-dextrin), and sufficient minerals and vitamins. The fat supplements were prepared from 10% olive oil and 10% was a mixture of various free fatty acid concentrates, (a) LA concentrate (derived from safflower oil, containing 79.5% 18:2n-6), (b) LN concentrate (derived from linseed oil, containing 62% 18:3n-3, and 20% 18:2n-6), (c) EPA concentrate (derived from fish oil concentrate containing 46% 20:5n-3 and 7.2% 22:6n-3), and (d) GLA concentrate (derived from evening primrose oil, containing 21.4% 18:2n-6 and 71% 18:3n-6). The final n-3 to n-6 fatty acid ratio was set at 0.25 in each case. This ratio was chosen based on our previous studies (6, 7) which showed that this was the optimum ratio for accumulation of 22:6n-3 in liver and brain. All fatty acid concentrates were supplied by Callanish Ltd (Breasclete, Isle of Lewis, Scotland). The detailed fatty acid compositions of the different fat supplements are shown in Table I. Extra vitamin E (α-tocopherol acetate, 4 IU/g) was also added to each fatty acid mixture to minimize the oxidation. During pregnancy, dams were fed daily at 0.65 kcal/g body wt/day. Fresh diet was prepared every other day, and the prepared diets and oils were stored in the refrigerator under nitrogen.

Birth occurred on Day 19 or 20 after conception, and litters were culled to six (three of each sex). During the next 12 days, dams were fed their respective diets at 0.975 kcal/g/day. As shown in our previous study (15), the levels fed results in body weight comparable to those of chow-fed animals. On Day 32 (12 days after birth), the dam and one male and one female pup from each litter were anesthetized with halothane, and were sacrificed by decapitation. The choice of 12-day-old mice was again based on our previous study (15), the results in which showed that behavioral development

Table I. Fatty Acid Composition (mg/100 mg total fatty acids) of Fat Mixtures Supplemented to the Maternal Diet

Fatty acid	Maternal dietary fat			
	LN/LA	LN/GLA	EPA/LA	EPA/GLA
16:0	6.5	5.1	6.3	6.4
16:1n-7	0.8	0.7	2.4	2.8
18:0	2.3	1.0	2.6	1.5
18:1n-9	33.1	25.4	31.1	29.2
18:2n-6	43.4	17.6	43.8	13.6
18:3n-6	—	34.9	—	32.4
18:3n-3	10.9	13.2	—	—
18:4n-3	—	—	0.4	0.4
20:5n-3	—	—	8.5	8.9
22:5n-3	—	—	0.7	0.8
22:6n-3	—	—	1.4	1.5
Others	2.9	2.1	2.8	2.5
n-3/n-6	0.25	0.25	0.25	0.25

was sensitive at this age. The brains, livers, kidneys, and hearts were removed, rinsed, blotted, frozen by dipping the tissues into liquid nitrogen, and stored in a freezer (-60°C) until lipid analysis (within 8 weeks).

Lipid Analysis. Tissue lipids were extracted by the method of Folch *et al.* (16). The quantity and distribution of major glycerophospholipid subfractions, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol, phosphatidylserine, sphingomyelin, and lysophospholipids, were determined by high-performance liquid chromatography (17). For fatty acid analysis, phospholipid subfractions were separated by thin-layer plates using a modified developing solvent system, chloroform-ethanol-water-triethylamine (4/5/1/4, by vol) (18). Two major glycerophospholipid subclasses (PC and PE) recovered from the plates were transmethylated following the method described by Morrison and Smith (19). The fatty acid methyl esters were then analyzed using a Hewlett-Packard (Avondale, PA) gas chromatograph (model 5890) equipped with a flame-ionization detector and either a glass column (2 mm i.d. × 180 cm) packed with 10% Silar 10C coated on Gas Chrom Q (Applied Science, State College, PA) or a fused silica capillary column (0.25 mm i.d. × 15 m) coated with SP-2230 (Supelco Canada Ltd, Oakville, Ontario) (3). Fatty acid identification was based on comparison of retention times with those of authentic standards (Nu-Chek Prep, Elysian, MN).

Statistical Analysis. Results are expressed as mean ± SD of 10 dams or 10 pooled samples (each pooled sample contains one male and one female pup from each litter). Statistical differences between the feeding groups were assessed by analysis of variance using the SYSTAT statistical system (20) general linear model procedure for factorial models. A 2 × 2 factorial arrangement was used to determine the effects of n-3 fatty acid sources (EPA versus LN) and n-6 fatty acid sources (GLA versus LA), and interactions. The differences between the dams and pups within each dietary group were assessed by two-tailed Student's *t* test.

Results

General. There were no significant differences in body weight, relative tissue weights (% body wt), tissue phospholipid concentration, and distribution of phospholipid subfractions among pups and among dams from different dietary groups (data not shown).

Liver. In liver PC and PE, the major n-3 fatty acids were 22:6n-3 in dams fed the LN-supplemented diet (LN/LA and LN/GLA groups), and 20:5n-3, 22:5n-3, and 22:6n-3 in those fed the EPA-supplemented diet (EPA/LA and EPA/GLA groups) (Table II). The major n-6 polyunsaturated fatty acids were 18:2n-6 and 20:4n-6 in dams fed the LA-supplemented diet (LN/LA and EPA/LA groups), and 18:3n-6, 20:3n-6, and 20:4n-6 in those fed the GLA-supplemented diet (LN/GLA and EPA/GLA groups). The overall incorporation of n-3 fatty acids (mainly 20:5n-3 and 22:6n-3) was significantly greater in the EPA-fed (EPA/LA and EPA/GLA) than in the LN-fed (LN/LA and LN/GLA) dams, and that of n-6 fatty acids (mainly 20:4n-6) was greater in the GLA-fed (LN/GLA and EPA/GLA) than in the LA-fed (LN/LA and EPA/LA) dams. The increase of n-3 fatty acid levels in EPA-fed dams was concomitant with a reduction of overall n-6 fatty acids (mainly 20:4n-6). Conversely, the increase of n-6 fatty acids in GLA-fed dams was accompanied by a decrease of n-3 fatty acids (mainly 20:5n-3 and 22:6n-3).

In pups, the distributions of n-3 and n-6 fatty acids in liver glycerophospholipids in response to maternal diets followed patterns similar to those seen in the dams, but to a lesser extent. Results in Table II show that pups from the dams fed a diet rich in 20:5n-3 (EPA/LA and EPA/GLA) as compared with those from dams fed a diet rich in 18:3n-3 (LN/LA and LN/GLA) had relatively higher levels of 20:5n-3, 22:5n-3, and 22:6n-3, whereas they had lower levels of 20:4n-6 and overall n-6 fatty acids. Pups from the GLA-fed dams (LN/GLA and EPA/GLA) as compared with those from the LA-fed dams (LN/LA and EPA/LA) had higher levels of 18:3n-6, 20:3n-6, and 20:4n-6. However, in liver PC, the increase of n-6 metabolites (mainly 20:4n-6) was negated by a reduction in the levels of 18:2n-6. The supplementation of either 18:2n-6 or 18:3n-6 in the maternal diet had no significant effect on the levels of n-3 fatty acids in pups.

Heart and Kidney. Tables III and IV show the effects of supplementation of different n-3 and n-6 fatty acid mixtures on the levels of polyunsaturated fatty acids in heart and kidney PC and PE. Since the compositions of n-3 and n-6 fatty acids in PC and PE in response to dietary modification in these two tissues were very similar to those in the liver, results are summarized as follows:

In LN/LA dams and their pups, the major n-3 fatty acids were 22:6n-3, followed by 22:5n-3 (in heart) and 20:5n-3 (in kidney), whereas the major n-6 fatty acids were 18:2n-6 and 20:4n-6. However, the levels of 20:3n-6, 20:4n-6 (except in kidney PE), 22:4n-6, overall n-6 fatty acids, and 22:5n-3 were consistently higher, whereas those of 18:2n-6, 22:6n-3, and overall n-3 fatty acids were lower in pups than in dams.

When 18:2n-6 was replaced with 18:3n-6 as the source of n-6 fatty acids in the maternal diet (LN/GLA versus LN/LA group), the levels of n-6 fatty acids (except 18:2n-6) in dam tissue glycerophospholipids were significantly increased, whereas those of 20:5n-3 (in kidney PC and PE) and 22:6n-3 (in heart PC and PE and in kidney PE) were reduced. The distributions

Table II. Effect of Maternal Diet Supplemented with Different Oil Mixtures on n-6 and n-3 Fatty Acid Composition (mg/100 mg total fatty acids) of Liver PC and PE in Dams and Their 12-Day-Old Suckling Pups (mean ± SD of 10 observations)

Fatty acid	Maternal dietary fat				P^a		
	LN/LA	LN/GLA	EPA/LA	EPA/GLA	ω3	ω6	ω3*ω6
PC							
Dam							
18:2n-6	12.1 ± 0.7	4.4 ± 0.2	12.1 ± 0.7	4.6 ± 3.2	—	0.001	—
18:3n-6	0.3 ± 0.0	3.4 ± 0.4	0.3 ± 0.0	3.1 ± 1.6	—	0.001	—
20:3n-6	3.2 ± 0.2	3.3 ± 0.4	2.4 ± 0.3	4.0 ± 0.7	0.001	0.001	—
20:4n-6	13.4 ± 1.0	25.4 ± 1.0	8.5 ± 1.3	19.4 ± 2.8	0.001	0.001	—
22:4n-6	0.1 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.001	0.001	—
22:5n-6	0.2 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.001	0.001	0.001
18:3n-3	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	—	0.001	—
20:5n-3	1.8 ± 0.5	0.4 ± 0.1	8.5 ± 1.6	2.5 ± 0.6	0.004	0.034	—
22:5n-3	0.4 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.5 ± 0.5	0.001	0.001	—
22:6n-3	14.9 ± 0.8	10.9 ± 0.7	17.3 ± 1.3	14.3 ± 0.8	0.001	0.001	—
Σ (n-6)	29.3 ± 1.0	37.4 ± 1.2	23.4 ± 1.4	31.2 ± 2.2	0.001	0.001	—
Σ (n-3)	17.3 ± 1.3	12.1 ± 0.6	26.9 ± 1.6	18.4 ± 1.0	0.001	0.001	—
n-3/n-6	0.59 ± 0.09	0.33 ± 0.05	1.15 ± 0.13	0.59 ± 0.07	0.001	0.001	—
Pup							
18:2n-6	13.7 ± 1.0	4.0 ± 0.4	13.8 ± 0.7	3.2 ± 0.4	0.033	0.001	—
18:3n-6	0.1 ± 0.0	0.8 ± 0.2	0.1 ± 0.1	0.8 ± 0.1	—	0.001	—
20:3n-6	2.0 ± 0.1	4.9 ± 0.4	1.9 ± 0.2	5.3 ± 0.6	—	0.001	—
20:4n-6	14.3 ± 0.8	20.2 ± 1.6	12.6 ± 0.8	17.4 ± 0.9	0.001	0.001	—
22:4n-6	0.2 ± 0.0	0.6 ± 0.2	0.1 ± 0.1	0.3 ± 0.1	0.023	0.001	0.001
22:5n-6	0.0 ± 0.0	0.5 ± 0.6	0.0 ± 0.0	0.0 ± 0.1	0.014	0.006	0.006
18:3n-3	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	—	—	—
20:5n-3	1.0 ± 0.1	0.2 ± 0.1	1.6 ± 0.7	0.3 ± 0.1	0.003	0.001	—
22:5n-3	0.9 ± 0.2	1.2 ± 0.2	1.2 ± 0.6	1.7 ± 0.2	0.001	0.001	—
22:6n-3	15.9 ± 0.7	16.7 ± 1.6	17.6 ± 1.2	19.8 ± 0.9	0.001	0.001	—
Σ (n-6)	30.3 ± 1.7	31.0 ± 1.2	28.5 ± 1.1	27.0 ± 1.2	0.016	—	0.001
Σ (n-3)	18.0 ± 1.0	18.2 ± 1.5	20.5 ± 1.5	21.9 ± 0.9	0.001	—	—
n-3/n-6	0.59 ± 0.13	0.59 ± 0.09	0.72 ± 0.11	0.81 ± 0.17	0.013	—	—
PE							
Dam							
18:2n-6	3.8 ± 0.3	1.8 ± 0.3	4.2 ± 0.5	1.2 ± 0.1	0.036	0.001	0.005
18:3n-6	0.0 ± 0.0	0.7 ± 0.1	0.1 ± 0.0	0.6 ± 0.1	—	0.001	—
20:3n-6	0.8 ± 0.0	1.6 ± 0.2	0.7 ± 0.0	1.6 ± 0.1	—	0.001	—
20:4n-6	20.1 ± 0.4	26.4 ± 0.7	12.3 ± 1.5	20.3 ± 0.8	0.001	0.001	0.039
22:4n-6	0.3 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.001	0.001	0.001
22:5n-6	0.3 ± 0.1	1.6 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.001	0.023	0.032
18:3n-3	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.1	—	0.001	—
20:5n-3	1.4 ± 0.2	0.3 ± 0.1	6.6 ± 0.8	2.5 ± 0.3	0.001	0.001	0.001
22:5n-3	0.7 ± 0.1	1.3 ± 0.3	1.5 ± 0.1	2.6 ± 0.3	0.001	0.009	0.001
22:6n-3	28.0 ± 1.0	23.3 ± 1.0	30.2 ± 1.3	28.1 ± 0.7	0.001	0.001	—
Σ (n-6)	25.3 ± 0.6	32.9 ± 1.0	17.5 ± 1.7	24.1 ± 0.9	0.001	0.001	—
Σ (n-3)	30.3 ± 1.1	24.9 ± 1.0	38.5 ± 1.6	33.2 ± 0.7	0.001	0.001	—
n-3/n-6	1.20 ± 0.07	0.76 ± 0.05	2.20 ± 0.26	1.38 ± 0.06	0.001	0.001	0.001
Pup							
18:2n-6	3.4 ± 0.3	1.0 ± 0.2	3.3 ± 0.4	0.8 ± 0.1	—	0.001	—
18:3n-6	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
20:3n-6	0.7 ± 0.2	2.3 ± 0.3	0.7 ± 0.2	2.2 ± 0.2	—	0.001	—
20:4n-6	23.1 ± 1.2	25.8 ± 1.9	19.4 ± 0.9	22.6 ± 1.4	0.001	0.001	—
22:4n-6	0.6 ± 0.1	1.1 ± 0.4	0.3 ± 0.1	0.7 ± 0.1	0.001	0.001	—
22:5n-6	0.1 ± 0.2	0.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.004	—	—
18:3n-3	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	—	—	—
20:5n-3	1.2 ± 0.2	0.1 ± 0.1	2.6 ± 0.3	0.5 ± 0.1	0.001	0.001	0.001
22:5n-3	1.6 ± 0.2	1.4 ± 0.5	2.1 ± 0.4	2.1 ± 0.2	0.001	—	—
22:6n-3	24.7 ± 1.3	25.0 ± 2.4	26.7 ± 0.7	26.5 ± 1.7	0.001	—	—
Σ (n-6)	27.9 ± 1.0	30.4 ± 1.7	23.7 ± 0.8	26.3 ± 1.3	0.001	0.001	—
Σ (n-3)	27.7 ± 1.5	26.6 ± 2.1	31.5 ± 0.7	29.2 ± 1.7	0.001	0.001	—
n-3/n-6	0.99 ± 0.07	0.88 ± 0.12	1.33 ± 0.04	1.11 ± 0.13	0.001	0.001	0.041

[a] —, Not significant ($P > 0.050$); ω3, ω6, and ω3*ω6 represent the n-3 effect (EPA versus LN), n-6 effect (GLA versus LA), and interaction between n-3 and n-6 sources, respectively.

Table III. Effect of Maternal Diet Supplemented with Different Oil Mixtures on n-6 and n-3 Fatty Acid Composition of Heart PC and PE in Dams and Their 12-Day-Old Suckling Pups (mean ± SD of 10 observations)

Fatty acid	Maternal dietary fat				P^a		
	LN/LA	LN/GLA	EPA/LA	EPA/GLA	ω3	ω6	ω3*ω6
	(mg/100 mg total fatty acids)						
PC							
Dam							
18:2n-6	12.2 ± 0.6	4.3 ± 0.4	8.5 ± 1.0	2.9 ± 0.2	0.001	0.001	0.001
18:3n-6	0.0 ± 0.0	1.9 ± 0.2	0.0 ± 0.0	1.6 ± 0.2	—	0.001	—
20:3n-6	0.7 ± 0.1	1.6 ± 0.2	0.4 ± 0.0	1.2 ± 0.1	0.001	0.001	—
20:4n-6	8.3 ± 0.5	16.4 ± 0.7	4.7 ± 0.5	9.4 ± 0.4	0.001	0.001	0.001
22:4n-6	0.4 ± 0.0	1.2 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	0.001	0.001	0.001
22:5n-6	0.5 ± 0.0	1.7 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.001	0.001	0.001
18:3n-3	0.3 ± 0.0	0.4 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.001	—	—
20:5n-3	0.2 ± 0.0	0.0 ± 0.0	0.9 ± 0.2	0.5 ± 0.3	0.001	0.001	—
22:5n-3	2.7 ± 0.2	2.8 ± 0.2	4.3 ± 0.2	4.8 ± 0.1	0.001	0.001	0.024
22:6n-3	19.5 ± 0.8	15.5 ± 1.5	27.2 ± 1.6	25.2 ± 0.9	0.001	0.001	—
Σ (n-6)	22.1 ± 0.6	27.1 ± 1.0	14.0 ± 0.9	15.8 ± 0.5	0.001	0.001	0.001
Σ (n-3)	22.7 ± 0.9	18.7 ± 1.6	32.5 ± 1.7	30.6 ± 0.8	0.001	0.001	0.001
n-3/n-6	1.03 ± 0.06	0.69 ± 0.07	2.32 ± 0.26	1.94 ± 0.11	0.001	0.001	0.001
Pup							
18:2n-6	5.3 ± 0.6	1.2 ± 0.1	4.2 ± 0.3	1.3 ± 0.7	0.001	0.001	0.001
18:3n-6	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	—	0.001	—
20:3n-6	1.3 ± 0.2	3.0 ± 0.4	1.2 ± 0.2	2.6 ± 0.3	0.010	0.001	—
20:4n-6	16.0 ± 1.1	19.7 ± 1.5	10.8 ± 0.6	14.7 ± 0.8	0.001	0.001	—
22:4n-6	1.5 ± 0.2	3.7 ± 0.4	0.6 ± 0.1	1.6 ± 0.1	0.001	0.001	0.001
22:5n-6	0.5 ± 0.2	1.6 ± 0.2	0.3 ± 0.0	0.5 ± 0.0	0.001	0.001	0.001
18:3n-3	0.2 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.2	—	—	—
20:5n-3	0.3 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.001	0.001	0.002
22:5n-3	6.3 ± 0.7	5.4 ± 0.4	8.3 ± 0.8	8.0 ± 0.4	0.001	0.003	—
22:6n-3	14.4 ± 2.3	11.3 ± 1.1	20.0 ± 1.2	18.7 ± 1.9	0.001	0.001	—
Σ (n-6)	24.6 ± 1.0	29.3 ± 1.7	17.1 ± 0.9	20.8 ± 1.5	0.001	0.001	—
Σ (n-3)	21.2 ± 2.8	16.9 ± 1.2	28.9 ± 1.2	27.0 ± 2.0	0.001	0.001	—
n-3/n-6	0.86 ± 0.12	0.58 ± 0.07	1.69 ± 0.15	1.30 ± 0.17	0.001	0.001	—
	(% total fatty acids)						
PE							
Dam							
18:2n-6	4.0 ± 0.3	1.6 ± 0.1	3.0 ± 0.4	1.2 ± 0.3	0.001	0.001	0.027
18:3n-6	0.0 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	—	0.001	—
20:3n-6	0.2 ± 0.0	0.7 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	—	0.001	—
20:4n-6	9.3 ± 0.0	13.6 ± 0.9	5.1 ± 0.3	8.0 ± 0.4	0.001	0.001	0.001
22:4n-6	0.6 ± 0.0	1.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.001	0.001	0.001
22:5n-6	0.8 ± 0.1	3.0 ± 0.2	0.3 ± 0.1	0.6 ± 0.3	0.001	0.001	0.001
18:3n-3	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.0	0.3 ± 0.0	—	—	—
20:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
22:5n-3	2.3 ± 0.1	2.4 ± 0.1	3.3 ± 0.1	3.4 ± 0.2	0.001	—	—
22:6n-3	37.3 ± 0.6	31.9 ± 1.4	41.8 ± 1.1	38.6 ± 1.8	0.001	0.001	0.001
Σ (n-6)	14.9 ± 0.3	20.7 ± 1.0	8.8 ± 0.7	11.2 ± 0.7	0.001	0.001	0.001
Σ (n-3)	40.0 ± 0.7	34.8 ± 1.3	45.4 ± 1.2	42.3 ± 1.8	0.001	0.001	0.026
n-3/n-6	2.68 ± 0.08	1.68 ± 0.13	5.16 ± 0.56	3.78 ± 0.37	0.001	0.001	—
Pup							
18:2n-6	1.8 ± 0.2	0.5 ± 0.1	1.6 ± 0.3	0.4 ± 0.2	0.009	0.001	—
18:3n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
20:3n-6	0.6 ± 0.1	1.6 ± 0.1	0.6 ± 0.1	1.4 ± 0.2	—	0.001	—
20:4n-6	17.2 ± 0.8	18.6 ± 1.0	12.1 ± 0.5	13.8 ± 0.8	0.001	0.001	—
22:4n-6	2.1 ± 0.1	4.8 ± 0.5	1.0 ± 0.1	2.1 ± 0.2	0.001	0.001	0.001
22:5n-6	0.9 ± 0.3	3.2 ± 0.3	0.4 ± 0.0	0.8 ± 0.2	0.001	0.001	0.001
18:3n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
20:5n-3	0.4 ± 0.2	0.1 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	0.001	0.001	0.001
22:5n-3	6.1 ± 0.3	5.2 ± 0.3	7.2 ± 0.5	6.3 ± 0.3	0.001	0.001	0.001
22:6n-3	27.0 ± 1.2	22.9 ± 1.9	32.4 ± 1.1	30.7 ± 1.8	0.001	0.001	0.014
Σ (n-6)	22.6 ± 1.0	28.7 ± 1.0	15.7 ± 0.7	18.5 ± 1.2	0.001	0.001	—
Σ (n-3)	33.5 ± 1.3	28.2 ± 1.8	40.4 ± 1.1	37.2 ± 1.7	0.001	0.001	—
n-3/n-6	1.48 ± 0.09	0.98 ± 0.08	2.57 ± 0.16	2.01 ± 0.20	0.001	0.001	—

[a] —, Not significant ($P > 0.050$); ω3, ω6, and ω3*ω6 represent the n-3 effect (EPA versus LN), n-6 effect (GLA versus LA), and interaction between n-3 and n-6 sources, respectively.

Table IV. Effect of Maternal Diet Supplemented with Different Oil Mixtures on n-6 and n-3 Fatty Acid Composition (mg/100 mg total fatty acids) of Kidney PC and PE in Dams and Their 12-Day-Old Suckling Pups (mean ± SD of 10 observations)

Fatty acid	Maternal dietary fat				P^a		
	LN/LA	LN/GLA	EPA/LA	EPA/GLA	ω3	ω6	ω3 * ω6
PC							
Dam							
18:2n-6	11.8 ± 0.9	3.0 ± 0.3	9.6 ± 0.6	2.5 ± 0.1	0.001	0.001	0.001
18:3n-6	0.1 ± 0.0	1.3 ± 0.2	0.1 ± 0.0	1.3 ± 0.0	—	0.001	—
20:3n-6	1.3 ± 0.1	2.4 ± 0.2	0.8 ± 0.0	2.6 ± 0.1	0.007	0.001	0.001
20:4n-6	12.1 ± 0.8	19.8 ± 1.7	7.6 ± 0.5	16.3 ± 0.7	0.001	0.001	—
22:4n-6	0.2 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.001	0.001	0.001
22:5n-6	0.1 ± 0.1	0.5 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.001	0.001	0.001
18:3n-3	0.6 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.001	—	—
20:5n-3	1.1 ± 0.2	0.2 ± 0.1	4.1 ± 0.4	2.3 ± 0.1	0.001	0.001	0.001
22:5n-3	1.6 ± 0.1	1.5 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	0.001	—	—
22:6n-3	14.7 ± 0.9	14.2 ± 1.6	16.8 ± 0.8	17.8 ± 0.5	0.001	—	—
Σ (n-6)	25.6 ± 1.6	27.5 ± 1.9	18.2 ± 0.7	23.0 ± 0.7	0.001	0.001	0.006
Σ (n-3)	18.0 ± 1.0	16.5 ± 1.6	23.3 ± 0.8	22.5 ± 0.4	0.001	0.008	—
n-3/n-6	0.70 ± 0.05	0.60 ± 0.03	1.28 ± 0.08	0.98 ± 0.03	0.001	0.001	0.001
Pup							
18:2n-6	8.2 ± 0.4	1.6 ± 0.2	8.1 ± 0.4	1.5 ± 0.1	—	0.001	—
18:3n-6	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.0	0.001	0.001	0.001
20:3n-6	1.6 ± 0.2	3.1 ± 0.3	1.6 ± 0.1	3.6 ± 0.3	0.001	0.001	0.005
20:4n-6	14.3 ± 0.5	23.8 ± 1.3	11.7 ± 0.5	21.7 ± 0.4	0.001	0.001	—
22:4n-6	0.5 ± 0.1	0.9 ± 0.1	0.3 ± 0.0	0.6 ± 0.1	0.001	0.001	0.001
22:5n-6	0.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.001	0.003	0.006
18:3n-3	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.001	—	—
20:5n-3	1.8 ± 0.1	0.4 ± 0.0	3.7 ± 0.3	1.1 ± 0.1	0.001	0.001	0.001
22:5n-3	1.4 ± 0.1	1.3 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	0.001	—	—
22:6n-3	13.0 ± 0.8	11.9 ± 0.9	13.9 ± 0.5	13.2 ± 0.5	0.001	0.001	—
Σ (n-6)	24.7 ± 0.6	29.8 ± 1.2	21.7 ± 0.5	27.6 ± 0.5	0.001	0.001	—
Σ (n-3)	16.5 ± 0.9	13.9 ± 1.8	19.6 ± 0.7	16.3 ± 0.5	0.001	0.001	—
n-3/n-6	0.67 ± 0.04	0.47 ± 0.02	0.90 ± 0.03	0.59 ± 0.02	0.001	0.001	0.001
PE							
Dam							
18:2n-6	4.4 ± 0.7	1.5 ± 0.2	4.0 ± 0.3	1.5 ± 0.7	—	0.001	—
18:3n-6	0.0 ± 0.0	0.7 ± 0.1	0.0 ± 0.0	0.6 ± 0.1	—	0.001	—
20:3n-6	0.6 ± 0.1	1.4 ± 0.1	0.6 ± 0.2	1.6 ± 0.2	—	0.001	—
20:4n-6	30.8 ± 1.2	35.5 ± 1.1	20.6 ± 0.9	31.1 ± 0.7	0.001	0.001	0.001
22:4n-6	0.4 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.001	0.001	0.001
22:5n-6	0.2 ± 0.0	0.8 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.001	0.001	0.001
18:3n-3	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.001	—	0.028
20:5n-3	2.1 ± 0.3	0.2 ± 0.2	9.7 ± 1.3	4.2 ± 0.4	0.001	0.001	0.001
22:5n-3	1.2 ± 0.1	1.0 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	0.001	0.001	—
22:6n-3	15.6 ± 0.6	12.9 ± 0.8	16.9 ± 0.9	16.7 ± 0.9	0.001	0.001	0.001
Σ (n-6)	36.4 ± 1.1	40.7 ± 1.3	25.5 ± 1.0	35.1 ± 1.0	0.001	0.001	0.001
Σ (n-3)	19.2 ± 0.7	14.4 ± 0.6	28.4 ± 1.4	22.5 ± 1.3	0.001	0.001	—
n-3/n-6	0.53 ± 0.02	0.35 ± 0.01	1.11 ± 0.07	0.64 ± 0.03	0.001	0.001	0.001
Pup							
18:2n-6	2.9 ± 0.4	0.7 ± 0.2	3.1 ± 0.5	0.6 ± 0.3	—	0.001	—
18:3n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
20:3n-6	0.9 ± 0.2	1.6 ± 0.1	1.2 ± 0.3	1.8 ± 0.3	0.008	0.001	—
20:4n-6	30.3 ± 1.0	35.5 ± 1.4	27.0 ± 0.9	35.8 ± 1.1	0.001	0.001	0.001
22:4n-6	1.5 ± 0.2	2.4 ± 0.1	0.9 ± 0.1	1.7 ± 0.2	0.001	0.001	—
22:5n-6	0.2 ± 0.2	0.5 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.001	0.001	0.001
18:3n-3	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.004	—	—
20:5n-3	3.1 ± 0.2	0.5 ± 0.1	7.1 ± 0.8	1.9 ± 0.5	0.001	0.001	0.001
22:5n-3	2.2 ± 0.1	1.7 ± 0.1	2.8 ± 0.1	2.2 ± 0.1	0.001	0.001	—
22:6n-3	13.9 ± 0.9	11.3 ± 0.6	15.0 ± 0.7	13.6 ± 0.4	0.001	0.001	0.008
Σ (n-6)	35.8 ± 0.9	40.7 ± 1.4	32.3 ± 0.9	40.0 ± 1.1	0.001	0.001	0.001
Σ (n-3)	19.4 ± 0.9	13.8 ± 0.7	25.0 ± 1.2	17.9 ± 0.6	0.001	0.001	0.009
n-3/n-6	0.54 ± 0.03	0.34 ± 0.02	0.77 ± 0.04	0.45 ± 0.02	0.001	0.001	0.001

[a] —, Not significant ($P > 0.050$); ω3, ω6, and ω3*ω6 represent the n-3 effect (EPA versus LN), n-6 effect (GLA versus LA), and interaction between n-3 and n-6 sources, respectively.

of n-3 and n-6 fatty acids in pup tissues in response to dietary modification were very similar to their dams. However, pups as compared with their dams had higher overall n-6 fatty acid but lower n-3 fatty acid levels.

Replacing 18:3n-3 with 20:5n-3 as the source of n-3 fatty acids (EPA/LA versus LN/LA group) significantly increased the levels of n-3 metabolites 20:5n-3, 22:5n-3, and 22:6n-3, whereas it suppressed the levels of 18:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6 in dams and their pups. Similarly, the overall levels of n-6 fatty acids were higher, whereas those of n-3 fatty acids were lower in pups than in their dams.

In EPA/GLA dams, 20:5n-3 was supplemented in the maternal diet as the major source of n-3 fatty acids, whereas 18:3n-6 was the major source of n-6 fatty acids. In comparison with LN/GLA dams, the levels of 20:5n-3, 22:5n-3, and 22:6n-3 were significantly increased, whereas those of 18:2n-6 and 20:4n-6 were suppressed. In comparison with dams that received the EPA/LA diet, the levels of 20:3n-6 and 20:4n-6 in dams that received the EPA/GLA diet were significantly higher, whereas those of 22:6n-3 were significantly lower. However, the levels of n-6 fatty acids were lower than those in the LN/GLA group. In pups, the changes of n-6 and n-3 fatty acid composition in heart (Table III) and kidney (Table IV) glycerophospholipids were very similar to those seen in their dams. There is an exception in that the levels of 22:6n-3 in the EPA-fed groups were not affected by either 18:2n-6 or 18:3n-6 supplementation.

Brain. In brain, the major n-3 fatty acid was 22:6n-3, whereas the major n-6 fatty acids were 20:4n-6 (in both PC and PE) and 22:4n-6 (in PE) (Table V). In brain PC, the feeding of an EPA-rich diet (EPA/LA and EPA/GLA) had no significant effect on the overall levels of n-3 fatty acids, nor on those of n-6 fatty acids. The feeding of a GLA-rich diet (LN/GLA and EPA/GLA), on the other hand, increased the levels of C_{20} and C_{22} n-6 fatty acids, more so in the pups than the dams. The overall levels of n-6 fatty acids were higher, whereas those of n-3 fatty acids were lower in pups than in their dams. In brain PE, the feeding of an EPA diet (EPA/LA and EPA/GLA) as compared with an LN diet (LN/LA and LN/GLA) had significantly increased the levels of 22:6n-3 in both dams and their pups, but it suppressed the levels of 20:4n-6, 22:4n-6, and 22:5n-6 in pups. Similar to brain PC, the overall levels of n-6 fatty acids were higher in pups than in their dams. However, the levels of n-3 fatty acids in pups were comparable to those in their dams.

Discussion

The present study compared in pups (12 day old) and their dams the responses of glycerophospholipid fatty acid compositions in different tissues with maternal dietary fats with constant n-3 to n-6 ratio (0.25), but varying in the types (parent acid or their post-Δ6-desaturation metabolites) of n-3 and n-6 fatty acids. The diet was fed to dams throughout gestation and during lactation.

Results show that the levels of n-3 metabolites in tissue glycerophospholipids were generally higher, whereas those of n-6 fatty acids were lower in EPA-fed dams than in LN-fed dams. On the other hand, GLA feeding as compared with LA feeding increased the levels of n-6 fatty acids, whereas it reduced those of n-3 fatty acids. These findings are consistent with the reports that 20:5n-3 is more readily converted to long-chain n-3 metabolites and incorporated into glycerophospholipids than is 18:3n-3 (21–24), whereas 18:3n-6 as compared with 18:2n-6 is more readily converted into long-chain n-6 metabolites (25–27). Thus, dietary supplementation of post-Δ6- desaturase n-3 or n-6 fatty acids in place of their parent essential fatty acids significantly enhanced the incorporation of n-3 or n-6 fatty acids into glycerophospholipids in dam tissues.

Although fatty acid composition in adult brain is known to resist changes (28, 29), results from the present study showed that EPA feeding increased the levels of n-3 metabolites (mainly in brain PE) (Table V, PE). GLA feeding increased the levels of n-6 metabolites in brain PC and PE in dams. GLA feeding also raised the levels of C_{20} and C_{22} n-6 metabolites in pups, but the extent of changes was either statistically not significant or lower than that seen in other tissues. These findings, nevertheless, are in agreement with the previous reports (6, 14, 30–32) in showing that maternal dietary composition can significantly influence the composition of the pup brain during the prenatal and suckling period, during which time major accumulation of 20:4n-6 and 22:6n-3 occurs (30).

In liver, it is generally agreed that both 18:3n-3 and 18:2n-6 are subjected to the same metabolic enzymes, e.g., desaturases (Δ6 and Δ5) and elongase, to form C_{20} and C_{22} n-3 and n-6 fatty acids, respectively (1). In comparison with the LN/LA group, replacing 18:3n-3 with 20:5n-3 (EPA/LA) elevated the n-3 to n-6 ratio in liver PC and PE by approximately 2-fold, whereas replacing 18:2n-6 with 18:3n-6 (LN/GLA) reduced the ratio by one half (Table II). Thus, replacing 18:3n-3 with 20:5n-3 in the diet, significantly suppressed the incorporation of total n-6 fatty acids, whereas replacing 18:2n-6 with 18:3n-6 reduced the incorporation of total n-3 fatty acids. In other words, 20:5n-3 in place of 18:3n-3 as the source of n-3 fatty acids reduced the suppressive effect of 18:3n-6 on the incorporation of n-3 fatty acids, and 18:3n-6 in place of 18:2n-6 as the source of n-6 fatty acids reduced the suppressive effect of 20:5n-3 on the incorporation of n-6 fatty acids into glycerophospholipids. These results demonstrated that

Table V. Effect of Maternal Diet Supplemented with Different Oil Mixtures on n-6 and n-3 Fatty Acid Composition (mg/100 mg total fatty acids) of Brain PC and PE in Dams and Their 12-Day-Old Suckling Pups (mean ± SD of 10 observations)

Fatty acid	Maternal dietary fat				P^a		
	LN/LA	LN/GLA	EPA/LA	EPA/GLA	ω3	ω6	ω3*ω6
PC							
Dam							
18:2n-6	0.6 ± 0.1	0.3 ± 0.2	0.7 ± 0.1	0.3 ± 0.1	—	0.001	—
18:3n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
20:3n-6	0.3 ± 0.1	0.6 ± 0.3	0.4 ± 0.1	0.8 ± 0.4	—	0.001	—
20:4n-6	5.0 ± 0.4	5.7 ± 0.5	4.5 ± 0.1	5.3 ± 0.3	0.003	0.001	—
22:4n-6	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	—	0.008	—
22:5n-6	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	—	—	—
18:3n-3	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	—	—	—
20:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	0.017	—
22:5n-3	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.001	0.007	—
22:6n-3	5.6 ± 0.5	5.8 ± 0.6	5.8 ± 0.2	5.7 ± 0.3	—	—	—
Σ (n-6)	6.5 ± 0.4	7.2 ± 0.7	6.0 ± 0.2	7.0 ± 0.4	—	0.001	—
Σ (n-3)	5.9 ± 0.5	5.9 ± 0.6	6.1 ± 0.2	6.0 ± 0.3	—	—	—
n-3/n-6	0.91 ± 0.04	0.82 ± 0.04	1.02 ± 0.04	0.86 ± 0.07	0.001	0.001	—
Pup							
18:2n-6	1.7 ± 0.1	0.4 ± 0.0	1.5 ± 0.1	0.4 ± 0.1	0.004	0.001	0.004
18:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	—	—	—
20:3n-6	0.6 ± 0.0	1.0 ± 0.1	0.7 ± 0.0	1.1 ± 0.2	0.004	0.001	—
20:4n-6	8.2 ± 0.3	9.2 ± 0.6	7.0 ± 0.3	8.7 ± 0.5	0.001	0.001	0.037
22:4n-6	0.6 ± 0.1	0.9 ± 0.1	0.6 ± 0.0	0.8 ± 0.1	—	0.001	—
22:5n-6	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.001	0.001	—
18:3n-3	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.8 ± 0.5	—	—	—
20:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
22:5n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.001	—	—
22:6n-3	4.1 ± 0.2	3.8 ± 0.4	3.8 ± 0.2	3.8 ± 0.3	—	—	—
Σ (n-6)	11.3 ± 0.3	11.8 ± 0.6	10.0 ± 0.3	11.3 ± 0.6	0.001	0.001	0.017
Σ (n-3)	4.8 ± 0.1	4.6 ± 0.3	4.6 ± 0.2	4.7 ± 0.4	—	—	—
n-3/n-6	0.42 ± 0.01	0.38 ± 0.02	0.46 ± 0.01	0.42 ± 0.02	0.001	0.001	—
PE							
Dam							
18:2n-6	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	—	0.001	—
18:3n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—	—	—
20:3n-6	0.4 ± 0.0	0.9 ± 0.1	0.5 ± 0.0	0.9 ± 0.0	—	0.001	—
20:4n-6	10.7 ± 0.6	11.8 ± 0.6	10.5 ± 0.4	11.5 ± 0.2	—	0.001	—
22:4n-6	4.9 ± 0.3	5.6 ± 0.3	4.8 ± 0.3	4.9 ± 0.1	0.002	0.001	0.010
22:5n-6	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	—	0.009	—
18:3n-3	0.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	—	—	—
20:5n-3	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.2	0.001	—	—
22:5n-3	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	0.001	0.001	0.001
22:6n-3	26.5 ± 1.0	24.8 ± 1.4	27.7 ± 1.0	26.5 ± 1.2	0.003	0.004	—
Σ (n-6)	16.5 ± 0.8	18.8 ± 1.2	16.3 ± 0.6	17.6 ± 0.5	—	0.001	—
Σ (n-3)	27.1 ± 0.9	25.6 ± 1.3	29.1 ± 1.1	27.5 ± 1.2	0.001	0.002	—
n-3/n-6	1.64 ± 0.04	1.36 ± 0.06	1.79 ± 0.03	1.56 ± 0.04	0.001	0.001	—
Pup							
18:2n-6	0.8 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	—	0.001	—
18:3n-6	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	—	—	—
20:3n-6	0.8 ± 0.0	1.0 ± 0.1	0.9 ± 0.0	1.1 ± 0.1	0.001	0.001	—
20:4n-6	18.5 ± 1.0	19.4 ± 1.2	17.9 ± 1.0	18.6 ± 0.7	0.034	0.015	—
22:4n-6	5.1 ± 0.2	6.3 ± 0.3	4.3 ± 0.2	5.8 ± 0.2	0.001	0.001	—
22:5n-6	0.8 ± 0.0	1.3 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.001	0.001	0.001
18:3n-3	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	—	—	—
20:5n-3	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	—	—	—
22:5n-3	0.6 ± 0.0	0.5 ± 0.0	0.9 ± 0.1	0.6 ± 0.0	0.001	0.001	0.001
22:6n-3	26.9 ± 1.1	25.1 ± 1.5	28.3 ± 1.6	25.6 ± 0.8	0.025	0.001	—
Σ (n-6)	26.3 ± 0.9	28.6 ± 1.0	24.6 ± 0.9	27.0 ± 0.5	0.001	0.001	—
Σ (n-3)	28.2 ± 1.1	26.2 ± 1.4	29.8 ± 1.6	26.8 ± 0.8	0.007	0.001	—
n-3/n-6	1.07 ± 0.02	0.92 ± 0.03	1.21 ± 0.05	0.99 ± 0.02	0.001	0.001	0.001

[a] —, Not significant ($P > 0.050$); ω3, ω6, ω3*ω6, represent the n-3 effect (EPA versus LN), n-6 effect (GLA versus LA), and interaction between n-3 and n-6 sources, respectively.

there existed an active competition for incorporation between n-3 and n-6 fatty acids. The incorporation into tissue was modulated by the supplemented fatty acid, whether a parent acid or a metabolite. It should be noted that differences in species, maturity, and duration of feeding could also determine the extent to which the n-6 fatty acid metabolism is influenced by the feeding of C_{20} and C_{22} n-3 fatty acids, and vice versa.

Interestingly, the n-3 to n-6 ratios in liver PC and PE from the EPA/GLA-fed dams (0.59 and 1.38, respectively) were similar to those from the LN/LA-fed dams (0.59 and 1.20, respectively). This result suggests that dietary supplementation with post-Δ6-desaturation metabolites produces an effect comparable to that shown by the parent acids even if the activity of Δ6-desaturase is suppressed by long-chain n-3 fatty acids (11, 12). Since the n-3 to n-6 ratio in the maternal diet in the EPA/GLA group was maintained at 0.25, the amount of n-6 fatty acids (18:2n-6 and 18:3n-6) in the diet was approximately 4-fold that of n-3 fatty acids (20:5n-3 and 22:6n-3) (Table I). The similarity of the n-3 to n-6 ratios in liver phospholipid observed in the EPA/GLA and in LN/LA groups suggests that the effect of 1 unit of dietary long-chain n-3 fatty acids (20:5n-3 and 22:6n-3) on liver n-3 fatty acid levels was equivalent to the effect of 4-fold of dietary n-6 fatty acids (18:2n-6 and 18:3n-6) on n-6 fatty acid levels. The greater competitive effect of EPA over GLA may be attributed to the following factors: (a) the fact that both 20:5n-3 and 22:6n-3 bypass two rate-limiting Δ6- and Δ5-desaturation steps, while 18:3n-6 bypasses only the Δ6-desaturation step; (b) the activity of an acyltransferase that favors C_{20} over C_{18} fatty acids as substrate (13); (c) the rate of oxidation, which decreases as fatty acid chain-length and degree of unsaturation increase (33). All these factors facilitate the incorporation of C_{20} n-3 fatty acids more than that of C_{18} n-6 fatty acids (e.g., 18:2n-6) into tissue glycerophospholipids.

As shown in the other report (34), the present study shows that the n-3 to n-6 ratios varied significantly among different tissues. In PE, heart had the highest and kidney had the lowest n-3 to n-6 ratio. In PC, heart had the highest n-3 to n-6 ratio. When EPA replaced LN, or GLA replaced LA in the maternal diet, the n-3 to n-6 ratio responded more substantially in the heart (particularly PE) than in other tissues. This may be attributed to a lack of desaturase activities (Δ5 and Δ6) in the heart, which obtains fatty acids from the blood and oxidizes them for the energy (8). Since the oxidation rate of long-chain fatty acids is significantly lower than that of short-chain fatty acids, this difference may contribute, at least in part, to the accumulation of long-chain polyunsaturated fatty acids in heart. An alternative explanation is that the n-3 fatty acids accumulated in the heart as compared with other tissues may be important for the heart function (35).

In pups as compared with dams, the distributions of fatty acid compositions in tissue glycerophospholipids in response to maternal diet were similar in pattern, but significantly less in magnitude. Sinclair (31) had shown that the liver long-chain polyunsaturated fatty acids in pups during the suckling period were derived from the dam's milk, and not synthesized from either 18:2n-6 or 18:3n-3 within the pups. Thus, the low response in pups might be attributed to the relative low proportions of n-3 fatty acids in fats transferred from dams to pups in comparison with that absorbed by the dams from the maternal diets. We have shown previously that the n-3 to n-6 ratio in milk fat was in direct relationship to, but lower in value than, that in the maternal dietary fat (6, 7). On the other hand, Scott and Bazan (36) have indicated that the liver in neonatal mice is involved in the conversion of 18:3n-3 to 22:6n-3, and its subsequent supply to the brain and retina.

Recently, Innis (37) has reviewed the essentiality of long-chain n-3 and n-6 fatty acids in the growth and development of neonates. Carlson *et al.* (38) have shown correlation between the growth and the level of 20:4n-6 in plasma phosphatidylcholine at birth. Koletzko and Braun (39) have also demonstrated a positive correlation between growth and levels of 20:4n-6 in plasma phospholipids and triacylglycerols in very low birth weight human infants. Leaf *et al.* (40) have shown that the level of 20:4n-6-containing PC in plasma is a good index of infant birth weight. All these observations suggest an essential role of long-chain n-6 fatty acids in neonatal growth. Thus, the metabolic regulation in pups favoring the n-6 fatty acids as shown in the present study may be related to the specific needs for growth. As both n-6 and n-3 fatty acids are important constituents of membrane phospholipids in all tissues examined, there must be a balance between the two fatty acid series and one must not be overemphasized at the expense of the other. This is currently a concern related to the question of whether long-chain n-3 fatty acids should be provided in preterm infant formulas. Recent evidence indicates that supplementation of preterm infant formula with n-3 fatty acids, particularly long-chain n-3 fatty acids, reduced the levels of 20:4n-6 in plasma and red blood cell phospholipids (21, 38, 41). This might slow infant growth. Arbuckle *et al.* (42) have advised caution in the use of fish oils low in n-6 long-chain fatty acids as a source of n-3 long-chain fatty acids for infant formula. Taking into consideration the metabolic differences that exist between species, the present data suggest that partial provision of the n-6 fatty acids also as long-chain metabolites may contribute to attaining an appropriate balance.

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