Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain

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Abstract **Fifteen weeks of dietary n-3 PUFA deprivation increases coefficients of conversion of circulating α-linolenic acid (α-LNA; 18:3n-3) to docosahexaenoic acid (DHA; 22:6n-3) in rat liver but not brain. To determine whether these increases reflect organ differences in enzymatic activities, we examined brain and liver expression of converting enzymes and of two of their transcription factors, peroxisome proliferator-activated receptor α (PPARα) and sterol-regulatory element binding protein-1 (SREBP-1), in rats fed an n-3 PUFA "adequate" (4.6% α-LNA of total fatty acid, no DHA) or "deficient" (0.2% α-LNA, no DHA) diet for 15 weeks after weaning. In rats fed the deficient compared with the adequate diet, mRNA and activity levels of Δ5 and Δ6 desaturases and elongases 2 and 5 were upregulated in liver but not brain, but liver PPARα and SREBP-1 mRNA levels were unchanged. In rats fed the adequate diet, enzyme activities generally were higher in liver than brain. Thus, differences in conversion enzyme expression explain why the liver has a greater capacity to synthesize DHA from circulating α-LNA than does the brain in animals on an adequate n-3 PUFA diet and why liver synthesis capacity is increased by dietary deprivation.** These data suggest that liver n-3 PUFA metabolism determines DHA availability to the brain when DHA is absent from the diet.—Igarashi, M., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. **Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain.** ***J. Lipid Res.*** **2007.** 48: **2463–2470.**

Supplementary key words β-oxidation • diet • docosahexaenoic acid • α-linolenic acid • polyunsaturated fatty acid

Adequate concentrations of docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (AA; 20:4n-6) in brain are required to maintain normal brain function and structure. These long-chain PUFAs regulate multiple processes, including membrane fluidity, cell signaling, and gene transcription, and they and their metabolites can influence neuropathological events (1–4). The PUFAs must be obtained directly through the diet or be converted from their respective shorter chain dietary precursors, linoleic acid (LA; 18:2n-6) or α-linolenic acid (α-LNA; 18:3n-3) (5–7), as they and their precursors cannot be synthesized de novo in vertebrate tissue.

We have quantified rates of conversion of circulating α-LNA to DHA in brain and liver of unanesthetized rats that had been fed, for 15 weeks starting at 21 days of age (after weaning), an n-3 PUFA "adequate" diet (4.6% α-LNA of total fatty acid, no DHA) or an n-3 PUFA "deficient" diet (0.2% α-LNA of total fatty acid, no DHA) (8–11). Rats on the adequate diet had α-LNA to DHA conversion coefficients 39-fold higher in the liver than brain, whereas placing rats on the deficient diet increased their liver conversion coefficients by an additional 7-fold without changing their brain coefficients.

We thought it of interest to determine whether differences in conversion capacities of liver and brain under the different dietary conditions were related to differences in the organ expression of enzymes that regulate conversion. **Figure 1** illustrates that conversion is catalyzed by a number of desaturases and elongases and by acyl-CoA oxidase (6, 7, 12, 13). These enzymes are expressed in many tissues but particularly in liver and brain (12, 14–16). High concentrations of DHA or α-LNA suppress the expression of liver desaturases and elongases and reduce DHA conversion from α-LNA in rat astrocytes (6, 12–15, 17).

Transcription of many of the enzymes shown in Fig. 1 may be regulated by sterol-regulatory element binding protein-1 (SREBP-1) or peroxisome proliferator-activated receptor α (PPARα) (12, 13, 18, 19). SREBP-1a and -1c are derived from

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Abbreviations: AA, arachidonic acid (20:4n-6); CPT-1, carnitine palmitoyltransferase-1; DHA, docosahexaenoic acid (22:6n-3); DPA, docosapentaenoic acid (22:5n-6); DTA, docosatetraenoic acid (22:4n-6); EPA, eicosapentaenoic acid (20:5n-3); FAME, fatty acid methyl ester; KPB, potassium phosphate buffer; LA, linoleic acid (18:2n-6); α-LNA, α-linolenic acid (18:3n-3); PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; SREBP, sterol-regulatory element binding protein.

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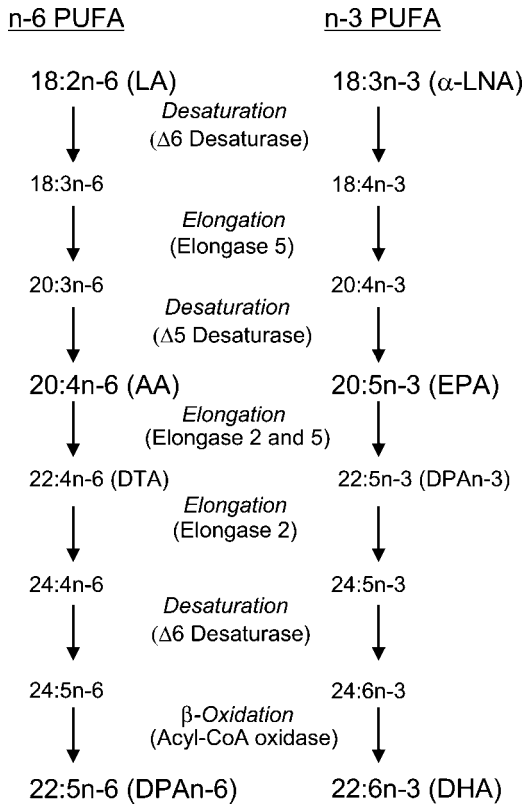

Fig. 1. Pathways of n-3 and n-6 PUFA metabolism. AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; α-LNA, α-linolenic acid.

a single gene through the involvement of different transcription start sites (20, 21). SREBP-1 is implicated in lipogenesis, and SREBP-1c is predominantly expressed in rat liver (20, 21). Liver SREBP-1 expression is reduced in rats and mice on a high dietary intake of fish oil containing DHA and eicosapentaenoic acid (EPA; 20:5n-3) (22–24), whereas n-3 PUFAs and their metabolites are ligands of PPARα (25, 26).

In this study, we tested the hypothesis that the reported effects of the 15 week n-3 PUFA deficient compared with adequate diet (see above) on measured conversion coefficients of circulating α-LNA to DHA in brain and liver (see above) would correlate with differences in expression of the relevant conversion enzymes and of their transcription factors (10, 11). Therefore, we measured activities and/or mRNA levels of these enzymes, and of SREBP-1 and PPARα, in brain and liver of rats fed an adequate or deficient diet for 15 weeks, starting at 21 days of age. We also examined mRNA levels of carnitine palmitoyltransferase-1 (CPT-1), which is a rate-limiting enzyme for mitochondrial β-oxidation (27, 28).

MATERIALS AND METHODS

Materials

[1-^{14}C]LA (51 mCi/mmol) was purchased from Perkin-Elmer Life Sciences, NEN Life Science Products (Boston, MA). [1-^{14}C]8,11,14-eicosatrienoic acid (52 mCi/mmol) and [2-^{14}C] malonyl-CoA (53 mCi/mmol) were obtained from Morvek (Brea, CA). Fatty acids and malonyl-CoA were purchased from Sigma-Aldrich (St. Louis, MO). Standards for general fatty acid methyl esters (FAMEs) for GC and HPLC were from NuChek Prep (Elysian, MN). Unique n-3 PUFAs (18:3n-6; 18:4n-3; 22:4n-6; and 22:5n-3) were purchased from Larodan Fine Chemicals (Malmö, Sweden). Liquid scintillation cocktail (Ready Safe™) was purchased from Beckman Coulter (Fullerton, CA). Solvents were HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ) or EMD Chemicals (Gibbstown, NJ). Other chemicals and reagents, unless noted otherwise, were purchased from Sigma-Aldrich or Fisher Scientific (Pittsburgh, PA).

Animals

The protocol was approved by the Animal Care and Use Committee of the National Institute of Child Health and Human Development and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 80-23). Male Fischer-344 (CDF) rat pups (18 days old) and their surrogate mothers were purchased from Charles River Laboratories (Portage, MI) and were housed in an animal facility having regulated temperature and humidity and a 12 h light/12 h dark cycle. The pups were allowed to nurse until 21 days of age. Lactating dams had free access to water and rodent chow formulation NIH-31 18-4, whose composition has been reported (29, 30).

Diets

After weaning, the pups were divided randomly into groups fed an n-3 PUFA adequate or deficient diet, with free access to food and water. Their food was replaced every 2 or 3 days, and their body weight was recorded every 2 or 3 days.

Table 1 presents the fatty acid composition of the n-3 PUFA adequate and deficient diets (10, 11, 31–33). Each was prepared by Dyets, Inc. (Bethlehem, PA), and was based on the AIN-93G formulation. Each diet contained 10% fat, and the adequate but not the deficient diet contained flaxseed oil. The adequate diet contained 7.8 μmol/g α-LNA (4.6% of total fatty acid), considered a minimum n-3 PUFA level for maintaining brain integrity in rodents (8, 9). The deficient diet contained 0.25 μmol/g α-LNA (0.2% of total fatty acid). There was no other n-3 PUFA in either diet. Both also contained 40 μmol/g LA (23–24% of total fatty acid), 110 μmol/g saturated fatty acid (65–69% of total), and 10 μmol/g monounsaturated fatty acid (5–6% of total). The rats were fed a diet for 15 weeks, starting when they were 21 days old.

We have reported the lipid composition of liver and brain in rats fed the n-3 PUFA adequate and deficient diets (10, 11, 32). The deficient diet reduces the liver DHA concentration by 97% and the brain DHA concentration by 30%.

Enzymes

After 15 weeks on a given diet, a rat was anesthetized by CO_2 inhalation and decapitated. Its liver and brain were removed rapidly, frozen in 2-methylbutane with dry ice at −50°C, and stored at −80°C until analyzed. We then determined mRNA and/or activity levels of elongases 2, 5, and 6; Δ5, Δ6 (EC 1.14.19.3) and Δ9 (EC 1.14.19.1) desaturases; acyl-CoA oxidase (EC 1.3.3.6); CPT-1 (EC 2.3.1.21); and PPARα and SREBP-1.

Total RNA isolation and real-time RT-PCR

Total RNA was isolated from liver and brain using commercial kits (RNeasy Lipid Tissue Kit; Qiagen, Valencia, CA). cDNA was prepared from total RNA using a high-capacity cDNA Archive Kit

TABLE 1. Fatty acid compositions of n-3 PUFA adequate and deficient diets

Fatty Acid	n-3 PUFA Adequate Diet			n-3 PUFA Deficient Diet		
	μmol/g food	*% of total fatty acid*	*% of energy*	*μmol/g food*	*% of total fatty acid*	*% of energy*
12:0	57.5 ± 6.2	34.0	2.6	60.5 ± 2.1	37.1	2.8
14:0	24.5 ± 2.2	14.5	1.3	25.6 ± 1.2	15.7	1.3
14:1n-5	0.02 ± 0.01	0.01	0.001	0.02 ± 0.01	0.01	0.001
16:0	16.0 ± 1.3	9.4	0.93	15.6 ± 1.0	9.6	0.9
16:1n-7	0.05 ± 0.01	0.03	0.003	0.05 ± 0.01	0.03	0.003
18:0	12.3 ± 0.8	7.3	0.8	12.0 ± 0.7	7.3	0.8
18:1n-9	10.6 ± 1.2	6.3	0.7	8.9 ± 0.8	5.5	0.6
18:2n-6	40.4 ± 1.4	23.9	2.6	40.2 ± 3.7	24.6	2.6
18:3n-3	7.8 ± 0.7	4.6	0.5	0.25 ± 0.01	0.2	0.016
20:4n-6	0.02 ± 0.01	0.01	0.001	0.02 ± 0.02	0.01	0.001
20:5n-3	ND	—	—	ND	—	—
22:6n-3	ND	—	—	ND	—	—
Total	169.2 ± 16.3	100	9.5	163.2 ± 9.4	100	9.0
Saturated	110.3 ± 10.4	65.2	5.6	113.8 ± 4.9	69.7	5.8
Monounsaturated	10.7 ± 1.2	6.3	0.7	9.0 ± 0.8	5.5	0.58
n-3 PUFA	7.8 ± 0.7	4.6	0.5	0.25 ± 0.01	0.2	0.016
n-6 PUFA	40.4 ± 4.4	23.9	2.6	40.2 ± 3.7	24.6	2.6

Values are means ± SD (n = 3).

(Applied Biosystems, Foster City, CA). mRNA levels of Δ5 desaturase (NM_053445), Δ6 desaturase (NM_031344), Δ9 desaturase (NM_139192), elongase 2 (AB071986), elongase 5 (NM_134382), elongase 6 (NM_134383), acyl-CoA oxidase (NM_017340), PPARα (NM_013196), SREBP-1 (XM_213329), CPT-1a (NM_031559), and CPT-1b (NM_013200) were measured with real-time quantitative RT-PCR using an ABI PRISM 7000 sequence detection system (Applied Biosystems). Specific primers and probes [TaqMan(r) MGB probe], and TaqMan(r) Universal PCR master Mix were purchased from Applied Biosystems and consisted of a 20× mix of unlabeled PCR primers and TaqMan minor groove binder probe (FAM™ dye-labeled). Data were analyzed with comparative cycle threshold (34). Data were expressed as the level of the target gene in animals fed the deficient diet normalized to the endogenous control (β-globulin) and relative to the level in animals fed the adequate diet.

Enzyme activities

Tissues were homogenized in 7 volumes of homogenizing buffer (10 mM Tris-HCl, pH 7.4, containing 0.25 M sucrose, 1 mM EDTA, 5 mM $MgCl_2$, and 1 mM DTT) and centrifuged at 500 *g* for 10 min. A small aliquot of supernatant was kept at 80°C to measure acyl-CoA oxidase activity. The remaining supernatant was centrifuged at 10,000 *g* for 30 min, and the resulting supernatant then was centrifuged at 105,000 *g* for 60 min. The pellet was suspended in homogenizing buffer and kept at −80°C until analyzed for desaturase and elongase activities. Protein concentrations were determined using the Bradford reagent (Sigma-Aldrich).

Δ5 and Δ6 desaturase activities were analyzed by the method of Kawashima, Musoh, and Kozuka (35) with slight modifications. [1-^{14}C]LA was used as the substrate for analysis of Δ6 desaturase activity, and γ-linolenic acid (18:3n-6) was detected as a product. To measure Δ5 desaturase activity, [1-^{14}C]8,11,14-eicosatrienoic acid (20:3n-6) was used, and AA was detected as a product. Each substrate was dissolved in 0.15% NaCl solution containing 10% BSA (described below). We did not determine the activities of Δ9 desaturase, elongase 6, or CPT-1.

To measure activities of the Δ5 and Δ6 desaturases, tissue microsomes (~1 mg of protein) were incubated with 100 μM fatty acid (~2.0 μCi/μmol), 2 mM NADH, 200 μM CoA, 5 mM ATP, and 5 mM $MgCl_2$ in 1 ml of 100 mM potassium phosphate buffer (KPB), pH 7.4. After preincubation for 1 min, the reaction was started by adding microsomes and continued for 5 min. The reaction was stopped by adding 2 ml of 10% KOH in 90% methanol, then saponifying for 1 h at 100°C. The solution was acidified with 1 ml of 12 N HCl, 1 ml of water was added, and then fatty acids were extracted two times with *n*-hexane. The hexane phase was dried with N_2 gas and methylated with 1% H_2SO_4 for 3 h at 70°C (32, 36). FAMEs were separated using reverse-phase HPLC as described below, peaks of substrate and product were corrected, and their radioactivities were counted using a liquid scintillation analyzer (2200CA, TRI-CARB®; Packard Instruments, Meriden, CT) with liquid scintillation cocktail (Ready Safe™ plus 1% glacial acetic acid) (37, 38). Activity was expressed as the synthesis rate of 18:3n-6 from LA for Δ6 desaturase and of AA from 20:3n-6 for Δ5 desaturase.

The elongation reactions were carried out as described by Moon et al. (39) with modifications. Microsomes (~0.2 mg of protein) were incubated in 0.2 ml of 50 mM KPB, pH 6.5, containing 5 μM rotenone, 20 μM fatty acid (bound with BSA; described below), 100 μM CoA, 1 mM ATP, 1 mM $MgCl_2$, 1 mM NADPH, and 150 μM [2-^{14}C]malonyl-CoA (~0.7 μCi/μmol). After preincubation for 1 min, the reaction was started by adding the microsomal fraction and then continued for 5 min. The reaction was stopped by adding 10% KOH in 90% methanol, and the solution was saponified. Fatty acids were extracted with *n*-hexane as described above, and radioactivity in the pooled hexane phase was counted. Enzyme activity was expressed as the rate of incorporation of malonyl-CoA into fatty acid. The fatty acids 18:3n-6, 20:4n-6, 22:4n-6, 18:4n-3, 20:5n-3, and docosapentaenoic acid (DPA; 22:5n-3) were used as substrates.

A 10 mM stock solution of each fatty acid was prepared according to the method of Hannah et al. (40) for enzyme activity analysis. Briefly, sodium salts of the fatty acid, produced by adding NaOH, were dissolved in 0.15 M NaCl containing 10% (w/v) BSA (fatty acid-free), and the solution was sonicated for 20 min at room temperature. Aliquots of the solution were kept at −80°C in a tube under nitrogen gas that was protected from light.

Acyl-CoA oxidase activity was measured using the method of Ide et al. (28, 41). The 500 *g* supernatant fraction was used for analysis, and palmitoyl-CoA was used as a substrate. The enzyme solution was incubated with 10 mM phenol, 0.82 mM 4-aminoantipyrine, 10 mM flavin adenine dinucleotide, 4 units of horseradish peroxidase, 0.05 mM palmitoyl-CoA, and 0.2 mg of BSA in 1 ml of 50 mM KPB, pH 7.4. After preincubation for 1 min at 30°C, the

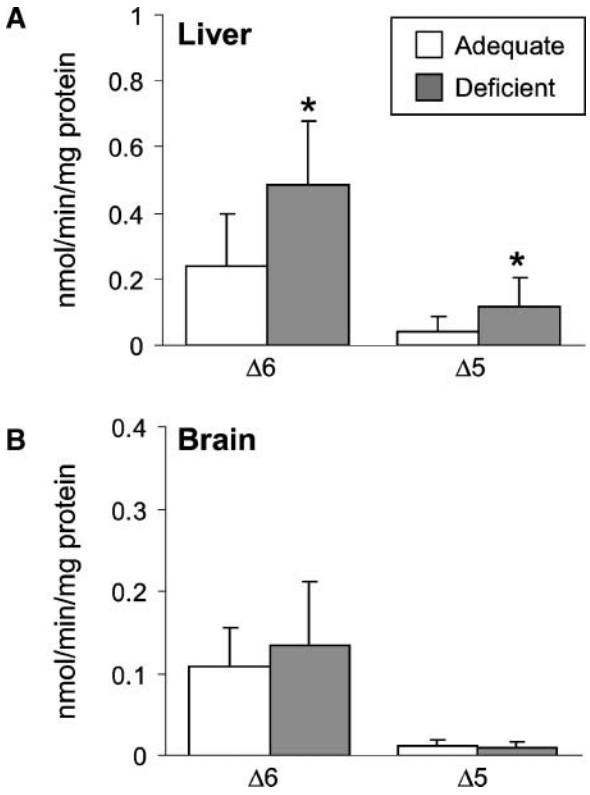

Fig. 2. Activities of Δ5 and Δ6 desaturases in liver (A) and brain (B) of rats fed an n-3 PUFA adequate or deficient diet. Values are means ± SD (n = 10 for each group). * $P < 0.05$ compared with the mean in the adequate group.

reaction was started by adding palmitoyl-CoA, and absorbance at 500 nm was monitored. An absorbance coefficient of quinoneimine dye to 6,390 M^{-1} cm^{-1} was used for calculations.

HPLC analysis

HPLC was used to determine desaturase activities. The FAMEs were dissolved in acetonitrile, and the solution was fractionated by reverse-phase column HPLC using a pump (System GOLD 126; Beckman Coulter) outfitted with an untraviolet light detector (UV/VIS-151; Gilson, Middleton, WI). The reverse-phase column, Luna 5 μC18 (2) (5 μM particle size, 4.6 × 250 mm), was purchased from Phenomenex (Torrance, CA). Chromatography was performed using a linear gradient system of water and acetonitrile at a flow rate of 1.0 ml/min with the detector set at 205 nm. The acetonitrile was held at 85% for 30 min, increased to 100% over 10 min, and held again at 100% for 20 min.

Statistical analysis

Data are expressed as means ± SD. An unpaired Student's *t*-test was used to compare means in two groups. Tukey's test was used for multiple comparisons. Statistical significance was taken at $P < 0.05$.

RESULTS

Activity and mRNA expression of desaturases in liver and brain

mRNA levels and activities of desaturases were determined in liver and brain from rats fed an n-3 PUFA adequate or deficient diet. As shown in **Fig. 2**, Δ6 and Δ5 desaturase activities were higher in the liver than in the brain of rats on the adequate diet. The activities of these desaturases were increased by 2-fold in the liver of rats fed the deficient compared with the adequate diet, but they were not changed significantly in the brain. In the liver of rats fed the deficient diet, furthermore, the mRNA levels of the Δ6 and Δ5 desaturases were increased by 2-fold and 1.3-fold, respectively (**Table 2**). No effect of dietary deprivation was noted in the brain.

Δ9 desaturase can catalyze the synthesis of monounsaturated fatty acids, and transcription of its gene is controlled by PPARα and SREBP-1 (42). However, its liver mRNA level was unaffected by the deficient diet (Table 2); therefore, its activity was not measured.

Activity and mRNA expression of elongases in liver and brain

mRNA and activity levels of elongases were analyzed in liver and brain of rats fed the n-3 PUFA adequate or deficient diet (**Fig. 3**). Elongase 2 can use C20–C22 PUFAs as substrates, whereas elongase 5 can act on a broad array

TABLE 2. mRNA expression of different desaturases, elongases, and oxidation enzymes, as well as of transcription factors, in liver and brain of rats fed an n-3 PUFA adequate or deficient diet

	Liver		Brain	
Constituent	Adequate	Deficient	Adequate	Deficient
Δ5 desaturase	1.00 ± 0.32	1.28 ± 0.20[a]	1.00 ± 0.39	0.95 ± 0.29
Δ6 desaturase	1.00 ± 0.83	2.07 ± 0.99[b]	1.00 ± 0.24	0.94 ± 0.21
Δ9 desaturase	1.00 ± 0.32	0.95 ± 0.25		
Elongase 2	1.00 ± 0.54	1.45 ± 0.67[a]	1.00 ± 0.25	0.96 ± 0.19
Elongase 5	1.00 ± 0.20	1.36 ± 0.52[a]	1.00 ± 0.17	1.00 ± 0.13
Elongase 6	1.00 ± 0.63	0.91 ± 0.56		
Acyl-CoA oxidase	1.00 ± 0.29	1.01 ± 0.24	1.00 ± 0.24	0.86 ± 0.23
Carnitine palmitoyltransferase-1a	1.00 ± 0.14	0.98 ± 0.26	1.00 ± 0.23	0.78 ± 0.12[b]
Carnitine palmitoyltransferase-1b			1.00 ± 0.45	0.67 ± 0.31[a]
Peroxisome proliferator-activated receptor α	1.00 ± 0.31	1.04 ± 0.15		
Sterol-regulatory element binding protein-1	1.00 ± 0.29	0.86 ± 0.24		

Values are means ± SD (n = 10 for each group).
[a] $P < 0.05$, differs significantly from the adequate mean.
[b] $P < 0.01$, differs significantly from the adequate mean.

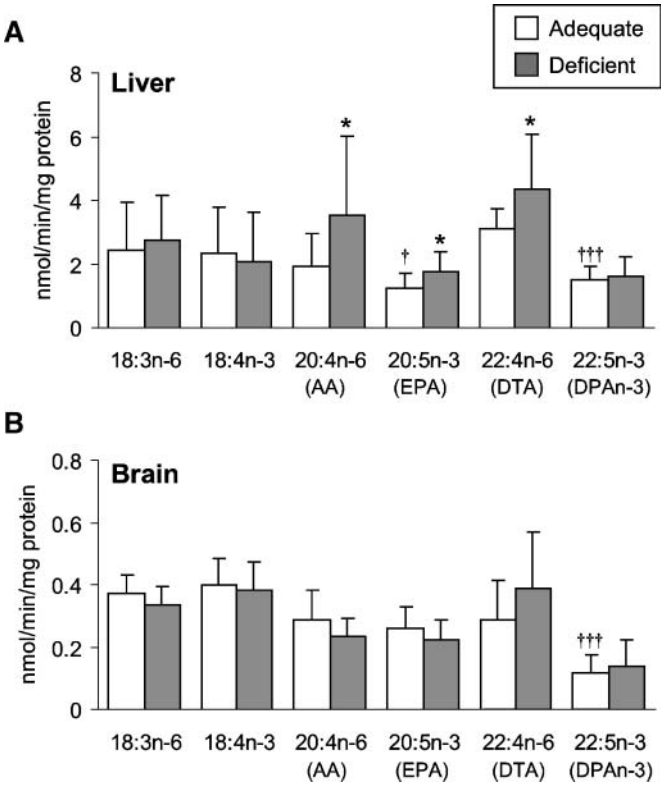

Fig. 3. Elongation activity in the liver (A) and brain (B) of rats fed an n-3 PUFA adequate or deficient diet. Values are means ± SD (n = 10 for both groups). * $P < 0.05$ compared with the mean in the adequate group; † $P < 0.05$ compared with elongation activity with AA in the adequate group; ††† $P < 0.001$ compared with elongation activity with docosatetraenoic acid (DTA) in the adequate group.

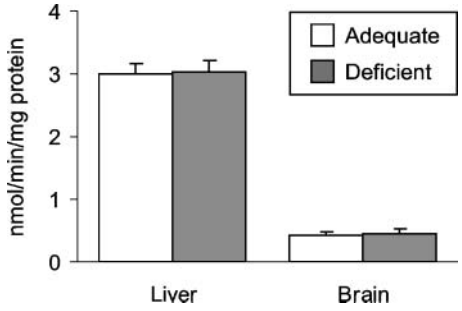

Fig. 4. Acyl-CoA oxidase activity in liver and brain of rats fed an n-3 PUFA adequate or deficient diet. Values are means ± SD (n = 10 for both groups).

of C16–C20 PUFAs (19, 43). We analyzed elongase activities using 18:3n-6, 20:4n-6 (AA), 22:4n-6 [docosatetraenoic acid(DTA)], 18:4n-3, 20:5n-3 (EPA), and 22:5n-3 (DPA n-3) as substrates. In the liver of the n-3 PUFA-deprived rats, elongation activity was increased when the substrate was 20:4n-6, 20:5n-3, or 22:4n-6, consistent with increased elongase 2 and 5 activities. 20:4n-6 and 22:4n-6 were elongated 1.5 and 2 times faster in the liver of the adequate diet rats than were 20:5n-3 and 22:5n-3, respectively.

In rats fed the adequate diet, brain elongase activities were one-fifth to one-tenth their respective activities in liver. In brain, 22:4n-6 was elongated 2.5 times faster than was 22:5n-3. In rats fed the deprived compared with the adequate diet, mRNA levels of liver elongases 2 and 5 were increased 1.5-fold and 1.4-fold, respectively (Table 2), whereas deprivation did not affect the mRNA level of either enzyme in brain. The liver mRNA level of elongase 6, which we also measured, was unaffected by the n-3 PUFA deficient diet, and we did not determine the activity of this enzyme.

Activity and mRNA expression of acyl-CoA oxidase in rat liver and brain

As illustrated in **Fig. 4** and Table 2, n-3 PUFA deprivation did not change the mRNA or activity level of acyl-CoA oxidase in either liver or brain. The activity of this enzyme in liver was six times higher than that in brain in rats fed the adequate diet.

Effects of the deficient diet on the expression of CPT-1

mRNA levels of CPT-1a and/or CPT-1b were determined in rat liver and brain to see if dietary deprivation affected this marker of mitochondrial β-oxidation. The deficient diet did not change the mRNA level of CPT-1a in liver but did significantly reduce levels of CPT-1a and CPT-1b in brain (Table 2).

Effects of diet on the expression of PPARα and SREBP-1 in rat liver

Although several studies have shown that SREBP-1 and PPARα can regulate the transcription of elongases, desaturases, and acyl-CoA oxidase for PUFAs (12, 13, 18, 19), there was no significant difference in the mRNA levels of PPARα or SREBP-1 between rats fed the deficient or adequate diet (Table 2).

DISCUSSION

In rats on the n-3 PUFA adequate diet, baseline activities of the enzymes involved in converting LA to AA and α-LNA to DHA (Fig. 1) were generally higher in liver than in brain. Additionally, mRNA and/or activity levels of elongases 2 and 5 but not of elongase 6, and of Δ5 and Δ6 desaturases but not of Δ9 desaturase, were upregulated in liver but not brain in rats fed the n-3 PUFA deficient compared with adequate diet for 15 weeks. Acyl-CoA oxidase activity in both organs was unaffected by dietary deprivation. The mRNA level of CPT-1a in the liver was unchanged, whereas mRNA levels of CPT-1a and CPT-1b were reduced in brain by deprivation. The mRNA levels of the transcription factors SREBP-1 and PPARα in liver also were unaffected by the n-3 PUFA deficient diet.

These differences with regard to enzyme expression correspond to kinetic differences that were measured after intravenous infusion of [1-^{14}C]α-LNA into unanesthetized rats that had been fed each of the two diet regimens for 15 weeks (10, 11, 37, 44). In those studies, baseline coefficients of the conversion of circulating α-LNA to DHA were higher in liver than in brain in rats on the n-3 PUFA

adequate diet, consistent with higher converting enzyme activities in the liver in rats on this diet (Figs. 1–3) (10, 11). Furthermore, placing rats on the deficient diet increased conversion coefficients by 10-fold in liver but did not change them in brain (10, 11, 37, 44), consistent with the upregulation of enzyme activities in liver but not in brain in rats on the deficient diet.

Desaturases and elongase 5 are expressed in many rodent tissues, but their expression is highest in liver and brain (12, 14, 15, 45, 46). Elongase 2 mRNA has been detected in liver, brain, lung, kidney, and white adipose tissue, with the liver having the highest expression, followed by the brain (12, 45). We determined enzyme activities only in the liver and brain in this study, to test the hypothesis that differences in activities would correspond to reported differences in conversion coefficients of α-LNA to DHA in the two organs under the different dietary conditions (10, 11, 29). Within brain, DHA biosynthesis occurs mainly in astrocytes (17, 47).

Dietary n-3 PUFA deprivation upregulated liver mRNA levels of Δ5 and Δ6 desaturases and elongases 2 and 5 without changing mRNA levels of elongase 6, Δ9 desaturase, or acyl-CoA oxidase (Table 2). Transcription of the affected liver enzymes can be controlled by PPARα and SREBP-1 (12, 18, 46), but because the liver mRNA levels of PPARα and SREBP-1 were unchanged by dietary deprivation, it is possible that their posttranslational regulation was altered (48–50). We did not examine this possibility. Expression of other transcription factors may have been changed, but we did not examine this either. These factors include liver X nuclear receptor, carbohydrate-regulatory element binding protein, MAX-like factor X, retinoid X receptor, hepatic nuclear factor-4, and thyroid hormone receptor (18, 51, 52).

The half-life of DHA in brain is prolonged by 3-fold in rats fed the deficient compared with adequate diet (32). This prolongation has been ascribed to downregulated expression of DHA-metabolizing enzymes, namely Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) and cyclooxygenase-1 (47, 53, 54). Our current results suggest that reduced brain expression of CPT-1a and CPT-1b also might contribute to the prolonged DHA half-life. These enzymes promote the entry into mitochondria of DHA and α-LNA from the brain acyl-CoA pool for subsequent β-oxidation (55, 56).

Others also have reported that the dietary PUFA composition affects desaturase and elongase activities in rat liver. In one study, feeding fish oil decreased liver elongase and desaturase activities (12, 57, 58), whereas in another study, a corn oil diet rich in LA suppressed liver Δ6 desaturase activity (14). A diet deficient in both LA and α-LNA increased desaturase and elongase activities in rat liver (59, 60), and we show here that a dietary n-3 PUFA deficiency alone increased liver desaturase and elongase activities. In one study, however, feeding a diet like ours, deficient in α-LNA, to rats for 2 and 6 months did not affect liver Δ6 desaturase activity (61).

It is well recognized that dietary n-3 PUFA deprivation leads to DPAn-6 accumulation in rat brain, liver, plasma, and heart (9–11, 62–68). DPAn-6 synthesis from AA can be catalyzed by elongases 2 and 5, Δ6 desaturase, and acyl-CoA oxidase (Fig. 1), and it has been suggested that DPA n-6 accumulates in the deprived brain because some of these enzymes are disinhibited by the reduced concentration of DHA (2, 69). On the other hand, a high DPAn-6 concentration in plasma of rats on the n-3 PUFA deficient diet could increase brain DPAn-6 (32).

In summary, 15 weeks of dietary n-3 PUFA deprivation starting at 21 days of age upregulated the expression and activity of Δ5 and Δ6 desaturases and of elongases 2 and 5 in rat liver but not brain, did not affect the expression of acyl-CoA oxidase in either organ, and downregulated the expression of CPT-1a and CPT-1b in brain. These changes likely explain why such deprivation increases coefficients of DHA synthesis from circulating unesterified α-LNA in liver but not brain (10, 11). Furthermore, lower enzyme activities in brain than liver of rats fed the adequate diet correspond to reported lower conversion coefficients in brain (10, 11, 29, 37).

In rats fed the adequate diet for 15 weeks, the estimated rate of DHA synthesis by the brain from circulating α-LNA is ~1% of the brain's DHA consumption rate (70, 71). In contrast, the estimated rate of secretion by the liver of the DHA that it has synthesized from α-LNA is 10-fold the brain's DHA consumption rate, more than sufficient to supply the brain's DHA requirements. This high synthesis-secretion rate is supported by the high expression of conversion enzymes in the liver.

Our kinetic and enzyme data show that the liver can maintain brain DHA composition when DHA is absent from the diet but a sufficient amount (4.6% of total fatty acid) of α-LNA is present and when dietary α-LNA is reduced to some extent (which has yet to be determined). This ability is attributable to high baseline liver converting enzyme activities and to increases in these activities in response to n-3 PUFA deprivation. This conclusion implies that conditions such as liver disease, diabetes, and aging, in which liver conversion enzymes can be downregulated (72–74), are risk factors for brain diseases involving disturbed PUFA metabolism.

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