Docosahexaenoic acid synthesis from α-linolenic acid by rat brain is unaffected by dietary n-3 PUFA deprivation[S]

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Abstract **Rates of conversion of α-linolenic acid (α-LNA, 18:3n-3) to docosahexaenoic acid (DHA, 22:6n-3) by the mammalian brain and the brain's ability to upregulate these rates during dietary deprivation of n-3 polyunsaturated fatty acids (PUFAs) are unknown. To answer these questions, we measured conversion coefficients and rates in post-weaning rats fed an n-3 PUFA deficient (0.2% α-LNA of total fatty acids, no DHA) or adequate (4.6% α-LNA, no DHA) diet for 15 weeks. Unanesthetized rats in each group were infused intravenously with [1-^{14}C]α-LNA, and their arterial plasma and microwaved brains collected at 5 minutes were analyzed. The deficient compared with adequate diet reduced brain DHA by 37% and increased brain arachidonic (20:4n-6) and docosapentaenoic (22:5n-6) acids. Only 1% of plasma [1-^{14}C]α-LNA entering brain was converted to DHA with the adequate diet, and conversion coefficients of α-LNA to DHA were unchanged by the deficient diet. In summary, the brain's ability to synthesize DHA from α-LNA is very low and is not altered by n-3 PUFA deprivation. Because the liver's reported ability is much higher, and can be upregulated by the deficient diet, DHA converted by the liver from circulating αLNA is the source of the brain's DHA when DHA is not in the diet.—**Igarashi, M., J. C. DeMar, Jr., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. **Docosahexaenoic acid synthesis from α-linolenic acid by rat brain is unaffected by dietary n-3 PUFA deprivation. *J. Lipid Res.* 2007.** 48: **1150–1158.**

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α-Linolenic acid (α-LNA, 18:3n-3), a nutritionally essential polyunsaturated fatty acid (PUFA), can be converted in vertebrate brain or liver to docosahexaenoic acid (DHA, 22:6n-3) (1) via different desaturases and elongases (2–5). DHA is found in high concentrations in brain, where it is critical for normal brain structure and function (6–9).

DHA can be obtained directly through the diet, and is found commonly in fish oils. However, dietary absence of DHA in monkeys, piglets, rats, and mice did not decrease brain DHA (10) when sufficient quantities of α-LNA were in the diet (6, 11, 12). In contrast, an all n-3 PUFA-deficient diet reduces rat brain DHA content and alters behavior, whereas n-3 PUFA dietary supplementation can have positive cognitive or behavioral effects in human neurodevelopment and certain human brain diseases (6, 8, 13–20). Thus, understanding how brain DHA is regulated by diet has important basic and clinical implications.

Cultured rat astrocytes and cerebrovascular endothelial cells can convert α-LNA to DHA (21–23), but the brain's capacity to perform this conversion in vivo, and whether this capacity can be upregulated when DHA is absent or low in the diet, remain controversial. In immature mice, data indicate that the brain cannot synthesize enough DHA from α-LNA to maintain a normal DHA composition, but must be provided dietary DHA or DHA that has been converted from α-LNA by the liver, to maintain its normal DHA concentration (24).

We have developed an in vivo method involving the intravenous infusion of [1-^{14}C]α-LNA to quantify tissue synthesis-incorporation (conversion) coefficients, $k^*_{i(\alpha-LNA\rightarrow j)}$ (ml/s/g tissue), of plasma α-LNA to longer-chain n-3 PUFAs *j* (including DHA) within stable tissue lipids *i* (25, 26). In rats fed a diet containing DHA as 2.3% of total fatty acid, we showed with this method that both brain and liver converted <1% of their plasma-derived unesterified α-LNA to esterified DHA, but that synthesis-incorporation coefficients and rates in liver were much higher than in brain (25–28).

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Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl ester; LA, linoleic acid; α-LNA, α-linolenic acid.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of a table.

We also reported that $k^*_{i(\alpha-LNA\rightarrow DHA)}$ was increased 6.6-, 8.4-, and 2.3-fold in i = liver triacylglycerol, phospholipid, and cholesteryl ester, respectively, in male rats fed, for 15 weeks after weaning, an n-3 PUFA-adequate diet without DHA (4.6% α-LNA of fatty acids, no DHA) compared with an n-3 PUFA-deficient diet (0.2% α-LNA, no DHA) (10, 16). Coefficients $k^*_{i(\alpha-LNA\rightarrow j)}$ for synthesis intermediates j = 20:4n-3, 20:5n-3, and 22:5n-3 in i = triacylglycerol also were elevated significantly by the deficient diet (25–28). That the liver can increase its capacity to synthesize DHA agrees with reports that high DHA-containing diets suppress expression in rat liver of the Δ5 and Δ6 desaturases and elongases that catalyze DHA synthesis from α-LNA (1–4, 29), that DHA conversion from α-LNA is reduced in rat astrocytes incubated with DHA (21), and that transcription of Δ5 and Δ6 desaturase and elongase genes is upregulated in rat liver by n-3 PUFA deprivation (unpublished observations).

To see whether brain synthesis-incorporation (conversion) coefficients $k^*_{i(\alpha-LNA\rightarrow j)}$ also are upregulated by n-3 PUFA dietary deprivation, in this paper, we determined these coefficients and related kinetic parameters in the brain of the rats whose liver metabolism we had already studied under the dietary n-3 PUFA-adequate and n-3 PUFA-deficient conditions noted above (26). The reader is referred to that study for experimental details and data on plasma fatty concentrations, plasma radioactive input functions, and diets.

MATERIALS AND METHODS

Materials

[1-^{14}C]α-LNA in ethanol was purchased from Perkin-Elmer Life Sciences, NEN Life Science Products (Boston, MA). Its specific activity was 54 mCi/mmol, and its purity was 98% [determined by high-performance liquid chromatography (HPLC) and scintillation counting]. Di-heptadecanoate phosphatidylcholine (di-17:0 PC), free heptadecanoic acid (17:0), heptadecanoyl-CoA (17:0-CoA), and acyl-CoA standards for HPLC, as well as thin-layer chromatography (TLC) standards for cholesterol, triglycerides, and cholesteryl esters, were purchased from Sigma-Aldrich (St. Louis, MO). Standards for general fatty acid methyl esters (FAMEs) for gas chromatography (GC) and HPLC were from NuChek Prep (Elysian, MN). FAMEs for unique n-3 PUFAs (20:4n-3, 22:5n-3, 24:5n-3, 24:6n-3, and 22:5n-6) were purchased from Larodan Fine Chemicals (Malmö, Sweden). 6-p-Toluidine-2-naphthalene sulfonic acid was from Acros Organics (Fair Lawn, NJ). 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.

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 (NIH Publication No. 80-23). Fischer-344 (CDF) male rat pups (18 days old) and their surrogate mothers were purchased from Charles River Laboratories (Portage, MI) and were housed in an animal facility with regulated temperature and humidity and a 12 h light/12 h dark cycle. The pups were allowed to nurse until 21 days old. Lactating rats had free access to water and rodent chow formulation NIH-31 18-4, which contained 4% (wt/wt) crude fat (Zeigler Bros., Gardners, PA) and whose fatty acid composition is reported elsewhere (25, 26). α-LNA, eicosapentaenoic acid (EPAn-3, 20:5n-3), and DHA contributed 5.1, 2.0, and 2.3% of total fatty acids, respectively, whereas linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6) contributed 47.9% and 0.02%, respectively. After weaning, the pups were divided randomly into n-3 PUFA-adequate and n-3 PUFA-deficient diet groups (see below). They had free access to food and water, and their food was replaced every 2 or 3 days. Body weight was recorded every 2 or 3 days.

n-3 PUFA-adequate and n-3 PUFA-deficient diets

The compositions of the n-3 PUFA-adequate and n-3 PUFA-deficient diets, each prepared by Dyets, Inc. (Bethlehem, PA), based on the AIN-93G formulation (30), are presented in **Table 1** (16, 26). Each diet contained 10% crude fat, and the adequate but not the deficient diet contained flaxseed oil. The fatty acid composition of both diets (μmol/g food, percent total fatty acid, and percent energy) are shown in supplementary Table I (16, 26). The adequate diet contained 7.8 μmol/g α-LNA (4.6% total fatty acid) (10, 31). The deficient diet contained 0.25 μmol/g α-LNA (0.2% total fatty acid). Other n-3 PUFAs were absent from both diets. Both contained 40 μmol/g diet LA (23–24% total fatty acid).

Surgery

A rat was anesthetized with 1–3% halothane. Polyethylene catheters (PE 50, Intramedic™, Clay Adams™; Becton Dickinson, Sparks, MD) filled with heparinized saline (100 IU/ml) were surgically implanted into the right femoral artery and vein (25), after which the skin was closed with staples and treated with 1% lidocaine for pain control. The rat was loosely wrapped in a fast-setting plaster cast taped to a wooden block, and allowed to recover from anesthesia for 3–4 h. Body temperature was maintained at 36–38°C using a feedback heating element (Indicating

TABLE 1. Composition of n-3 PUFA-adequate and n-3 PUFA-deficient diets

Component	n-3 PUFA-adequate Diet	n-3 PUFA-deficient Diet
	g/100 g diet	
Protein	20.0	20.0
Casein	20.0	20.0
Carbohydrate	60.0	60.0
Cornstarch	15.0	15.0
Sucrose	10.0	10.0
Dextrose	20.0	20.0
Maltose dextrin	15.0	15.0
Fat	10.0	10.0
Hydrogenated coconut oil	6.0	6.6
Safflower oil	3.2	3.4
Flaxseed oil	0.8	0.0
Additives	10.0	10.0
Cellulose	4.95	4.95
Salts	3.5	3.5
Vitamins	1.0	1.0
L-cystine	0.3	0.3
Choline chloride	0.25	0.25
TBQH	0.002	0.002

PUFA, polyunsaturated fatty acid; TBHQ, tertiary-butylhydroquinone (antioxidant).

Temperature Controller; Yellow Springs Instruments, Yellow Springs, OH). Animals were provided food and water the night prior to surgery.

Tracer infusion

Each rat was infused via the femoral vein catheter with 500 μCi/kg [1-^{14}C]α-LNA (25, 26, 32). An aliquot of [1-^{14}C]α-LNA in ethanol was dried under nitrogen, and the residue was dissolved in HEPES buffer (pH 7.4) containing 50 mg/ml fatty acid-free BSA, to a final volume of 1.3 ml. The mixture was sonicated at 40°C for 20 min and mixed by vortexing. A computer-controlled variable-speed pump (No. 22; Harvard Apparatus, South Natick, MA) was used to infuse 1.3 ml tracer at a rate of 0.223 $(1 - e^{-1.92t})$ ml/min (t in min) to rapidly establish a steady-state plasma radioactivity (33). Arterial blood was collected in centrifuge tubes (polyethylene-heparin lithium fluoride-coated, Beckman) at 0, 0.25, 0.5, 0.75, 1.5, 3, 4, and 5 min after starting infusion. At 5 min, each rat was euthanized by an overdose of sodium pentobarbital (100 mg/kg i.v.), and its head and torso were immediately subjected to high-energy focused-beam microwave irradiation (5.5 kW, 3.4 s) (Model S6F; Cober Electronics, Stamford, CT). The brain was removed and confirmed visually to be browned; if not browned, it was discarded. Brain weight was recorded, and the brain was stored at −80°C until analyzed. Arterial blood samples were centrifuged at 13,000 rpm for 5 min, and plasma was collected and frozen at −80°C.

Separation of brain lipid

Total brain lipid was extracted by the procedure of Folch, Lees, and Sloane Stanley (34), which produces a negligible (<0.5%) loss of lipid. The aqueous extraction phases were washed once with an equal volume of chloroform to remove residual lipid, and aqueous and total lipid radioactivity was counted (see below). Total lipid extracts were separated into neutral lipid subclasses by TLC on silica gel 60 plates (EM Separation Technologies; Gibbstown, NJ) using heptane-diethyl ether-glacial acetic acid (60:40:3; v/v/v) (35). Authentic standards of triacylglycerol, phospholipids, cholesterol, and unesterified fatty acids were run on the plates to identify the lipids. The plates were sprayed with 0.03% 6-p-Toluidine-2-naphthalene sulfonic acid in 50 mM Tris buffer (pH 7.4) (w/v), and the lipid bands were visualized under ultraviolet (UV) light. The bands were scraped, and the silica gel was used to directly quantify radioactivity by scintillation counting and to prepare FAMEs for quantifying fatty acid concentrations and radioactivities (see below).

Quantification of radioactivity

Samples for measuring radioactivity were placed in scintillation vials and dissolved in liquid scintillation cocktail (Ready Safe™ plus 1% glacial acetic acid). Their radioactivity was determined using a liquid scintillation analyzer (2200CA, TRI-CARB®; Packard Instruments, Meriden, CT).

FAME preparation

The FAMEs were quantified by GC and HPLC. Unesterified and esterified fatty acids were methylated with 1% H_2SO_4-methanol for 3 h at 70°C (36, 37). Before the sample was methylated for GC analysis, appropriate quantities of di-17:0 PC (for phospholipids and triacylglycerol) or unesterified 17:0 (for unesterified fatty acids) were added as internal standards.

GC analysis

Fatty acid concentrations (nmol/g brain wet wt) in brain lipids were determined using a gas chromatograph (6890N; Agilent Technologies, Palo Alto, CA) equipped with an SP™-2330 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Supelco; Bellefonte, PA) and a flame ionization detector (37). Concentrations were calculated by proportional comparison of peak areas to the area of the 17:0 internal standard.

HPLC analysis

To determine fatty acid radioactivity in brain lipids, FAMEs from brain lipids were quantified by HPLC using the method of Aveldano, VanRollins, and Horrocks (38) with modifications. 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 a UV detector (UV/VIS-151; Gilson, Middleton, WI). The reverse-phase column, Luna 5 μ C18 (2) (5 μM particle size, 4.6 × 250 mm) was from Phenomenex (Torrance, CA). HPLC eluates were collected every 30 s and subjected to liquid scintillation counting to obtain radioactivity profiles. Chromatography was performed using a linear gradient system of water and acetonitrile. The acetonitrile was held at 85% for 30 min, increased to 100% over 10 min, and held again at 100% for 20 min. The flow rate was 1.0 ml/min. The UV detector was set at 205 nm.

HPLC profiles of FAMEs were obtained using equally pooled samples from each group (10 animals for the adequate group and 7 animals for the deficient group). Percent radioactivities of [1-^{14}C]α-LNA, [^{14}C]DHA, and [^{14}C]intermediates of DHA synthesis in brain lipid fractions were determined from these HPLC profiles.

Analysis of long-chain acyl-CoAs

Long-chain acyl-CoAs were extracted from microwaved brains using an affinity chromatography method with slight modifications (39). After 5 nmol heptadecanoyl-CoA (17:0-CoA) was added as an internal standard to ~0.8 g of brain, the sample was homogenized in isopropanol-25 mM KH_2PO_4-acetonitrile (1:1:2; v/v/v), then sonicated with a probe sonicator (Model W-225; Misonix, Farmingdale, NY). A small volume (~3% of total) of saturated $(NH_4)_2SO_4$ was added to the homogenate to precipitate proteins, then the sample was mixed vigorously for 5 min and centrifuged. The supernatant was diluted with a 1.25-fold volume of 25 mM KH_2PO_4. The solution was passed three times through an oligonucleotide purification cartridge (ABI Masterpiece™, OPC®; Applied Biosystems, Foster City, CA), and the cartridge was washed with 25 mM KH_2PO_4. Acyl-CoA species were eluted with a small volume of isopropanol-1 mM glacial acetic acid (75:25; v/v).

Extracted acyl-CoAs were separated on a reverse-phase HPLC column (Symmetry; 5 μm particle size, 4.6 mm × 250 mm; Waters Corporation, Milford, MA) using a pump coupled with a UV/VIS detector (System Gold, Model 168, Beckman). Chromatography was performed using a linear gradient system of 75 mM KH_2PO_4 and acetonitrile. At the start, acetonitrile was 44% and held for 1 min, then increased to 49% over 25 min, increased to 68% over 10 min, held at 100% for 4 min, returned to 44% over 6 min, and held for 6 min (52 min total run time). The flow rate was 1.0 ml/min. UV detection was set at 260 nm for integration of concentrations and at 280 nm for identifying acyl-CoAs (260/280 = 4:1) (39). Peaks were identified from retention times of acyl-CoA standards. The acyl-CoA standards for α-LNA, EPA, docosapentaenoic acid (DPA)n-3, DPAn-6, and DHA were prepared from the free fatty acid and free CoA by an enzymatic method (40). Endogenous acyl-CoA concentrations (nmol/g brain) were calculated by direct proportional comparison with the peak area of the 17:0-CoA internal standard. Each acyl-CoA peak was collected, and its radioactivity was determined by liquid scintillation count-

ing. Radioactivity (nCi/g brain) was corrected for loss by normalization against percent recovery of the 17:0-CoA internal standard.

In this HPLC system, 14:0-CoA, α-LNA-CoA, and EPA-CoA coeluted as a single peak (25). This peak was collected and saponified with 2% KOH/EtOH (wt/v) at 100°C for 45 min and acidified with HCl, and the fatty acids were extracted with n-hexane. The fatty acids were converted to FAMEs and separated on HPLC as described above. The FAME derivatives of 14:0, α-LNA, and EPA were completely separated on the HPLC system. Each peak was collected, and its radioactivity was measured by scintillation counting. The concentrations of the FAMEs that came from the acyl-CoA species also were determined by GC, as described above. Thus, the concentrations of 14:0, α-LNA, and EPA in the original acyl-CoA peak were determined by proportional comparison of their GC peak areas.

Calculations

Equations for determining the in vivo kinetics of α-LNA in brain and liver, following a 5 min intravenous infusion of radiolabeled α-LNA to produce a steady-state plasma radioactivity, have been described elsewhere (25, 26, 32). Briefly, with regard to brain, following the 5 min [1-^{14}C]α-LNA infusion, incorporation coefficients $k^*_{i(\alpha-LNA)}$ (ml/s/g brain), representing transfer of unesterified [1-^{14}C]α-LNA from plasma into stable brain lipid *i* (phospholipid or triacylglycerol), were calculated as follows:

$$k^*_{i(\alpha-LNA)} = \frac{c^*_{brain,i(\alpha-LNA)}(T)}{\int_0^T c^*_{plasma(\alpha-LNA)}\,dt} \qquad (Eq.\ 1)$$

where $c^*_{brain,i(\alpha-LNA)}$ (T) (nCi/g brain) is brain α-LNA radioactivity in *i* at time *t* (5 min) after starting tracer infusion, *t* is time after starting infusion, and $c^*_{plasma(\alpha-LNA)}$ (nCi/ml plasma) is plasma radioactivity of unesterified α-LNA. Coefficients $k^*_{i(\alpha-LNA\rightarrow j)}$ (ml/s/g brain), representing synthesis from α-LNA of *j* = DHA or its n-3 intermediates, and subsequent incorporation into stable lipid *i*, were calculated as follows:

$$k^*_{i(\alpha-LNA\rightarrow j)} = \frac{c^*_{brain,i(j)}(T)}{\int_0^T c^*_{plasma(\alpha-LNA)}\,dt} \qquad (Eq.\ 2)$$

where $c^*_{brain,i(j)}$ (T) (nCi/g brain) is radioactivity of the n-3 PUFA *j* in stable lipid *i* at *T* = 5 min.

Rates of incorporation of unlabeled unesterified α-LNA from plasma into lipid *i*, $J_{in,i(\alpha-LNA)}$, and the rate of synthesis of *j* from α-LNA followed by its incorporation into *i* (nmol/s/g brain), were calculated as

$$J_{in,i(\alpha-LNA)} = k^*_{i(\alpha-LNA)}\,c_{plasma(\alpha-LNA)} \qquad (Eq.\ 3)$$

$$J_{in,i(\alpha-LNA\rightarrow j)} = k^*_{i(\alpha-LNA\rightarrow j)}\,c_{plasma(\alpha-LNA)} \qquad (Eq.\ 4)$$

where $c_{plasma(\alpha-LNA)}$ is the plasma concentration (nmol/ml) of unlabeled unesterified α-LNA.

The brain takes up unesterified fatty acids from plasma by simple diffusion after they are released from circulating albumin (41–43). A "dilution factor," $\lambda_{\alpha-LNA-CoA}$, represents the unesterified α-LNA flux from plasma into the brain α-LNA-CoA pool, divided by the sum of fluxes of unesterified plasma α-LNA and of the α-LNA released (recycled) from stable lipids (41, 44, 45). $\lambda_{\alpha-LNA-CoA}$ is determined as the steady-state ratio of brain α-LNA-CoA-specific activity to the specific activity of unesterified α-LNA in plasma:

$$\lambda_{\alpha-LNA-CoA} = \frac{c^*_{brain(\alpha-LNA-CoA)}/c_{brain(\alpha-LNA-CoA)}}{c^*_{plasma(\alpha-LNA)}/c_{plasma(\alpha-LNA)}} \qquad (Eq.\ 5)$$

The rate of incorporation $J_{FA,i(\alpha-LNA)}$ (nmol/s/g) of unlabeled α-LNA from the precursor brain α-LNA-CoA pool into stable lipid *i* equals

$$J_{FA,i(\alpha-LNA)} = J_{in,i(\alpha-LNA)}/\lambda_{\alpha-LNA-CoA} \qquad (Eq.\ 6)$$

whereas the rate of conversion of α-LNA to *j* (including DHA), followed by incorporation of *j* into *i*, equals $J_{FA,i(\alpha-LNA\rightarrow j)}$ (nmol/s/g)

$$J_{FA,i(\alpha-LNA\rightarrow j)} = J_{in,i(\alpha-LNA\rightarrow j)}/\lambda_{\alpha-LNA-CoA} \qquad (Eq.\ 7)$$

Statistical analysis

Data are expressed as means ± SD (n = 10 for n-3 PUFA-adequate group; n = 7 for n-3 PUFA-deficient group). Student's *t*-tests were used to determine significance of differences between means, taken as $P \leq 0.05$.

RESULTS

Brain fatty acid concentrations

Table 2 presents concentrations of unesterified fatty acids and of fatty acids esterified in phospholipid and triacylglycerol in microwaved brains from the n-3 PUFA diet-adequate and diet-deficient rats. In the deficient compared with adequate group, the brain DHA concentration was reduced significantly by 37%, and α-LNA, EPAn-3 (20:5n-3), and DPAn-3 (22:5n-3) were not detected. Brain concentrations of AA (20:4n-6) and DPAn-6 (22:5n-6) were increased 1.1-fold and 30-fold, respectively, whereas the brain LA (18:2n-6) concentration was decreased by 26%. Concentrations of unesterified AA and total unesterified n-6 PUFAs were not affected significantly by deprivation, whereas unesterified DHA was decreased by 86%.

Distribution of brain radioactivity

Figure 1 summarizes the distribution of radioactivity and metabolic pathways between different brain lipid compartments in the two dietary groups. After the 5 min [1-^{14}C]α-LNA infusion, total brain (lipid plus aqueous phase) radioactivity did not differ significantly between the diet-deficient (124.7 ± 19.8 nCi/g brain) and diet-deprived (135.2 ± 24.0 nCi/g brain) group. Additionally, total lipid radioactivity following deprivation (44.2 ± 16.0 nCi/g brain, 33% of net radioactivity) did not differ significantly from radioactivity in the diet-adequate group (33.2 ± 4.1 nCi/g brain 29.5% of net radioactivity). Deprivation did not significantly affect radioactivity in stable brain lipids; radioactivity equaled 25.08 ± 3.96 and 34.59 ± 15.32 nCi/g in phospholipid of the diet-adequate and diet-deprived groups, respectively; 3.16 ± 1.17 and 4.41 ± 2.09 nCi/g, respectively, in triacylglycerol; and 4.94 ± 0.73 and 5.21 ± 2.28 nCi/g, respectively, in cholesterol.

Studies indicate that α-LNA is elongated and desaturated to 24:6n-3 by the following steps: 18:3→18:4→20:4→20:5→22:5→24:5→24:6, after which 24:6n-3 is shortened to DHA (22:6n-3) by one round of β-oxidation in peroxisomes (1, 23). An additional pathway of α-LNA conversion has been reported, in which 20:3n-3, 22:4n-3, and 24:4n-3

TABLE 2. Fatty acid concentrations in brain lipids of n-3 PUFA-adequate and n-3 PUFA-deficient rats

	Phospholipids		Triacylglycerol		Unesterified Fatty Acid	
Fatty Acid	Adequate	Deprived	Adequate	Deprived	Adequate	Deprived
			nmol/g brain			
14:0	1,408 ± 228	12,160 ± 119	15.7 ± 12.6	14.6 ± 8.3	3.9 ± 2.2	2.2 ± 1.8
16:0	21,088 ± 1,120	20,816 ± 1559	74.8 ± 29.7	77.8 ± 27.9	27.1 ± 12.5	20.0 ± 11.7
16:1n-7	306 ± 38	275 ± 66	4.7 ± 3.7	4.6 ± 1.6	0.7 ± 0.5	0.7 ± 0.6
18:0	21,436 ± 1,665	20,966 ± 750[a]	35.7 ± 14.1	44.7 ± 26.3	27.5 ± 11.6	23.2 ± 14.7
18:1n-7	6,077 ± 670	5,727 ± 902	16.3 ± 8.0	16.3 ± 3.3	2.9 ± 1.8	2.5 ± 2.2
18:1n-9	19,994 ± 3,742	17,237 ± 671	29.2 ± 15.7	26.2 ± 7.8	9.3 ± 6.5	11.0 ± 6.4
18:2n-6	518 ± 105	382 ± 46[a]	15.0 ± 12.6	11.3 ± 3.4	2.3 ± 1.1	2.6 ± 2.1
18:3n-3	ND	ND	ND	ND	ND	ND
20:4n-6	8,740 ± 599	9,304 ± 490[a]	8.3 ± 5.6	10.2 ± 3.5	5.6 ± 3.3	5.4 ± 2.4
20:5n-3	ND	ND	ND	ND	ND	ND
22:5n-6	157 ± 22	4,792 ± 208[c]	ND	ND	ND	1.2 ± 1.0
22:5n-3	162 ± 31	ND	ND	ND	0.5 ± 0.3	ND
22:6n-3	11,395 ± 819	7,138 ± 238[c]	7.3 ± 5.3	3.3 ± 2.1[a]	2.6 ± 1.8	0.42 ± 0.36[b]
Total	95,287 ± 8,568	87,897 ± 4,259	207 ± 90	209 ± 70	82.5 ± 36.2	69.1 ± 34.4
Total n-6	94,163 ± 669	14,479 ± 714[c]	23.3 ± 13.9	21.5 ± 6.3	7.9 ± 4.1	9.2 ± 3.5
Total n-3	11,558 ± 843	7,138 ± 238[c]	7.3 ± 5.3	3.3 ± 2.1[a]	3.2 ± 1.9	0.42 ± 0.36[c]
Total saturated	43,932 ± 2976	43,042 ± 2,231	126 ± 51	137 ± 53	58.5 ± 25.8	45.4 ± 27.1
Total monounsaturated	26,377 ± 4,260	23,239 ± 1,278[a]	50.1 ± 26.7	47.1 ± 15.3	12.9 ± 7.0	14.1 ± 7.2

Values are means ± SD (n = 10 and 7 for adequate and deficient groups, respectively). ND = not detected; <0.1 nmol/g brain.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$; differs significantly from mean in adequate group.

intermediates are converted to 24:5n-3 and then to DHA in the usual manner (46). [1-^{14}C]α-LNA, [^{14}C]DHA, and [^{14}C]n-3 PUFA intermediates (18:4, 20:4, 20:5, 22:5, 24:5, and 20:3) along the pathways of conversion of α-LNA to DHA could be detected in brain triacylglycerol and phospholipid in the diet-adequate and diet-deficient groups (data not shown) (26). Radiolabeled 16:0 and 18:0, which would have come from recycling of radiolabeled carbon from [1-^{14}C]α-LNA, also were detected in brains from both groups.

Table 3 presents values for radioactivity of α-LNA, DHA, and n-3 synthesis intermediates in total brain lipids, phospholipids, and triacylglycerol. Of the intermediates, radioactivities of 18:4n-3 and of 22:5n-3 (DPAn-3) were increased significantly in the diet-deficient compared with diet-adequate rats.

In our study on liver kinetics in these same rats (26), unesterified plasma α-LNA concentrations before infusion equaled 27.0 ± 6.0 nmol/ml and 1.0 ± 0.5 nmol/ml in the n-3 PUFA-adequate and n-3 PUFA-deficient groups, respectively (26). Furthermore, mean integrated plasma radioactivity over the 5 min [1-^{14}C]α-LNA infusion (input function), which was due entirely to [1-^{14}C]α-LNA, equaled 452,235 ± 75,337 nCi/ml plasma/s and 500,384 ± 77,308 nCi/ml plasma/s, respectively (26). Inserting individual experimental integrals of plasma radioactivity into

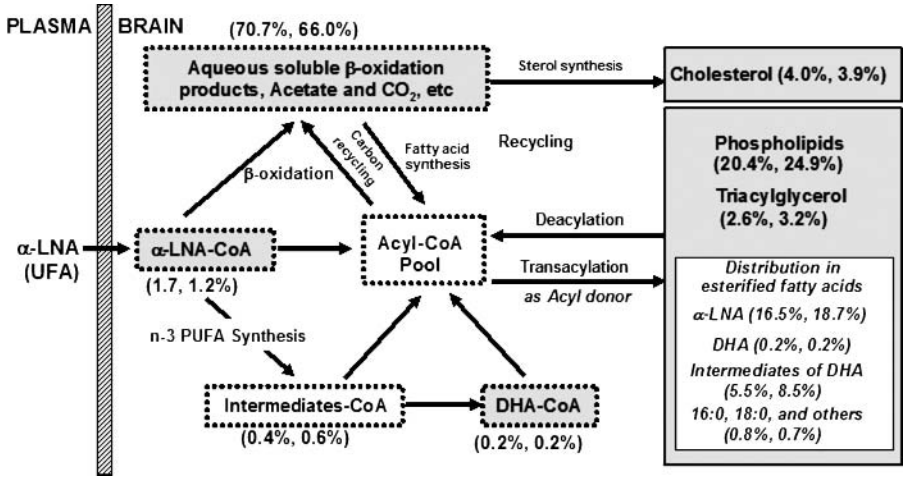

Fig. 1. Percent radioactivity in brain lipid compartments of rats after a 5 min intravenous infusion of [1-^{14}C]α-LNA. Percentages were calculated by dividing radioactivity in each compartment by net brain (excluding unesterified fatty acid) radioactivity. The left number in parentheses is for the n-3 polyunsaturated fatty acid-adequate group, the right number for the deprived group. Arrows show metabolic pathways.

TABLE 3. Measurements and calculations in dietary n-3 PUFA-adequate and n-3 PUFA-deficient rats following 5 min of intravenous [1-^{14}C]α-LNA infusion

	Radioactivity		$k^*_{i(\alpha-LNA)}$ or $k^*_{i(\alpha-LNA\rightarrow j)}$		J_{in}	
	Adequate	Deficient	Adequate	Deficient	Adequate	Deficient
	nCi/g brain		*ml/s/g* $\times 10^{-4}$		*nmol/s/g* $\times 10^{-4}$	
Total lipids						
α-LNAn-3	20.2 ± 2.8	26.0 ± 11.4	0.460 ± 0.105†	0.521 ± 0.210		
DHAn-3	0.235 ± 0.032	0.262 ± 0.115	0.00534 ± 0.00122††	0.00526 ± 0.00212		
Phospholipid						
α-LNAn-3	16.8 ± 2.7	20.9 ± 9.3	0.380 ± 0.089†	0.419 ± 0.170	10.4 ± 3.9	0.498 ± 0.454^{c}
DHAn-3	0.276 ± 0.044	0.250 ± 0.113	0.0063 ± 0.0015††	0.00510 ± 0.00207	0.172 ± 0.065	0.00606 ± 0.00553^{c}
18:4n-3	0.536 ± 0.085	0.806 ± 0.357^{a}	0.0122 ± 0.0028††	0.0161 ± 0.0066		
20:4n-3	0.763 ± 0.121	0.967 ± 0.428	0.0173 ± 0.0040††	0.0194 ± 0.0079		
20:5n-3	1.16 ± 0.18	1.64 ± 0.73	0.0264 ± 0.0062††	0.0328 ± 0.0133		
22:5n-3	1.26 ± 0.20	4.88 ± 2.16^{b}	0.0287 ± 0.0067††	0.0977 ± 0.0397^{b}		
24:5n-3	2.57 ± 0.41	3.40 ± 1.51	0.0582 ± 0.0136††	0.0682 ± 0.0277		
24:6n-3	ND	ND				
20:3n-3	0.823 ± 0.130	1.14 ± 0.50	0.0187 ± 0.0044††	0.0228 ± 0.0093		
Triacylglycerol						
α-LNAn-3	2.15 ± 0.79	2.82 ± 1.33	0.0494 ± 0.0217†	0.0566 ± 0.0247	1.34 ± 0.63	0.0644 ± 0.0562^{c}
DHAn-3	0.0336 ± 0.0124	0.0445 ± 0.0210	0.000772 ± 0.000338††	0.000894 ± 0.000391	0.0209 ± 0.0098	0.00102 ± 0.00089^{c}

Values are means ± SD (n = 10 and 7 for adequate and deficient groups, respectively). †, $k^*_{i(\alpha\text{-}LNA)}$; ††, $k^*_{i(\alpha\text{-}LNA\rightarrow j)}$.
$^{a}P < 0.05$.
$^{b}P < 0.01$.
$^{c}P < 0.001$; differs significantly from mean in adequate group.

equations 1 and 2 provided the values for $k^*_{i(\alpha-LNA)}$ and $k^*_{i(\alpha-LNA\rightarrow j)}$ presented in the third and fourth data columns of Table 3. With the exception of a significant 3.4-fold elevation in $k^*_{i(\alpha-LNA\rightarrow j)}$ for j = 22:5n-3 (DPAn-3) in brain phospholipid, no incorporation or synthesis-incorporation (conversion) coefficient was altered significantly by the n-3 PUFA-deficient diet.

Additionally, inserting preinfusion values for unesterified plasma α-LNA concentrations in equations 3 and 4, incorporation rates $J_{in,i(\alpha-LNA)}$ and synthesis-incorporation rates $J_{in,i(\alpha-LNA\rightarrow DHA)}$ in brain phospholipid and triacylglycerol were markedly reduced by dietary deprivation (fifth and sixth data columns of Table 3). For example, $J_{in,i(\alpha-LNA\rightarrow DHA)}$ equaled 0.172 and 0.00606 nmol/s/g $\times 10^{-4}$ in phospholipids, and 0.0209 and 0.00102 nmol/s/g $\times 10^{-4}$ in triacylglycerol, respectively, for the diet-adequate and diet-deficient groups.

Table 4 presents mean unlabeled and labeled acyl-CoA concentrations in brains from the diet-adequate and diet-deficient rats. Concentrations of unlabeled DHA-CoA and 18:3n-3-CoA were reduced significantly by n-3 PUFA deprivation, whereas concentrations of unlabeled 20:4n-6-CoA and 22:5n-6-CoA were increased significantly. No significant difference was evident in radioactivity in any of the measured acyl-CoAs.

Steady-state plasma and brain α-LNA-CoA radioactivities were achieved within the first minute of controlled [1-^{14}C]α-LNA infusion, because plasma lipid radioactivity had reached an approximate plateau by 0.5 min and brain acyl-CoA radioactivity stabilizes within 1 min of infusion (26, 33, 37). Accordingly, we calculated $\lambda_{\alpha-LNA-CoA}$, the steady-state ratio of brain α-LNA-CoA to plasma α-LNA-specific activity (equation 5), as equaling 0.71 ± 0.28 and 0.59 ± 0.17, respectively, in the diet-adequate and diet-deprived rats. The unesterified plasma α-LNA concentrations at the end of infusion were 53.6 ± 5.3 nmol/ml and 32.5 ± 8.4 nmol/ml, respectively, in the adequate and deprived rats, which has been reported (26). These concentrations were elevated compared with preinfusion concentrations due to the low specific activity and large quantity of infused [1-^{14}C]α-LNA necessary to get easily measured brain radioactivities (26).

Inserting individual experimental values for $\lambda_{\alpha-LNA-CoA}$ into equations 6 and 7 provided net α-LNA incorporation rates $J_{FA,i(\alpha-LNA)}$ from the precursor acyl-CoA pool (Fig. 1) (41, 44, 45) for i = phospholipid plus triacylglycerol equal to 16.5 and 0.953 nmol/s/g $\times 10^{-4}$ in diet-adequate and diet-deficient rats, respectively, and net synthesis-incorporation rates of DHA, $J_{FA,i(\alpha-LNA\rightarrow DHA)}$, equal to

TABLE 4. Acyl-CoA concentrations and corresponding radioactivities in rat following 5 min of [1-^{14}C]α-LNA infusion in n-3 PUFA-adequate and n-3 PUFA-deficient rats

	Unlabeled Concentration		Radioactivity	
Fatty acyl-CoA	Adequate	Deficient	Adequate	Deficient
	nmol/g brain		*nCi/g brain*	
18:3n-3-CoA	0.085 ± 0.044	0.046 ± 0.012^{a}	2.2 ± 0.9	1.6 ± 0.2
22:5n-3-CoA	0.17 ± 0.08	<0.069^{b}	0.53 ± 0.46	0.78 ± 0.32
22:6n-3-CoA	1.4 ± 0.49	0.28 ± 0.13^{a}	0.31 ± 0.20	0.28 ± 0.19
14:0-CoA	1.0 ± 0.31	1.0 ± 0.33	0.49 ± 0.43	0.58 ± 0.16
16:0-CoA	16.7 ± 2.7	15.3 ± 4.7	1.0 ± 0.6	0.99 ± 0.48
18:0-CoA	4.9 ± 2.2	4.3 ± 1.3	0.63 ± 0.55	0.87 ± 0.54
18:1-CoA	20.1 ± 4.0	18.6 ± 6.0	0.77 ± 0.26	0.90 ± 0.40
18:2n-6-CoA	0.62 ± 0.27	0.78 ± 0.39		
20:4n-6-CoA	1.6 ± 0.8	2.1 ± 0.7^{a}		
22:5n-6-CoA	<0.032^{b}	0.94 ± 0.39		

Values are means ± SD (n = 10 and 7 for adequate and deficient groups, respectively).
$^{a}P < 0.001$; differs significantly from mean in adequate group.
^{b}DPAn-3 CoA and DPAn-6 CoA were detected in two samples, and these values are the average of two samples.

10.4 and 0.498 nmol/s/g × 10^{-4}, respectively. These synthesis-incorporation rates are equivalent to 2.35 and 0.104 nmol/day/g brain, respectively.

DISCUSSION

Synthesis-incorporation (conversion) coefficients $k^*_{i(\alpha-LNA\rightarrow DHA)}$ of α-LNA to DHA within brain phospholipid and triacylglycerol were not altered significantly in male postweaning rats that had been fed an n-3 PUFA-deficient diet (0.2% α-LNA of total fatty acids, no DHA) for 15 weeks, compared with rats fed an adequate diet (4.6% α-LNA of total fatty acids, no DHA). On the other hand, $k^*_{i(\alpha-LNA\rightarrow j)}$ for j = 22:5n-3 (DPAn-3) in brain phospholipid was elevated 3.4-fold by deprivation. Deprivation did not significantly alter any direct incorporation coefficient $k^*_{i(\alpha-LNA)}$ of plasma α-LNA into brain phospholipid or triacylglycerol.

These observations indicate that brain conversion of α-LNA to 22:5n-3 was upregulated by n-3 PUFA deprivation, whereas conversion to longer-chain PUFAs, including DHA, was unaffected. Elongation of 22:5n-3 to 24:5n-3 is catalyzed by Elongase 2, which, together with Elongase 5, has been identified in rat liver and brain, but to a lesser extent in brain (4, 47). Thus, it is likely that brain Elongase 2 activity is relatively insensitive to dietary n-3 PUFA restriction. Our results are consistent with evidence that [^{14}C]22:5n-3 accumulated to a greater extent than [^{14}C]20:5n-3 or [^{14}C]22:6n-3 (DHA) in rat brain astrocytes incubated with [1-^{14}C]α-LNA (21).

That the 15 week n-3 PUFA-deficient diet decreased brain DHA by 37% (Table 2) agrees with our earlier reports (16, 37), as do the increased brain concentrations of AA and DPAn-6, and of AA-CoA and DPAn-6-CoA, following deprivation. Brain DPAn-6 accumulation also has been noted in longer-term n-3 PUFA deprivation paradigms (48–50), and may arise from disinhibition of AA elongation by Elongase 5, due to reduced brain DHA (7). Alternatively, increased brain DPAn-6 may have been derived from the plasma, as dietary deprivation increased the unesterified DPAn-6 plasma concentration from undetected to 8.7 ± 1.2 nmol/ml (26).

Of the n-3 PUFA intermediates in the conversion of α-LNA to DHA (1, 23, 46), we detected, in addition to radiolabeled DHA, radiolabeled 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3, 24:5n-3, and 20:3n-3 in brain from both dietary groups. These radioactive n-3 PUFAs were largely synthesized in brain, inasmuch as none were detected in plasma during the 5 min [1-^{14}C]α-LNA infusion (26).

We also found that 77.0% and 69.9% of the [1-^{14}C]α-LNA that entered brain was converted to β-oxidation products (aqueous soluble β-oxidation products plus cholesterol) in the diet-adequate and diet-deprived rats, respectively (Fig. 1), ignoring loss of [^{14}C]CO_2. Radiolabeled 14:0-CoA, 16:0-CoA, 18:0-CoA, and 18:1-CoA constituted approximately 0.4% of net (lipid and aqueous) brain radioactivity, and radiolabeled esterified 16:0 and 18:0 were identified. For comparison, in brains of rats fed the α-LNA plus DHA-containing diet, approximately 67% of α-LNA was β-oxidized (25), and in a human study, high α-LNA- and EPA+DHA-containing diets did not change the fraction of α-LNA that was β-oxidized (51). α-LNA is reported to be rapidly β-oxidized within liver mitochondria (52).

Values of $k^*_{i(\alpha-LNA\rightarrow DHA)}$ in the brains of the rats on the n-3 PUFA-adequate diet of this study (Table 3), which contains no DHA, were much less than in the liver (e.g., 0.0063 ml/s/g brain × 10^{-4} compared with 0.0528 ml/s/g liver × 10^{-4} in phospholipid; 0.00077 ml/s/g brain × 10^{-4} compared with 0.219 ml/s/g liver × 10^{-4} in triacylglycerol) (25–28), demonstrating that the liver is the major source of the brain's DHA in rats fed the adequate diet. Furthermore, in contrast to the lack of a deprivation effect on brain coefficients of DHA synthesis in the liver of these same rats, deprivation increased $k^*_{i(\alpha-LNA\rightarrow DHA)}$ by 6.6-, 8.4-, and 2.3-fold in triacylglycerol, phospholipid, and cholesteryl ester, respectively (26). These increases correspond to upregulated transcription of liver Δ5 and Δ6 desaturase and elongase genes in the DHA synthesis pathways (unpublished results).

Taking $\lambda_{\alpha-LNA-CoA}$ as 0.71 and 0.59 in the diet-adequate and diet-deficient rats (see Results) gave DHA synthesis rates $J_{FA,i(\alpha-LNA\rightarrow DHA)}$ equal to 2.35 and 0.104 nmol/day/g brain, respectively (equation 7). Under comparable conditions, brain DHA consumption rates are 0.257 and 0.058 μmol/day/g brain, respectively (36, 37), about 100-fold greater than the synthesis rates. Thus, with α-LNA as the only dietary n-3 PUFA, the brain cannot synthesize sufficient DHA to satisfy its requirements under either the n-3 PUFA-adequate or n-3 PUFA-deficient conditions of this study. These requirements are met by the liver (26), whose DHA secretion rates, 2.19 and 0.82 μmol/day in n-3 PUFA-adequate and n-3 PUFA-deprived rats, respectively, are 6- and 10-fold higher than the respective brain DHA consumption rates.

Our data show that the liver is a major site of DHA synthesis from α-LNA and can maintain a normal brain DHA concentration by this synthesis when the diet contains 4.6% α-LNA but no DHA (10, 53). Thus, reduced liver DHA synthesis with age or liver damage (15, 54), associated with reduced desaturase or elongase activities (55–57), may be a risk factor for brain disease in the absence of dietary DHA supplementation (14, 15).

In rats on a DHA-containing diet (2.3% of total fatty acids), $k^*_{i(\alpha-LNA\rightarrow DHA)}$ is 0.03 ml/s/g liver × 10^{-4} compared with 0.0055 ml/s/g brain × 10^{-4} in phospholipid, and 0.1 ml/s/g liver × 10^{-4} compared with 0.00040 ml/s/g brain × 10^{-4} in triacylglycerol (25–28), somewhat lower than in rats on the DHA-free n-3 PUFA-adequate diet of this study. The DHA-fed rats also had more DHA in their brain phospholipid, 17.63 ± 0.82 μmol/g brain, than did the diet-adequate rats of this study (11.29 ± 2.78 μmol/g brain) (Table 2), suggesting that high dietary DHA can inhibit both brain and liver conversion of α-LNA to DHA.

In summary, n-3 PUFA deprivation for 15 weeks following weaning did not affect coefficients for DHA synthesis from α-LNA in rat brain phospholipid or triacylglycerol,

while upregulating the coefficient for 22:5n-3 synthesis in phospholipid. Elongation of 22:5n-3 to 24:5-n-3 becomes rate limiting in the brain's ability to synthesize DHA during deprivation. The liver, in contrast, can increase its DHA synthesis-incorporation (conversion) coefficients during n-3 PUFA deprivation. In view of this and the higher synthesis-incorporation coefficients in liver than brain in both the n-3 PUFA-adequate and n-3 PUFA-deficient conditions, the liver must be the source of most brain DHA in either condition.

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REFERENCES

1. Sprecher, H. 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim. Biophys. Acta.* **1486:** 219–231.
2. Cho, H. P., M. Nakamura, and S. D. Clarke. 1999. Cloning, expression, and fatty acid regulation of the human delta-5 desaturase. *J. Biol. Chem.* **274:** 37335–37339.
3. Cho, H. P., M. T. Nakamura, and S. D. Clarke. 1999. Cloning, expression, and nutritional regulation of the mammalian delta-6 desaturase. *J. Biol. Chem.* **274:** 471–477.
4. Wang, Y., D. Botolin, B. Christian, J. Busik, J. Xu, and D. B. Jump. 2005. Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases. *J. Lipid Res.* **46:** 706–715.
5. Zheng, X., D. R. Tocher, C. A. Dickson, J. G. Bell, and A. J. Teale. 2005. Highly unsaturated fatty acid synthesis in vertebrates: new insights with the cloning and characterization of a delta6 desaturase of Atlantic salmon. *Lipids.* **40:** 13–24.
6. Innis, S. M. 2000. The role of dietary n-6 and n-3 fatty acids in the developing brain. *Dev. Neurosci.* **22:** 474–480.
7. Uauy, R., P. Mena, and C. Rojas. 2000. Essential fatty acids in early life: structural and functional role. *Proc. Nutr. Soc.* **59:** 3–15.
8. Salem, N., Jr., B. Litman, H. Y. Kim, and K. Gawrisch. 2001. Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids.* **36:** 945–959.
9. Youdim, K. A., A. Martin, and J. A. Joseph. 2000. Essential fatty acids and the brain: possible health implications. *Int. J. Dev. Neurosci.* **18:** 383–399.
10. Bourre, J. M., M. Francois, A. Youyou, O. Dumont, M. Piciotti, G. Pascal, and G. Durand. 1989. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J. Nutr.* **119:** 1880–1892.
11. Connor, W. E., M. Neuringer, and S. Reisbick. 1992. Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. *Nutr. Rev.* **50:** 21–29.
12. Salem, N., Jr., and G. R. Ward. 1993. Are omega 3 fatty acids essential nutrients for mammals? *World Rev. Nutr. Diet.* **72:** 128–147.
13. McGahon, B. M., D. S. Martin, D. F. Horrobin, and M. A. Lynch. 1999. Age-related changes in synaptic function: analysis of the effect of dietary supplementation with omega-3 fatty acids. *Neuroscience.* **94:** 305–314.
14. Conquer, J. A., M. C. Tierney, J. Zecevic, W. J. Bettger, and R. H. Fisher. 2000. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids.* **35:** 1305–1312.
15. Suzuki, H., Y. Morikawa, and H. Takahashi. 2001. Effect of DHA oil supplementation on intelligence and visual acuity in the elderly. *World Rev. Nutr. Diet.* **88:** 68–71.
16. DeMar, J. C., Jr., K. Ma, J. M. Bell, M. Igarashi, D. Greenstein, and S. I. Rapoport. 2006. One generation of n-3 polyunsaturated fatty acid deprivation increases depression and aggression test scores in rats. *J. Lipid Res.* **47:** 172–180.
17. Horrocks, L. A., and Y. K. Yeo. 1999. Health benefits of docosahexaenoic acid (DHA). *Pharmacol. Res.* **40:** 211–225.
18. Freeman, M. P. 2000. Omega-3 fatty acids in psychiatry: a review. *Ann. Clin. Psychiatry.* **12:** 159–165.
19. Mischoulon, D., and M. Fava. 2000. Docosahexanoic acid and omega-3 fatty acids in depression. *Psychiatr. Clin. North Am.* **23:** 785–794.
20. Stoll, A. L., W. E. Severus, M. P. Freeman, S. Rueter, H. A. Zboyan, E. Diamond, K. K. Cress, and L. B. Marangell. 1999. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. *Arch. Gen. Psychiatry.* **56:** 407–412.
21. Williard, D. E., S. D. Harmon, T. L. Kaduce, M. Preuss, S. A. Moore, M. E. Robbins, and A. A. Spector. 2001. Docosahexaenoic acid synthesis from n-3 polyunsaturated fatty acids in differentiated rat brain astrocytes. *J. Lipid Res.* **42:** 1368–1376.
22. Moore, S. A. 2001. Polyunsaturated fatty acid synthesis and release by brain-derived cells in vitro. *J. Mol. Neurosci.* **16:** 195–200.
23. Moore, S. A., E. Yoder, S. Murphy, G. R. Dutton, and A. A. Spector. 1991. Astrocytes, not neurons, produce docosahexaenoic acid (22:6 omega-3) and arachidonic acid (20:4 omega-6). *J. Neurochem.* **56:** 518–524.
24. Scott, B. L., and N. G. Bazan. 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc. Natl. Acad. Sci. USA.* **86:** 2903–2907.
25. DeMar, J. C., Jr., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. 2005. alpha-Linolenic acid does not contribute appreciably to docosahexaenoic acid within brain phospholipids of adult rats fed a diet enriched in docosahexaenoic acid. *J. Neurochem.* **94:** 1063–1076.
26. Igarashi, M., J. C. Demar, Jr., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. 2007. Upregulated liver conversion of alpha-linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet. *J. Lipid Res.* **48:** 152–164.
27. Igarashi, M., K. Ma, L. Chang, J. M. Bell, J. C. DeMar, Jr., and S. I. Rapoport. 2005. Alpha-linolenic acid is minimally converted to docosahexaenoic acid in brain and liver of adult rats fed a DHA-containing diet. *Soc. Neurosci. Abstr.* **35:** 188.14.
28. Contreras, M. A., R. S. Greiner, M. C. Chang, C. S. Myers, N. Salem, Jr., and S. I. Rapoport. 2000. Nutritional deprivation of alpha-linolenic acid decreases but does not abolish turnover and availability of unacylated docosahexaenoic acid and docosahexaenoyl-CoA in rat brain. *J. Neurochem.* **75:** 2392–2400.
29. Nakamura, M. T., and T. Y. Nara. 2003. Essential fatty acid synthesis and its regulation in mammals. *Prostaglandins Leukot. Essent. Fatty Acids.* **68:** 145–150.
30. Reeves, P. G., K. L. Rossow, and J. Lindlauf. 1993. Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. *J. Nutr.* **123:** 1923–1931.
31. Bourre, J. M., G. Durand, G. Pascal, and A. Youyou. 1989. Brain cell and tissue recovery in rats made deficient in n-3 fatty acids by alteration of dietary fat. *J. Nutr.* **119:** 15–22.
32. Igarashi, M., K. Ma, L. Chang, J. M. Bell, S. I. Rapoport, and J. C. Demar, Jr. 2006. Low liver conversion rate of alpha-linolenic to docosahexaenoic acid in awake rats on a high-docosahexaenoate-containing diet. *J. Lipid Res.* **47:** 1812–1822.
33. Washizaki, K., Q. R. Smith, S. I. Rapoport, and A. D. Purdon. 1994. Brain arachidonic acid incorporation and precursor pool specific activity during intravenous infusion of unesterified [3H]arachidonate in the anesthetized rat. *J. Neurochem.* **63:** 727–736.
34. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
35. Skipski, V. P., J. J. Good, M. Barclay, and R. B. Reggio. 1968. Quantitative analysis of simple lipid classes by thin-layer chromatography. *Biochim. Biophys. Acta.* **152:** 10–19.
36. Makrides, M., M. A. Neumann, R. W. Byard, K. Simmer, and R. A. Gibson. 1994. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am. J. Clin. Nutr.* **60:** 189–194.
37. DeMar, J. C., Jr., K. Ma, J. M. Bell, and S. I. Rapoport. 2004. Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids. *J. Neurochem.* **91:** 1125–1137.
38. Aveldano, M. I., M. VanRollins, and L. A. Horrocks. 1983. Separation and quantitation of free fatty acids and fatty acid methyl esters by reverse phase high pressure liquid chromatography. *J. Lipid Res.* **24:** 83–93.
39. Deutsch, J., E. Grange, S. I. Rapoport, and A. D. Purdon. 1994. Isolation and quantitation of long-chain acyl-coenzyme A esters in brain tissue by solid-phase extraction. *Anal. Biochem.* **220:** 321–323.

40. Taylor, D. C., N. Weber, L. R. Hogge, and E. W. Underhill. 1990. A simple enzymatic method for the preparation of radiolabeled erucoyl-CoA and other long-chain fatty acyl-CoAs and their characterization by mass spectrometry. *Anal. Biochem.* **184:** 311–316.
41. Robinson, P. J., J. Noronha, J. J. DeGeorge, L. M. Freed, T. Nariai, and S. I. Rapoport. 1992. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: review and critical analysis. *Brain Res. Brain Res. Rev.* **17:** 187–214.
42. Purdon, D., T. Arai, and S. Rapoport. 1997. No evidence for direct incorporation of esterified palmitic acid from plasma into brain lipids of awake adult rat. *J. Lipid Res.* **38:** 526–530.
43. Smith, Q. R., and H. Nagura. 2001. Fatty acid uptake and incorporation in brain: studies with the perfusion model. *J. Mol. Neurosci.* **16:** 167–172.
44. Rapoport, S. I. 2001. In vivo fatty acid incorporation into brain phospholipids in relation to plasma availability, signal transduction and membrane remodeling. *J. Mol. Neurosci.* **16:** 243–261.
45. Rapoport, S. I. 2005. In vivo approaches and rationale for quantifying kinetics and imaging brain lipid metabolic pathways. *Prostaglandins Other Lipid Mediat.* **77:** 185–196.
46. Sauerwald, T. U., D. L. Hachey, C. L. Jensen, H. Chen, R. E. Anderson, and W. C. Heird. 1997. Intermediates in endogenous synthesis of C22:6 omega 3 and C20:4 omega 6 by term and preterm infants. *Pediatr. Res.* **41:** 183–187.
47. Jump, D. B., D. Botolin, Y. Wang, J. Xu, B. Christian, and O. Demeure. 2005. Fatty acid regulation of hepatic gene transcription. *J. Nutr.* **135:** 2503–2506.
48. Contreras, M. A., M. C. Chang, T. A. Rosenberger, R. S. Greiner, C. S. Myers, N. Salem, Jr., and S. I. Rapoport. 2001. Chronic nutritional deprivation of n-3 alpha-linolenic acid does not affect n-6 arachidonic acid recycling within brain phospholipids of awake rats. *J. Neurochem.* **79:** 1090–1099.
49. Greiner, R. S., J. N. Catalan, T. Moriguchi, and N. Salem, Jr. 2003. Docosapentaenoic acid does not completely replace DHA in n-3 FA-deficient rats during early development. *Lipids.* **38:** 431–435.
50. Moriguchi, T., S. Y. Lim, R. Greiner, W. Lefkowitz, J. Loewke, J. Hoshiba, and N. Salem, Jr. 2004. Effects of an n-3-deficient diet on brain, retina, and liver fatty acyl composition in artificially reared rats. *J. Lipid Res.* **45:** 1437–1445.
51. Burdge, G. C., Y. E. Finnegan, A. M. Minihane, C. M. Williams, and S. A. Wootton. 2003. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [^{13}C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. *Br. J. Nutr.* **90:** 311–321.
52. Gavino, V. C., S. Cordeau, and G. Gavino. 2003. Kinetic analysis of the selectivity of acylcarnitine synthesis in rat mitochondria. *Lipids.* **38:** 485–490.
53. Bourre, J. M., O. Dumont, G. Pascal, and G. Durand. 1993. Dietary alpha-linolenic acid at 1.3 g/kg maintains maximal docosahexaenoic acid concentration in brain, heart and liver of adult rats. *J. Nutr.* **123:** 1313–1319.
54. Watanabe, A., S. Saito, T. Tsuchida, K. Higuchi, and M. Okita. 1999. Low plasma levels of docosahexaenoic acid in patients with liver cirrhosis and its correction with a polyunsaturated fatty acid-enriched soft oil capsule. *Nutrition.* **15:** 284–288.
55. Bourre, J. M., and M. Piciotti. 1992. Delta-6 desaturation of alpha-linolenic acid in brain and liver during development and aging in the mouse. *Neurosci. Lett.* **141:** 65–68.
56. Nakamura, M. T., A. B. Tang, J. Villanueva, C. H. Halsted, and S. D. Phinney. 1994. Selective reduction of delta 6 and delta 5 desaturase activities but not delta 9 desaturase in micropigs chronically fed ethanol. *J. Clin. Invest.* **93:** 450–454.
57. Gerster, H. 1998. Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)? *Int. J. Vitam. Nutr. Res.* **68:** 159–173.