

Upregulated liver conversion of α -linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet[§]

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Abstract We quantified incorporation rates of plasma-derived α -linolenic acid (α -LNA, 18:3n-3) into “stable” liver lipids and the conversion rate of α -LNA to docosahexaenoic acid (DHA, 22:6n-3) in male rats fed, after weaning, an n-3 PUFA-adequate diet (4.6% α -LNA, no DHA) or an n-3 PUFA-deficient diet (0.2% α -LNA, no DHA) for 15 weeks. Unanesthetized rats were infused intravenously with [1 - 14 C] α -LNA, and arterial plasma was sampled until the liver was micro-irradiated at 5 min. Unlabeled α -LNA and DHA concentrations in arterial plasma and liver were reduced >90% by deprivation, whereas unlabeled arachidonic acid (20:4n-6) and docosapentaenoic acid (22:5n-6) concentrations were increased. Deprivation did not change α -LNA incorporation coefficients into stable liver lipids but increased synthesis-incorporation coefficients of DHA from α -LNA by 6.6-, 8.4-, and 2.3-fold in triacylglycerol, phospholipid, and cholesteryl ester, respectively. Assuming that synthesized-incorporated DHA eventually would be secreted within lipoproteins, calculated liver DHA secretion rates equaled 2.19 and 0.82 μ mol/day in the n-3 PUFA-adequate and -deprived rats, respectively. These rates exceeded the published rates of brain DHA consumption by 6- and 10-fold, respectively, and should be sufficient to maintain normal and reduced brain DHA concentrations, respectively, in the two dietary conditions.—Igarashi, M., J. C. DeMar, Jr., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. Upregulated liver conversion of α -linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet. *J. Lipid Res.* 2007. 48: 152–164.

Supplementary key words deprivation • incorporation • turnover • synthesis • pulse labeling • diet • brain • polyunsaturated fatty acid

α -Linolenic acid (α -LNA, 18:3n-3) is a nutritionally essential PUFA that must be obtained through the diet. It can be converted in vertebrate tissue to docosahexaenoic acid (DHA, 22:6n-3) through serial steps of desaturation and elongation with final peroxisomal chain shortening (1), by means of desaturases and elongases (2–5).

Controversy exists regarding the extent of DHA synthesis from α -LNA in brain and liver. In immature rats, Scott and Bazan (6) suggested that the brain does not synthesize enough DHA to maintain its DHA composition but must receive the DHA that has been converted from α -LNA by the liver to do so. To address issues such as this, we recently developed pulse-labeling methods involving the intravenous infusion of [1 - 14 C] α -LNA to estimate conversion rates of plasma α -LNA to DHA in brain and liver of unanesthetized rats and showed that each of these organs converted <1% of their plasma-derived unesterified α -LNA to DHA (7, 8). However, on a per gram basis, in rats fed a high-DHA diet (2.3% of total fatty acids), conversion rates of plasma α -LNA to DHA were 0.24 and 6.6 nmol/s/g tissue $\times 10^{-4}$ in brain and liver, respectively (7, 9, 10). Thus, the liver has a 27.5-fold greater conversion rate per gram than does brain in such rats.

In this study, we examined the effect of altering dietary n-3 PUFA composition on liver composition and conversion rates of α -LNA to DHA in unanesthetized rats. Based on evidence that high amounts of dietary DHA or α -LNA suppress the expression in rat liver of $\Delta 5$ and $\Delta 6$ desaturases and elongases that catalyze DHA synthesis from α -LNA (1–4, 11) and that DHA synthesis from α -LNA is reduced in rat astrocytes incubated with DHA (12), we hypothesized that conversion would be upregulated in rats fed a diet with low compared with adequate n-3 PUFA content.

Abbreviations: AA, arachidonic acid (20:4n-6); DHA, docosahexaenoic acid (22:6n-3); DPA, docosapentaenoic acid (22:5); EPA, eicosapentaenoic acid (20:5n-3); FAME, fatty acid methyl ester; LA, linoleic acid (18:2n-6); α -LNA, α -linolenic acid (18:3n-3); UV, ultraviolet.

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We measured the kinetics of intravenously infused [$1\text{-}^{14}\text{C}$]α-LNA in the liver of rats fed an n-3 PUFA-adequate diet (4.6% α-LNA of total fatty acids, no DHA) or an n-3 PUFA-deficient diet (0.2% α-LNA of total fatty acids, no DHA) for 15 weeks after weaning (13–16). Compared with rats fed the adequate diet, rats on the deficient diet have a one-third reduction in their brain DHA content and abnormal scores on behavioral tests of depression and aggression (15). An abstract of part of our work has been published (9).

MATERIALS AND METHODS

Materials

[$1\text{-}^{14}\text{C}$]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 HPLC and scintillation counting). Diheptadecanoate phosphatidylcholine, free heptadecanoic acid (17:0), heptadecanoyl-CoA (17:0-CoA), and acyl-CoA standards for HPLC, as well as 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 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 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, approved by the Animal Care and Use Committee of the National Institute of Child Health and Human Development, followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 80-23). Fischer-344 (CDF) male rat pups (18 days old) and their surrogate mothers were purchased from Charles River Laboratories (Portage, MI) and 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 to rodent chow formulation NIH-31 18-4, which contained 4% crude fat (w/w) (Zeigler Bros., Gardeners, PA) and whose fatty acid composition has been reported (7, 10). α-LNA, eicosapentaenoic acid (EPA, 20:5n-3), and DHA contributed 5.1, 2.0, and 2.3% of total fatty acids, respectively, whereas n-6 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 -deficient diet groups (see below). They had free access to food and water, with their food being replaced every 2 or 3 days. Body weight was recorded every 2 or 3 days.

n-3 PUFA-adequate and -deficient diets

The n-3 PUFA-adequate and -deficient diets, whose composition is given in supplementary Table I, were prepared by Dyets, Inc. (Bethlehem, PA), based on the AIN-93G formulation (15, 17, 18). Each diet contained 10% fat. The adequate diet but not the deficient diet contained flaxseed oil. The fatty acids (μmol/g

food, percentage of total fatty acid, and percentage of food energy) in the diets are shown in supplementary Table II. To analyze each diet, total lipids were extracted from random ~0.6 g samples ($n = 3$) of each diet and methylated, and the resulting FAMES were separated by GC as described below for the liver. The n-3 PUFA-adequate diet contained 7.8 μmol/g α-LNA (4.6% total fatty acid), a minimum level for diet n-3 PUFA adequacy in rodents (13–15). The deficient diet contained 0.25 μmol/g α-LNA (0.2% total fatty acid). Other n-3 PUFAs were absent in both diets. Both contained 40 μmol/g diet 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).

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 a right femoral artery and vein (10), after which the skin was closed and treated with 1% lidocaine for pain control. The rat was loosely wrapped in a fast-setting plaster cast that was 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 (YSI Indicating Temperature Controller; Yellow Springs Instruments, Yellow Springs, OH). Surgery, which took ~20 min, was performed between 10:00 AM and 12:00 noon. Animals were provided food the night before surgery.

Radiotracer infusion

Each rat was infused via the femoral vein catheter with 500 μCi/kg [$1\text{-}^{14}\text{C}$]α-LNA (10). An aliquot of [$1\text{-}^{14}\text{C}$]α-LNA in ethanol was dried under nitrogen gas, and the residue was dissolved in 5 mM 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 of tracer at a rate of $0.223 (1 - e^{-1.92t})$ ml/min (t in min) to rapidly establish a steady-state plasma radioactivity (19). 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, the rat was euthanized by an overdose of sodium pentobarbital (100 mg/kg iv), and its head and torso were immediately subjected to high-energy focused-beam microwave irradiation (5.5 kW, 3.4 s) (model S6FI; Cober Electronics, Stamford, CT). Liver weight was recorded, and tissue samples that were confirmed visually to be browned or “cooked” were removed and stored at –80°C until analyzed. The arterial blood samples were centrifuged at 13,000 rpm for 5 min, and plasma was collected and frozen at –80°C.

Separation of plasma and liver lipid

Total liver and plasma lipids were extracted by the procedure of Folch, Lees, and Sloane Stanley (20). The aqueous extraction phases were washed once with an equal volume of chloroform to remove residual lipid, and aqueous and total lipid radioactivities were 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) (21). Authentic standards of triacylglycerol, phospholipids, cholesterol, cholesteryl ester, and unesterified fatty acids were run on the plates to identify the lipids. The plates were sprayed with 0.03% (w/v) 6-*p*-toluidine-2-naphthalene sulfonic acid in 50 mM Tris buffer (pH 7.4), and the lipid bands were visualized under ultraviolet

(UV) light. The bands were scraped, and the silica gel was used directly to quantify radioactivity by scintillation counting and to prepare FAMES (see below).

To measure total phospholipid concentration, an aliquot of total lipid extract was added to a tube and dried in a SpeedVac to prepare for digestion. To measure individual phospholipids, total lipid extracts were separated into phospholipid classes by TLC on silica gel 60 plates using chloroform-methanol-glacial acetic acid-water (60:40:1:4, v/v/v/v) (22). The bands were scraped and added to the tube. The silica gel was used directly to analyze phospholipid concentrations. The digestion was carried out by adding 0.5 ml of water and 0.65 ml of perchloric acid (70%) to all material, the scraping and dried extract, and all were digested at 180°C for 1 h (23). After the sample was cooled to room temperature, 0.5 ml of ascorbic acid (10%, w/v), 0.5 ml of ammonium molybdate (2.5%, w/v), and 3.0 ml of water were added. The mixture was boiled for 5 min to develop color, and after cooling, its absorbance was read at 797 nm. Standards for this assay were purchased from Sigma, and phospholipid concentrations were determined using standard curves.

To quantify concentrations of total and free cholesterol and triacylglycerol, lipid extract was dried using a SpeedVac, and the residue was dissolved in isopropanol. Total cholesterol and free cholesterol were determined with a commercial kit (BioVision Research Products, Mountain View, CA), as was the triacylglycerol concentration (Sigma-Aldrich).

Quantification of radioactivity

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

FAME preparation

The FAMES were analyzed by GC and HPLC. Unesterified and esterified fatty acids were methylated with 1% H₂SO₄-methanol for 3 h at 70°C (16, 24). Before the sample was methylated for GC analysis, appropriate quantities of diheptadecanoate phosphatidylcholine and 17:0 fatty acid were added as internal standards for liver lipids and plasma esterified lipids. A 17:0 fatty acid also was added as an internal standard before lipid extraction for the analysis of plasma unesterified fatty acid.

GC analysis

Fatty acid concentrations of liver and plasma 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 inner diameter, 0.25 μm film thickness) (Supelco, Bellefonte, PA) and a flame ionization detector (16). Fatty acid concentrations (nmol/g liver or nmol/ml plasma) were calculated by proportional comparison of peak areas to the area of the 17:0 internal standard.

HPLC analysis

FAMES from plasma and liver lipids were determined by HPLC by the method of Avelano, VanRollins, and Horrocks (25) with modifications. They 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 light detector (UV/VIS-151; Gilson, Middleton, WI) and an on-line continuous scintillation counter β-RAM detector (β-RAM model 2; IN/US Systems). The reverse-phase column, Luna 5 μ C18 (2) (5 μm particle size, 4.6 × 250 mm), was from Phono-

menex (Torrance, CA). 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 light detector was set at 205 nm.

HPLC profiles for FAMES (see Figs. 1 and 4 below) were pooled from all samples from each group (10 animals for the adequate diet group and 7 animals for the deficient diet group). Percentage radioactivities of [¹⁴C]α-LNA, [¹⁴C]DHA, and ¹⁴C-labeled intermediates of DHA synthesis in the liver lipid fractions (see Fig. 4 below) were determined from these HPLC profiles (see Table 6 below).

Analysis of long-chain acyl-CoAs

Long-chain acyl-CoAs were extracted from microwaved liver using an affinity chromatography method with slight modifications (26). After 5 nmol of 17:0-CoA was added as an internal standard to ~1 g of liver, the sample was homogenized in 25 mM KH₂PO₄ (Tissuemizer; Tekmar, Cincinnati, OH). The homogenate was adjusted with isopropanol and acetonitrile to isopropanol/25 mM KH₂PO₄/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 (NH₄)₂SO₄ solution was added to the homogenate to precipitate proteins, after which the sample was mixed vigorously for 5 min and centrifuged. The supernatant was washed with hexane (equal volume) to remove nonpolar lipids, then diluted with a 1.25-fold volume of 25 mM KH₂PO₄. 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 KH₂PO₄. 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 by reverse-phase column HPLC using a pump coupled with a UV/VIS detector (System Gold, model 168; Beckman). The reverse-phase column was from Waters Corp. (Milford, MA; Symmetry, 5 μm particle size, 4.6 mm × 250 mm). Chromatography was performed using a linear gradient system of 75 mM KH₂PO₄ 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 light detection was set at 260 nm for integration of concentrations and at 280 nm for identifying acyl-CoAs (260:280, 4:1) (26). 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 (27). Endogenous acyl-CoA concentrations (nmol/g liver) 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 counting. Radioactivity (nCi/g liver) was corrected for the loss by normalization against percentage recovery of the 17:0-CoA internal standard.

In this HPLC system, 14:0-CoA, α-LNA-CoA, and EPA-CoA co-eluted as a single peak (10). This peak was collected and saponified with 2% KOH/ethanol (w/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 by HPLC as described above. The FAME derivatives of 14:0, α-LNA, and EPA were completely resolved by HPLC. 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 with each other.

Calculations

The pulse-labeling equations for determining the *in vivo* kinetics of α -LNA in liver or brain, after a 5 min intravenous infusion of radiolabeled α -LNA to produce a steady-state plasma radioactivity, have been introduced elsewhere (7, 10). As illustrated in Fig. 5 below, unesterified plasma α -LNA crosses the vascular endothelium into the liver unesterified α -LNA pool (data not shown) and then is activated to α -LNA-CoA by an acyl-CoA synthetase. The α -LNA-CoA can be converted to DHA-CoA or intermediate n-3 acyl-CoAs by elongation and desaturation enzymes, transacylated into phospholipid, triacylglycerol, or cholesteryl ester (called "stable" lipids), or transferred by carnitine α -palmitoyl transferase-1 to mitochondria for β -oxidation (28). Aqueous radiolabeled β -oxidation fragments (predominantly acetyl-CoA) then can be recycled into cholesterol, saturated long-chain fatty acids, or other products. Additionally, fatty acids esterified in stable lipids can be returned to the acyl-CoA pool or can be secreted into blood within VLDLs or other types of lipoproteins (29, 30). As we are not measuring VLDL or other lipoprotein secretion directly, we will refer only to VLDLs in subsequent discussions.

After 5 min of intravenous [$1\text{-}^{14}\text{C}$] α -LNA, the incorporation coefficient $k_{i(\alpha\text{-LNA})}^*$ (ml/s/g liver), representing the transfer of unesterified [$1\text{-}^{14}\text{C}$] α -LNA from plasma to stable liver lipid *i*, was calculated as follows:

$$k_{i(\alpha\text{-LNA})}^* = \frac{c_{\text{liver},i(\alpha\text{-LNA})}^*(T)}{\int_0^T c_{\text{plasma}(\alpha\text{-LNA})}^* dt} \quad (\text{Eq. 1})$$

where $c_{\text{liver},i(\alpha\text{-LNA})}^*(T)$ (nCi/g liver) is α -LNA radioactivity in *i* at time *T* (5 min) after starting tracer infusion, *t* is the time after starting infusion, and $c_{\text{plasma}(\alpha\text{-LNA})}^*$ (nCi/ml plasma) is plasma radioactivity of unesterified α -LNA. The coefficient $k_{i(\alpha\text{-LNA} \rightarrow \text{DHA})}^*$ (ml/s/g liver), representing the synthesis of DHA from α -LNA and its subsequent incorporation into stable lipids *i*, was calculated as follows:

$$k_{i(\alpha\text{-LNA} \rightarrow \text{DHA})}^* = \frac{c_{\text{liver},i(\text{DHA})}^*(T)}{\int_0^T c_{\text{plasma}(\alpha\text{-LNA})}^* dt} \quad (\text{Eq. 2})$$

where $c_{\text{liver},i(\text{DHA})}^*(T)$ (nCi/g liver) is DHA radioactivity in *i* at time *T* (5 min). A comparable coefficient [$k_{i(\alpha\text{-LNA} \rightarrow j)}^*$] was calculated for the n-3 synthesis intermediate *j* (see Table 5 below).

The rate of incorporation of unlabeled unesterified α -LNA from plasma into liver lipid *i*, $J_{\text{in},i(\alpha\text{-LNA})}$, and the rate of DHA synthesis from α -LNA followed by DHA incorporation into *i* (nmol/s/g liver) were calculated as follows:

$$J_{\text{in},i(\alpha\text{-LNA})} = k_{i(\alpha\text{-LNA})}^* c_{\text{plasma}(\alpha\text{-LNA})} \quad (\text{Eq. 3})$$

$$J_{\text{in},i(\alpha\text{-LNA} \rightarrow \text{DHA})} = k_{i(\alpha\text{-LNA} \rightarrow \text{DHA})}^* c_{\text{plasma}(\alpha\text{-LNA})} \quad (\text{Eq. 4})$$

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

The liver can take up unesterified fatty acids from plasma by simple diffusion or by facilitated transport (31, 32) or it can take up fatty acids esterified in circulating lipoproteins via hepatocyte lipoprotein receptors by a process that may involve lipases (33–38). A "dilution factor," $\lambda_{\alpha\text{-LNA-CoA}}$, represents unesterified α -LNA flux from plasma into the liver α -LNA-CoA pool, divided by the sum of fluxes into α -LNA-CoA of unesterified and esterified plasma α -LNA and of α -LNA released (recycled) from

stable lipids (39–41). $\lambda_{\alpha\text{-LNA-CoA}}$ is given as the steady-state ratio of liver α -LNA-CoA specific activity to the specific activity of unesterified α -LNA in plasma:

$$\lambda_{\alpha\text{-LNA-CoA}} = \frac{c_{\text{liver}(\alpha\text{-LNA-CoA})}^* / c_{\text{liver}(\alpha\text{-LNA-CoA})}}{c_{\text{plasma}(\alpha\text{-LNA})}^* / c_{\text{plasma}(\alpha\text{-LNA})}} \quad (\text{Eq. 5})$$

In rats fed a high-DHA diet (2.3% of fatty acid), $\lambda_{\alpha\text{-LNA-CoA}} = 0.42 \pm 0.28$ (SD) (7).

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

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

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

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

Comparable equations can be written for the elongation and incorporation of DHA synthesis intermediates.

The net rate of synthesis followed by esterification for *i* = triacylglycerol, phospholipid, and cholesteryl ester thus equals $\sum_i J_{\text{FA},i(\alpha\text{-LNA} \rightarrow \text{DHA})}$. If we assume that this sum approximates the net rate of secretion by liver of α -LNA-derived DHA within VLDLs, $J_{\text{secretion}(\text{DHA})}$ (nmol/s/g), then we have

$$J_{\text{secretion}(\text{DHA})} \approx \sum_i J_{\text{FA},i(\alpha\text{-LNA} \rightarrow \text{DHA})} \quad (\text{Eq. 8})$$

The validity of equation 8 depends on how much DHA is recycled and metabolized rather than recycled and reincorporated into stable lipids, so that our pulse-labeling calculations are not limited by time delays in the synthesis of DHA and its n-3 precursors (see Discussion).

Statistical analysis

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

RESULTS

Body and liver weights

Mean initial body weights of the dietary adequate and deficient rats equaled 29.2 ± 2.6 and 29.4 ± 2.2 g (*P* > 0.05), respectively, whereas after 15 weeks on the diet, their weights equaled 364 ± 13 and 366 ± 19 g (*P* > 0.05), respectively. Mean liver weights (11.47 ± 0.94 and 11.92 ± 0.91 g, respectively) also did not differ significantly.

Plasma fatty acid composition

Table 1 presents plasma lipid composition in the dietary adequate and deficient groups. Before [$1\text{-}^{14}\text{C}$] α -LNA infusion, mean plasma concentrations of unesterified α -LNA in the groups equaled 27.0 ± 6.0 and 1.0 ± 0.45 nmol/ml, respectively (*P* < 0.0001), whereas after infusion, they equaled 53 ± 5.3 and 33 ± 8.4 nmol/ml, respectively. The increments above preinfusion concentrations reflected the effect of infusing [$1\text{-}^{14}\text{C}$] α -LNA with a low specific activity.

The n-3 PUFA deprivation decreased net plasma concentrations of α -LNA and DHA by 93% and 91%, respec-

TABLE 1. Fatty acid composition of plasma lipids in dietary n-3 PUFA-adequate and -deficient rats

Fatty Acid	Unesterified Fatty Acid		Phospholipid		Triacylglycerol		Cholesteryl Ester	
	Adequate	Deprived	Adequate	Deprived	Adequate	Deprived	Adequate	Deprived
<i>nmol/ml plasma</i>								
14:0	57 ± 11	64 ± 7.6	12 ± 3.9	13 ± 2.5	32 ± 24	43 ± 40	10 ± 2.4	11 ± 1.6
16:0	459 ± 108	488 ± 60	551 ± 63	522 ± 74	567 ± 322	574 ± 485	100 ± 25	92 ± 8.9
16:1n-7	84 ± 25	95 ± 15	12 ± 3.7	18 ± 4.7 ^a	62 ± 51	98 ± 93	30 ± 9.1	36 ± 6.5
18:0	96 ± 20	95 ± 8.5	809 ± 104	784 ± 125	179 ± 39	149 ± 77	33 ± 14	41 ± 19
18:1n-7	49 ± 12	56 ± 18	60 ± 13	72 ± 9.0 ^b	80 ± 45	92 ± 85	12 ± 5.5	10 ± 3.5
18:1n-9	240 ± 66	244 ± 36	67 ± 14	76 ± 13	317 ± 203	340 ± 319	63 ± 23	62 ± 8.7
18:2n-6	259 ± 49	253 ± 36	313 ± 51	255 ± 43 ^b	366 ± 212	352 ± 307	164 ± 33	162 ± 19
18:3n-3	27 ± 6.0	1.0 ± 0.45 ^c	ND	ND	23 ± 11	2.5 ± 0.93 ^c	ND	ND
20:4n-6	25 ± 4.8	34 ± 5.9 ^c	630 ± 72	730 ± 110 ^b	81 ± 38	124 ± 69	624 ± 154	710 ± 63
20:5n-3	3.3 ± 1.1	ND	8.7 ± 3.4	ND	14 ± 9.2	ND	7.3 ± 4.1	ND
22:5n-6	ND	8.7 ± 1.2	ND	107 ± 23	ND	21 ± 19	ND	ND
22:5n-3	3.6 ± 1.3	ND	11 ± 4.6	ND	4.7 ± 1.9	ND	ND	ND
22:6n-3	6.5 ± 2.6	0.23 ± 0.10 ^c	106 ± 18	11 ± 5.3 ^c	26 ± 19	1.3 ± 0.92 ^a	12.5 ± 3.6	1.3 ± 0.6 ^c
Total	1,312 ± 292	1,338 ± 171	2,600 ± 323	2,588 ± 384	1,751 ± 938	1,847 ± 1,356	1,056 ± 212	1,126 ± 63
Total n-6	285 ± 53	295 ± 41	943 ± 96	1,092 ± 166 ^b	448 ± 246	498 ± 386	788 ± 178	871 ± 61
Total n-3	40.7 ± 9.7	1.3 ± 0.4 ^c	117 ± 19	11 ± 5.3 ^c	68 ± 38	3.8 ± 0.97 ^c	20 ± 7.2	1.3 ± 0.55 ^c
Total saturated	613 ± 134	647 ± 73	1,371 ± 162	1,319 ± 197	779 ± 371	766 ± 522	143 ± 39	145 ± 16
Total monounsaturated	374 ± 101	395 ± 61	139 ± 29	166 ± 25 ^b	460 ± 296	518 ± 496	104 ± 31	108 ± 10
18:3n-3 ^d	53 ± 5.3	33 ± 8.4						

α-LNA, α-linolenic acid; ND, not detected. Values are means ± SD (n = 10 and 7 for adequate and deficient groups, respectively).

^a P < 0.01, differs significantly from mean in the adequate group.

^b P < 0.05, differs significantly from mean in the adequate group.

^c P < 0.001, differs significantly from mean in the adequate group.

^d Unesterified 18:3n-3 concentration in plasma after 5 min of intravenous [1-¹⁴C]α-LNA.

tively, and decreased the total n-3 PUFA concentration [α-LNA, EPA, DPA n-3 (22:5n-3), and DHA] by 93% (Table 1). DPA n-6 (22:5n-6) was detected in plasma of the diet deprived but not adequate rats, and unesterified and phospholipid AA concentrations were increased significantly by deprivation. The net concentration of n-6 PUFAs [LA, AA, and DPA n-6] was not changed significantly.

Plasma radioactivity

For both dietary groups, 98% of total lipid radioactivity in plasma after 5 min of [1-¹⁴C]α-LNA infusion was labeled unesterified fatty acid, with the remaining 2% in phospholipid and cholesterol (Fig. 1A). HPLC chromatograms showed that >98% of plasma fatty acid radioactivity was [1-¹⁴C]α-LNA in both groups (Fig. 1B,

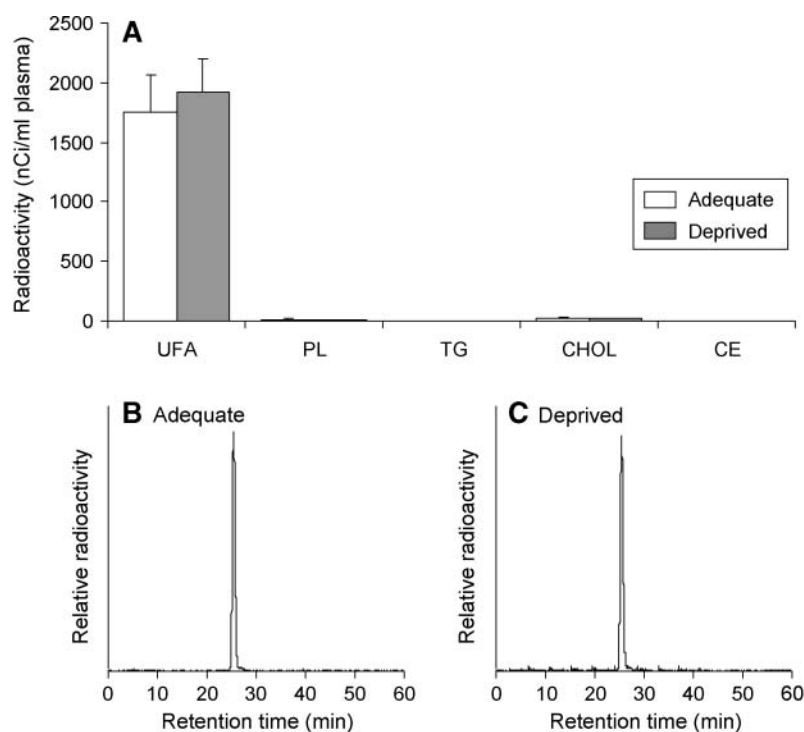


Fig. 1. Plasma lipid radioactivity in n-3 PUFA-adequate and -deficient rats after a 5 min intravenous infusion of [1-¹⁴C]α-linolenic acid (α-LNA). A: Distribution of radioactivity. B, C: HPLC chromatograms of fatty acid methyl esters (FAMES) from plasma total lipids. CE, cholesteryl ester; CHOL, cholesterol; PL, phospholipid; TG, triacylglycerol; UFA, unesterified fatty acid. Values are means ± SD. Profiles were generated from pooled samples from 10 animals for the adequate group and 7 animals for the deprived group.

C). Radioactive elongation or β -oxidation products were not detected.

Figure 2 summarizes radioactivity in the total lipid and aqueous phases of plasma during 5 min of $[1-^{14}\text{C}]\alpha$ -LNA infusion in the dietary adequate and deficient rats. Steady-state lipid radioactivity was achieved within ~ 1 min with the controlled infusion in both groups, and at 5 min, $\sim 80\%$ of plasma radioactivity was in the total lipid phase. During the 5 min infusion, the integral of radioactivity of plasma total lipids (representing 98% $[1-^{14}\text{C}]\alpha$ -LNA; see above) equaled $452,235 \pm 75,337$ and $500,384 \pm 77,308$ nCi/ml/s in the n-3 PUFA-adequate and -deficient groups, respectively. Individual values of integrated radioactivity were used to calculate $k_{i(\alpha\text{-LNA})}^*$ and $k_{i(\alpha\text{-LNA} \rightarrow \text{DHA})}^*$ by equations 1 and 2.

Unlabeled concentrations of stable liver lipids

Concentrations of stable lipids in microwaved liver of rats fed n-3 PUFA-adequate or -deficient diets are presented in supplementary Table III. Triacylglycerol and total phospholipid concentrations did not differ significantly between groups, but cholesterol (115%) and cholesteryl ester (122%) concentrations were increased by deprivation. Concentrations of phosphatidylinositol (-7%) and sphingomyelin (-19%) were decreased significantly, whereas concentrations of phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine did not differ significantly between groups.

Liver fatty acid composition

Table 2 presents liver fatty acid concentrations in the two groups. In the deficient rats, concentrations of α -LNA, EPA, DPAn-3, DHA, and total n-3 PUFAs in total lipid were reduced significantly by 95, 94, 95, 92, and 93%, respectively, compared with the respective concentrations in the adequate group. Mean AA and DPAn-6 concentrations were increased by 1.1-fold and 22-fold, respectively, whereas the 18:2n-6 concentration was decreased significantly by 17%.

Distribution of liver radioactivity

Figure 3 provides values for radioactivity in different liver compartments after 5 min of $[1-^{14}\text{C}]\alpha$ -LNA infusion. Total lipid radioactivity was calculated when ignoring

unesterified fatty acid radioactivity, as we could not distinguish intravascular from parenchymal contributions. Total lipid radioactivity was significantly higher ($3,201 \pm 545$ vs. $2,694 \pm 357$ nCi/g liver, 19%; $P < 0.05$) in the deficient than in the adequate group, as was radioactivity in phospholipid and cholesterol.

$[1-^{14}\text{C}]\alpha$ -LNA, $[^{14}\text{C}]\text{DHA}$, and $[^{14}\text{C}]\text{n-3}$ intermediates along expected pathways of the conversion of α -LNA to DHA could be detected in HPLC chromatograms of triacylglycerol (**Fig. 4A, B**), phospholipid (**Fig. 4C, D**), and cholesteryl ester (chromatogram not shown) in the adequate (**Fig. 4A, C**) and deficient (**Fig. 4B, D**) groups. Radiolabeled 14:0, 16:0, 18:0, or 18:1, which would have come from the recycling of radiolabeled carbon from $[1-^{14}\text{C}]\alpha$ -LNA, was not detected in either group.

As illustrated in **Table 3**, percentage radioactivity attributable to α -LNA in triacylglycerol, phospholipid, and cholesteryl ester was not affected significantly by dietary deprivation, whereas percentage DHA radioactivity was increased by 2.6- to 9.4-fold. Radioactive intermediates in the synthesis of DHA from α -LNA, with the exception of 18:4, also were increased.

Unlabeled liver acyl-CoA concentrations and associated radioactivity

HPLC separation of aqueous liver extract yielded unlabeled concentrations and radioactivities of individual acyl-CoA species (**Table 4**). The concentrations of unlabeled α -LNA-CoA and DHA-CoA were decreased significantly ($P < 0.05$) by deprivation, whereas the concentration of AA-CoA was increased by 184%. Radioactive 20:5n-3-CoA and DPAn-3-CoA also were increased significantly. Unlabeled concentrations of 14:0-CoA, 16:0-CoA, 18:0-CoA, 18:1-CoA, and 18:2-CoA were unaffected by deprivation, whereas concentrations of AA-CoA and DPAn-6-CoA were increased significantly. There was no group difference in labeled 14:0-CoA, 16:0-CoA, 18:0-CoA, or 18:1-CoA, markers of the recycling of radiolabeled carbon from oxidized $[1-^{14}\text{C}]\alpha$ -LNA.

Fractional distribution of liver radioactivity

Figure 5 summarizes the mean percentage disposition of radioactivity in liver compartments of dietary adequate

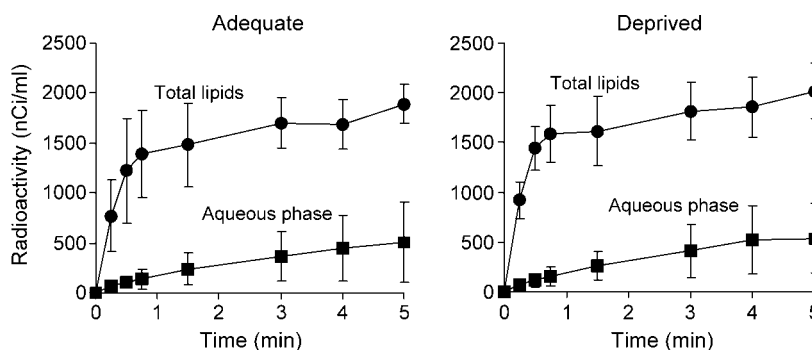


Fig. 2. Radioactivity in total lipid and aqueous phases from plasma of n-3 PUFA-adequate and -deficient rats during intravenous infusion of $[1-^{14}\text{C}]\alpha$ -LNA. $n = 10$ and 7 in the adequate and deficient groups, respectively. Values are means \pm SD.

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

Fatty Acid	Triacylglycerol		Phospholipid		Cholesteryl Ester		Unesterified Fatty Acid	
	Adequate	Deprived	Adequate	Deprived	Adequate	Deprived	Adequate	Deprived
	<i>nmol/g liver</i>							
14:0	1,627 ± 428	1,969 ± 398	194 ± 30	211 ± 12	7.7 ± 6.0	14 ± 7.0 ^a	14 ± 14	8.0 ± 2.9
16:0	22,685 ± 6,840	27,150 ± 5,423	11,050 ± 1,013	10,632 ± 731	158 ± 38	255 ± 65 ^b	185 ± 89	146 ± 78
16:1n-7	4,059 ± 1,602	5,597 ± 1,436 ^a	581 ± 98	694 ± 49 ^c	51 ± 29	103 ± 29 ^c	14 ± 10	14 ± 7.6
18:0	1,295 ± 268	1,468 ± 297	17,224 ± 1,590	17,174 ± 1797	43 ± 15	62 ± 18 ^c	99 ± 43	84 ± 59
18:1n-7	2,624 ± 566	4,125 ± 1,768 ^a	1,848 ± 237	2,256 ± 208 ^c	46 ± 19	60 ± 15	24 ± 15	27 ± 20
18:1n-9	15,545 ± 4,601	17,403 ± 3,921	1,411 ± 153	1,452 ± 154	131 ± 41	234 ± 64 ^b	59 ± 34	47 ± 22
18:2n-6	13,309 ± 3,196	11,528 ± 1,707	6,734 ± 860	5,220 ± 508 ^b	118 ± 41	89 ± 31	78 ± 51	50 ± 29
18:3n-3	1,005 ± 314	40 ± 7.8 ^b	58 ± 12	14 ± 1.8 ^b	6.0 ± 2.2	0.77 ± 0.27 ^b	6.7 ± 5.6	0.57 ± 0.55 ^c
20:4n-6	1,042 ± 323	15,73 ± 327 ^c	18,452 ± 1,935	20,646 ± 1,539 ^a	167 ± 54	214 ± 47 ^a	42 ± 31	51 ± 30
20:5n-3	233 ± 69	11 ± 3.2 ^b	296 ± 65	21 ± 15 ^b	2.9 ± 1.1	ND	4.4 ± 2.4	ND
22:5n-6	47 ± 31	293 ± 72 ^b	194 ± 27	5,045 ± 482 ^b	0.80 ± 0.023	12 ± 4.4 ^b	0.64 ± 0.33	8.3 ± 5.0 ^c
22:5n-3	390 ± 186	10 ± 2.0 ^b	851 ± 114	48 ± 6.4 ^b	1.2 ± 0.7	ND	6.2 ± 3.7	ND
22:6n-3	470 ± 145	12 ± 3.1 ^b	5,977 ± 631	509 ± 57 ^b	7.4 ± 2.3	0.31 ± 0.21 ^b	15 ± 8.3	0.65 ± 0.40 ^b
Total	64,205 ± 15,796	71,270 ± 13,642	64,871 ± 5,579	63,921 ± 4,709	739 ± 187	1,081 ± 157 ^c	549 ± 285	430 ± 245
Total n-6	14,398 ± 3,454	13,394 ± 1,975	25,381 ± 2,395	30,911 ± 2,296 ^c	285 ± 71	316 ± 41	121 ± 76	110 ± 60
Total n-3	2,098 ± 684	73 ± 14 ^b	7,183 ± 642	592 ± 73 ^b	17 ± 5.2	1.1 ± 0.45 ^b	22 ± 18	1.2 ± 0.79 ^b
Total saturated	25,608 ± 7,332	30,588 ± 6,009	28,438 ± 2,445	28,016 ± 2,376	109 ± 47	332 ± 72 ^b	299 ± 138	238 ± 135
Total monounsaturated	22,228 ± 6,499	27,125 ± 5,636	3,840 ± 409	4,402 ± 204 ^c	228 ± 70	397 ± 105 ^b	97 ± 57	89 ± 48

ND, not detected. Values are means ± SD (n = 10 and 7 for adequate and deficient groups, respectively).

^a *P* < 0.05, differs significantly from mean in the adequate group.

^b *P* < 0.001, differs significantly from mean in the adequate group.

^c *P* < 0.01, differs significantly from mean in the adequate group.

and deprived rats after 5 min of [1-¹⁴C]α-LNA infusion. Of net radioactivity in the adequate rats, 78.0% was in the lipid fraction and 22.0% was in the aqueous phase. Of stable lipid radioactivity, 58.6% was in triacylglycerol, 12.7% was in phospholipid, 5.7% was in cholesterol, 0.5% was in cholesteryl ester, and 0.5% was in other lipids. Of cholesteryl ester radioactivity (0.5%), the sterol body and acyl chain components contained 0.2% and 0.3%, respectively. Of fatty acid radioactivity in stable lipids, [1-¹⁴C]α-LNA, [1-¹⁴C]DHA, and [1-¹⁴C]n-3 synthesis intermediates constituted 50.1, 0.3, and 21.8%, respectively, whereas radiolabeled α-LNA-CoA, EPAn-3-CoA + DPAn-3-CoA, and DHA-CoA contributed 0.1, 0.02, and 0.05%, respectively.

Of total liver radioactivity in the n-3 PUFA-deficient group, 80.5% was in the lipid fraction and 19.5% was in the aqueous phase. Of stable lipid radioactivity, 57.8% was in triacylglycerol, 13.3% was in phospholipid, 8.5% was in cholesterol, 0.4% was in cholesteryl ester, and 0.4% was in other lipids (Fig. 5). Of cholesteryl ester radioactivity (0.4%), the sterol body and acyl chain components contained 0.2% and 0.2%, respectively. Of percentage fatty acid radioactivity within stable lipids, [1-¹⁴C]α-LNA, [1-¹⁴C]DHA, and [1-¹⁴C]n-3 acyl intermediates involved in [1-¹⁴C]DHA synthesis constituted 42.3, 1.9, and 27.7%, respectively, of net liver lipid radioactivity, whereas radiolabeled α-LNA-CoA, EPA-CoA, and DHA-CoA contributed 0.1, 0.06, and 0.07%, respectively.

In both dietary groups, radioactive saturated and monounsaturated fatty acids were not detected (Table 3), but 0.4% of net radioactivity was in saturated and monounsaturated acyl-CoA species (14:0, 16:0, 18:0, and 18:1n-9).

α-LNA incorporation and synthesis-incorporation coefficients and rates

Using individual experimental values for integrated plasma radioactivity, we calculated by equation 1 that the incorporation coefficient $k_{i(\alpha\text{-LNA})}^*$ of plasma-derived unesterified α-LNA into liver triacylglycerol, phospholipid, and cholesteryl ester was not significantly changed by n-3 PUFA deprivation (Table 5). By equation 2, we calculated that $k_{i(\alpha\text{-LNA} \rightarrow \text{DHA})}^*$, the coefficient for the conversion of α-LNA to esterified DHA in these stable lipids, was increased by 6.6-, 8.4-, and 2.3-fold, respectively. The conversion-incorporation coefficient $k_{i(\alpha\text{-LNA} \rightarrow j)}^*$ [*j* = 20:4n-3, 20:5n-3, and 22:5n-3] also was increased significantly by deprivation (Table 5).

Using unesterified plasma α-LNA concentrations before [1-¹⁴C]α-LNA infusion in equations 3 and 4, values for $\sum_i J_{in,i(\alpha\text{-LNA})}$ (summed over *i* = triacylglycerol, phospholipid, and cholesteryl ester) equaled 911 and 28.5 nmol/s/g × 10⁻⁴ (deprived-adequate ratio = 0.031) in the dietary adequate and deficient rats, respectively, whereas $\sum_i J_{in,i(\alpha\text{-LNA} \rightarrow \text{DHA})}$ equaled 7.45 and 1.99 nmol/s/g × 10⁻⁴ (ratio = 0.26). The higher ratio during deprivation reflects the increased conversion coefficient $k_{i(\alpha\text{-LNA} \rightarrow \text{DHA})}^*$ but no change in $k_{i(\alpha\text{-LNA})}^*$ (see above).

$\lambda_{\alpha\text{-LNA-CoA}}$, the ratio of steady-state liver α-LNA-CoA specific activity to plasma α-LNA specific activity (equation 5), equaled 0.35 ± 0.26 and 0.24 ± 0.11, respectively, in the n-3 PUFA-adequate and -deprived rats at the end of the 5 min tracer infusion. These values likely were reached within 1 min after starting the programmed [1-¹⁴C]α-LNA infusion, when plasma lipid radioactivity had reached an approximate plateau (Fig. 2). Indeed, in a previous study,

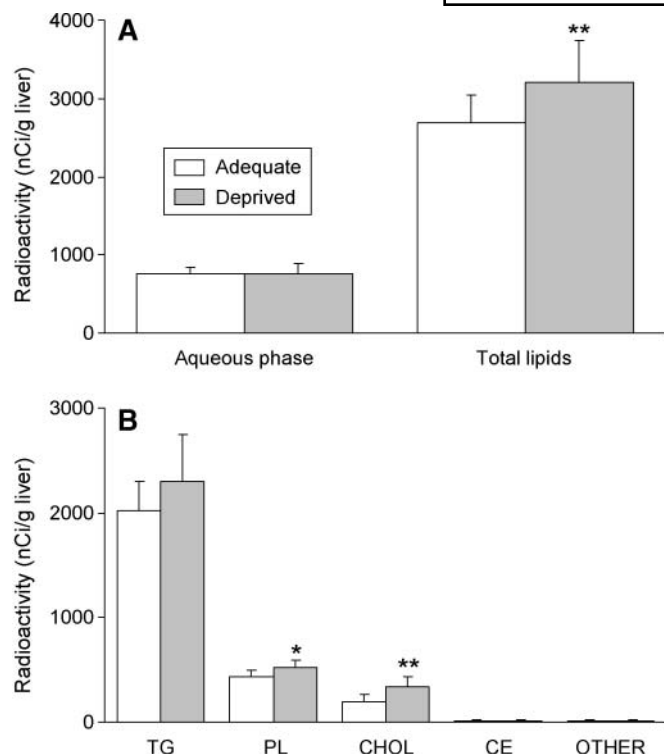


Fig. 3. Radioactivity in liver lipids in n-3 PUFA-adequate and -deficient rats after intravenous infusion of [$1\text{-}^{14}\text{C}$] α -LNA for 5 min. A: Radioactivities of total lipid and aqueous phases in both groups. B: Radioactivity in individual lipids. CE, cholesteryl ester; CHOL, cholesterol; PL, phospholipid; TG, triacylglycerol. Values are means \pm SD ($n = 10$ and 7 in the adequate and deficient groups, respectively). * $P < 0.05$, ** $P < 0.01$, significant difference of means.

we showed that $\lambda_{\alpha\text{-LNA-CoA}}$ did not differ significantly between 3 and 5 min of infusion (7). Therefore, we used 0.35 and 0.24 for $\lambda_{\alpha\text{-LNA-CoA}}$ in equation 7 for the diet-adequate and -deprived rats, respectively, to obtain net synthesis rates of DHA, $\sum_i J_{\text{FA},i(\alpha\text{-LNA}\rightarrow\text{DHA})}$, equal to $7.45/0.35 = 21.3$ and $1.99/0.24 = 8.3 \text{ nmol/s/g} \times 10^{-4}$, respectively. Multiplying these rates by the respective liver weights of 11.9 and 11.5 g gave net daily synthesis rates of 2.19 and 0.82 $\mu\text{mol/day}$, respectively.

DISCUSSION

We used our published pulse-labeling method (7) to quantify the distribution of intravenously infused [$1\text{-}^{14}\text{C}$] α -LNA in different liver compartments of unanesthetized rats fed an n-3 PUFA-adequate or -deprived diet for 15 weeks after weaning. From these data, we estimated rates of α -LNA delivery from plasma to different liver compartments, including synthesis rates of DHA from α -LNA.

Rats fed the deficient compared with the adequate diet had significant reductions in n-3 PUFA concentrations in the plasma and liver unesterified and esterified fatty acid pools (Tables 1, 2) and increased liver concentrations of cholesterol and cholesteryl ester but decreased concentra-

tions of phosphatidylinositol and sphingomyelin (see supplementary Table III). Concentrations of esterified n-6 PUFAs (20:4n-6 and 22:5n-6) were increased in both plasma and liver (Tables 1, 2). The concentration of AA (20:4n-6)-CoA was increased by 184%, and DPA (22:5n-6)-CoA was easily measured in the liver (Table 4), consistent with reports of upregulated AA elongation to DPA n-6 in tissues of rats subjected to n-3 PUFA deprivation (15, 16, 42–44).

A major finding of this study is that dietary deprivation increased the coefficient $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^*$, which represents the conversion of unesterified plasma α -LNA to esterified liver DHA, by 6.6-, 8.4-, and 2.3-fold for $i = \text{triacylglycerol}$, phospholipids, and cholesteryl ester (Table 5). Values of $k_{i(\alpha\text{-LNA}\rightarrow j)}^*$ for the n-3 synthesis intermediates $j = 20:4\text{n-3}$, $20:5\text{n-3}$, and $22:5\text{n-5}$ also were increased significantly. Consistent with upregulated conversion, radiolabeled intermediates in the conversion of α -LNA to DHA along the pathway $\alpha\text{-LNA}$ (18:3) \rightarrow 18:4 \rightarrow 20:4 \rightarrow 20:5 \rightarrow 22:5 \rightarrow 24:5 \rightarrow 24:6 \rightarrow DHA (22:6) (1, 45) were increased in the deficient rats (Table 3). An additional pathway of α -LNA metabolism exists, in which 20:3n-3, 22:4n-3, and 24:4n-3 intermediates are converted to 24:5n-3 and then to DHA in the usual manner (46), and we also detected increased radiolabeled 20:3n-3 from this pathway in stable liver lipid (Fig. 4, Table 3).

These results, which show that n-3 PUFA deprivation caused the liver to upregulate its ability to convert plasma-derived α -LNA to DHA and its n-3 synthesis intermediates, are consistent with preliminary evidence (M. Igarashi, unpublished results) that the transcription of $\Delta 5$ and $\Delta 6$ desaturase and elongase genes also is upregulated in the liver of n-3 PUFA-deprived compared with -adequate rats, and with data that feeding rats a diet high in DHA or α -LNA downregulates the transcription of liver $\Delta 5$ and $\Delta 6$ desaturase and elongase genes (3) as well as the liver expression of sterol-regulatory element binding protein-1, which positively affects their transcription (47).

Multiplying the coefficients $k_{i(\alpha\text{-LNA})}^*$ and $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^*$ by preinfusion plasma concentrations of unesterified α -LNA (equations 3, 4) showed that the incorporation rate of unesterified plasma α -LNA, $J_{\text{in},i(\alpha\text{-LNA})}$, was decreased by 97% into triacylglycerol, phospholipid, and cholesteryl ester, whereas the conversion-incorporation rate of α -LNA to DHA, $J_{\text{in},i(\alpha\text{-LNA}\rightarrow\text{DHA})}$, was decreased by 68–91% (Table 5). The lesser percentage decreases in $J_{\text{in},i(\alpha\text{-LNA}\rightarrow\text{DHA})}$ than in $J_{\text{in},i(\alpha\text{-LNA})}$ reflected the upregulated synthesis-incorporation coefficients $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^*$ in animals on the deficient diet (see above), with no change in the α -LNA incorporation coefficients $k_{i(\alpha\text{-LNA})}^*$. It can be seen that the decreased conversion-incorporation rate $J_{\text{in},i(\alpha\text{-LNA}\rightarrow\text{DHA})}$, despite an upregulated capacity for conversion, reflected the markedly reduced plasma α -LNA concentrations in the deprived rats (Table 1). The effects of lesser degrees of dietary n-3 PUFA deprivation might be studied to determine the lowest plasma α -LNA concentration at which upregulated values of $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^*$ can still maintain normal DHA synthesis rates.

We estimated that the liver of the diet-adequate and -deficient rats synthesized DHA derived from plasma

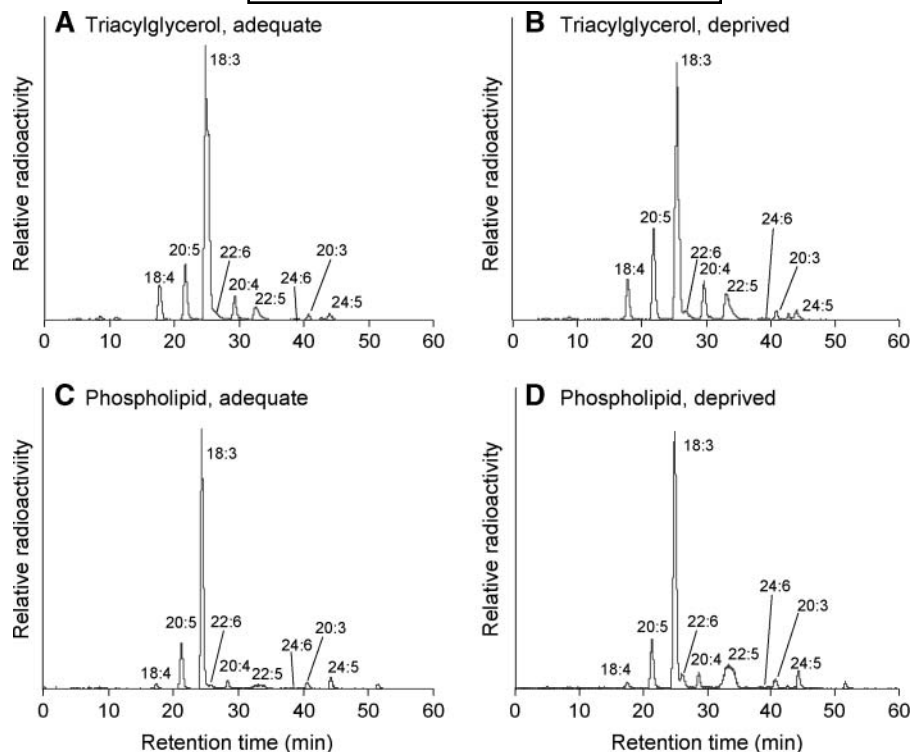


Fig. 4. HPLC chromatograms of FAMES from triacylglycerol radioactivity (A, B) and phospholipid radioactivity (C, D) in n-3 PUFA-adequate (A, C) and n-3 PUFA-deficient (B, D) rats after a 5 min intravenous infusion of [1-¹⁴C]α-LNA. Samples from 10 animals for the adequate group and 7 animals for the deprived group were pooled to generate profiles.

unesterified and esterified α-LNA (using $\lambda_{\alpha\text{-LNA-CoA}}$ in equation 7 takes both forms into account) at respective net rates of $\sum J_{\text{FA},i(\alpha\text{-LNA} \rightarrow \text{DHA})} = 2.19$ and $0.82 \mu\text{mol/day}$. We propose (equation 8) that these synthesis rates approximate the rates of DHA secretion within VLDLs. The validity of this proposal depends on the extent to which DHA recycled from stable liver lipids will be reincorporated into them rather than degraded, and on the extent to which the 5 min pulse-labeling condition approximates

the steady-state uptake-secretion condition. Indeed, our secretion rates may be underestimates, because VLDL secretion requires the translocation of apolipoprotein B across the endoplasmic reticulum membrane (29), which is slow compared with the 5 min tracer infusion period [only slight radioactivity (2% of total) appeared in stable plasma lipids after infusion (Fig. 1)]. Furthermore, we did not take into account time-dependent substrate-product relations in the serial desaturation and elongation reactions involving the conversion of α-LNA to DHA (41, 48), nor did we consider that n-3 synthesis intermediates during [1-¹⁴C]α-LNA infusion could later have been converted to esterified DHA. We also approximated values for $\lambda_{\alpha\text{-LNA-CoA}}$ in relation to changing plasma levels of unesterified α-LNA during tracer perfusion. More accurate secretion rates of DHA derived from plasma α-LNA might be determined using longer intravenous [1-¹⁴C] α-LNA infusions or in long-term in situ or in vivo liver perfusion studies (48–51).

In our n-3 PUFA-adequate and -deprived rats, brain DHA consumption rates determined by measuring DHA half-lives in phospholipid after intracerebral injection of [4,5-³H]DHA are 0.25 and 0.06 $\mu\text{mol/g/day}$ (0.38 and 0.08 $\mu\text{mol/day}$ for 1.5 g of brain) (16). Thus, our estimated liver DHA secretion rates within VLDLs, 2.19 and 0.82 $\mu\text{mol/day}$, respectively, are 6- and 10-fold higher than the respective reported brain DHA consumption rates and should be sufficient to maintain brain DHA con-

TABLE 3. Percentage total fatty acid radioactivity in stable liver lipids from n-3 PUFA-adequate and -deficient rats

Fatty Acid	Triacylglycerol		Phospholipid		Cholesteryl Ester	
	Adequate	Deprived	Adequate	Deprived	Adequate	Deprived
% of total						
18:3n-3	56.2	44.2	70.8	57.5	92.1	89.2
18:4n-3	10.3	8.5	1.7	1.3	0.9	0.9
20:4n-3	7.0	8.9	2.3	3.1	1.4	2.0
20:5n-3	15.4	19.4	15.2	10.7	2.0	3.4
22:5n-3	6.4	10.5	3.8	16.8	0.9	1.3
24:5n-3	2.3	2.6	3.6	3.6	1.4	1.5
24:6n-3	0.2	0.4	0.1	0.1	—	—
22:6n-3	0.5	3.1	0.5	4.1	0.4	0.9
20:3n-3	1.5	1.7	2.0	2.0	1.0	0.8
Others	0.3	0.8	—	0.4	—	—

Analysis of fatty acid methyl esters from pooled samples from 10 adequate and 7 deficient rats.

TABLE 4. Acyl-CoA concentrations and radioactivity in liver from n-3 PUFA-adequate and -deficient rats after 5 min of [1^{14} C] α -LNA infusion

Acyl-CoA	Concentration		Radioactivity	
	Adequate	Deprived	Adequate	Deprived
	<i>nmol/g liver</i>		<i>nCi/g liver</i>	
18:3n-3-CoA	0.75 \pm 0.52	0.35 \pm 0.15 ^a	6.0 \pm 3.0	5.8 \pm 4.0
Eicosapentaenoic acid (20:5n-3)-CoA	0.52 \pm 0.27	0.35 \pm 0.13	1.3 \pm 0.76	5.4 \pm 4.5 ^b
DPA(22:5n-3)-CoA	1.12 \pm 0.48	<0.47	2.2 \pm 1.7	4.1 \pm 2.6 ^a
DHA(22:6n-3)-CoA	1.5 \pm 0.73	0.54 \pm 0.23 ^a	1.4 \pm 0.81	2.6 \pm 1.8
14:0-CoA	1.5 \pm 1.5	0.98 \pm 0.57	3.4 \pm 1.1	4.4 \pm 1.9
16:0-CoA	11.7 \pm 9.5	9.8 \pm 4.8	3.6 \pm 2.1	4.1 \pm 2.2
18:0-CoA	7.6 \pm 4.4	10.7 \pm 6.6	3.8 \pm 2.4	4.0 \pm 2.3
18:1-CoA	22.9 \pm 14.5	20.5 \pm 14.9	5.4 \pm 2.9	7.0 \pm 4.2
18:2n-6-CoA	6.6 \pm 4.0	6.1 \pm 5.2		
20:4n-6-CoA	17.7 \pm 6.6	32.5 \pm 16.8 ^a		
DPA(22:5n-6)-CoA	<0.52	2.5 \pm 1.7		

DHA, docosahexaenoic acid; DPA, docosapentaenoic acid. Values are means \pm SD (n = 10 and 7 for adequate and deficient groups, respectively).

^a P < 0.05, differs significantly from mean in the adequate group.

^b P < 0.01, differs significantly from mean in the adequate group.

centrations, which are reported to be 12.0 and 7.6 μ mol/g brain under the two dietary conditions, respectively (16).

We also do not know how much of the DHA secreted within VLDLs will ultimately be taken up by brain, although some of the DHA taken up may have been derived from residual body stores. VLDL receptors are found on brain capillaries, but in vivo feeding and infusion studies indicate that long-chain fatty acids within plasma are largely taken up by brain in their unesterified form and not within lipoproteins (52, 53). Thus, DHA within secreted VLDLs would first have to be hydrolyzed to unesterified DHA in adipose tissue, in the circulation, or at the blood-brain barrier by appropriate lipases before being incorporated into brain (54–56). With regard to unesteri-

fied circulating DHA, tracer injection studies suggest that \sim 0.5% and \sim 4.6% of the intravenously injected amount will be taken up by brain in adult rats and fetal baboons, respectively (57, 58).

In a previous study in rats on a high-DHA diet, we suggested that the conversion rates of plasma α -LNA to DHA by liver might be insufficient to supply significant DHA to the brain (7). However, we did not use the extended model of this paper, nor did we take into account the relative weights of the two organs. Doing so now leads us to conclude that our estimated liver conversion-secretion rate is quite high compared with the brain consumption rate and might be sufficient to maintain a normal brain DHA concentration in rats fed

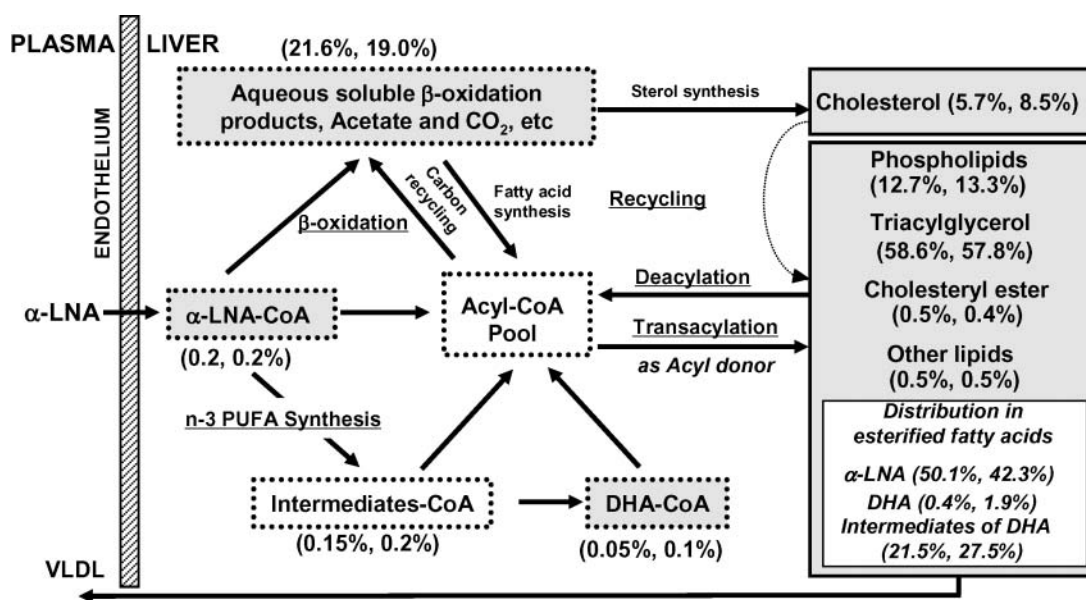


Fig. 5. Percentage radioactivity in liver lipid compartments of rats after a 5-min intravenous infusion of [1^{14} C] α -LNA. Percentage was calculated by dividing radioactivity in each compartment by net liver (excluding unesterified fatty acid) radioactivity. The back arrow to blood represents secretion within VLDLs. In parentheses, the left number is for the n-3 PUFA-adequate group and the right number is for the n-3 PUFA-deprived group. DHA, docosahexaenoic acid.

TABLE 5. Radioactivity, incorporation coefficients, and incorporation rates of unesterified plasma α -LNA into stable liver lipids in n-3 PUFA-adequate and -deficient rats

Variable	Radioactivity		Incorporation Coefficients, k_i^*		Incorporation rates, $J_{in,i}$	
	Adequate	Deprived	Adequate	Deprived	Adequate	Deprived
	<i>nCi/g liver</i>		<i>ml/s/g $\times 10^{-4}$</i>		<i>nmol/s/g $\times 10^{-4}$</i>	
Total lipids						
α -LNA (18:3n-3)	1,731 \pm 220	1,878 \pm 320				
DHA(22:6n-3)	11 \pm 1.5	85 \pm 15 ^a				
Triacylglycerol						
α -LNA	1,137 \pm 156	1,019 \pm 194	26.1 \pm 7.14	20.7 \pm 4.51	709 \pm 244	21.7 \pm 9.99 ^a
DHA	9.55 \pm 1.31	71.5 \pm 13.6 ^a	0.219 \pm 0.0601	1.45 \pm 0.318 ^a	5.97 \pm 2.05	1.52 \pm 0.699 ^a
18:4n-3	209 \pm 28.7	196 \pm 37.4	4.80 \pm 1.32	3.97 \pm 0.872		
20:4n-3	143 \pm 19.5	204 \pm 39.0 ^a	3.27 \pm 0.896	4.14 \pm 0.909 ^b		
20:5n-3	312 \pm 42.7	446 \pm 85.1 ^a	7.15 \pm 1.96	9.03 \pm 1.99 ^b		
22:5n-3	129 \pm 17.6	241 \pm 46.0 ^a	2.95 \pm 0.809	4.89 \pm 1.07 ^a		
24:5n-3	45.9 \pm 6.29	59.8 \pm 11.4 ^c	1.05 \pm 0.289	1.21 \pm 0.266		
24:6n-3	4.66 \pm 0.638	6.16 \pm 1.18 ^c	0.107 \pm 0.0293	0.125 \pm 0.0274		
20:3n-3	29.6 \pm 4.06	38.5 \pm 7.34 ^c	0.680 \pm 0.187	0.779 \pm 0.171		
Phospholipid						
α -LNA	310 \pm 43.5	301 \pm 41.8	7.16 \pm 2.09	6.19 \pm 1.75	195 \pm 76.9	6.57 \pm 3.17 ^a
DHA	2.29 \pm 0.322	21.6 \pm 3.00 ^a	0.0528 \pm 0.0155	0.444 \pm 0.126 ^a	1.45 \pm 0.569	0.471 \pm 0.228 ^a
Cholesteryl ester						
α -LNA	14.2 \pm 5.52	11.9 \pm 4.19	0.280 \pm 0.121	0.236 \pm 0.0593	7.40 \pm 3.48	0.245 \pm 0.103 ^a
DHA	0.0476 \pm 0.0213	0.127 \pm 0.0446 ^c	0.00108 \pm 0.000466	0.00252 \pm 0.000632 ^a	0.0285 \pm 0.0134	0.00261 \pm 0.0110 ^a

Values are means \pm SD (n = 10 and 7 for adequate and deficient groups, respectively). k_i^* represents $k_{i(\alpha\text{-LNA})}^*$, $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^*$, or $k_{i(\alpha\text{-LNA}\rightarrow j)}^*$, where i is stable lipid and j is intermediate in DHA synthesis (see text).

^a $P < 0.001$, differs significantly from mean in the adequate group.

^b $P < 0.05$, differs significantly from mean in the adequate group.

^c $P < 0.01$, differs significantly from mean in the adequate group.

an adequate diet containing α -LNA but not other n-3 PUFAs (13–16).

Our calculated values for $k_{i(\alpha\text{-LNA})}^*$ in the diet-adequate rats are roughly comparable to values in rats fed a high-DHA-containing diet (2.3% of total fatty acid), in which $k_{i(\alpha\text{-LNA})}^*$ equaled 22.5 ± 7.7 ml/s/g $\times 10^{-4}$ for triacylglycerol, 7.6 ± 2.2 ml/s/g $\times 10^{-4}$ for phospholipid, and 0.1 ± 0.02 ml/s/g $\times 10^{-4}$ for cholesteryl ester (7); our values for the incorporation rate $J_{in,i(\alpha\text{-LNA})}$ are also roughly comparable (reported values were $1,000 \pm 622$, 312 ± 94 , and 3.7 ± 1.9 nmol/s/g $\times 10^{-4}$, respectively). Our values for $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^*$ are somewhat higher than the respective published values in high-DHA diet rats, 0.1 ± 0.04 , 0.03 ± 0.01 , and 0.0001 ± 0.00002 ml/s/g $\times 10^{-4}$ (7), agreeing with evidence that high dietary DHA suppresses the expression of enzymes involved in liver DHA synthesis from α -LNA (see below and Introduction). Unesterified plasma concentrations of α -LNA and DHA in the high-DHA diet-fed rats were 41.3 ± 13.2 and 26.0 ± 12.2 nmol/ml, respectively (7, 10), compared with 27 ± 6.0 and 6.5 ± 3.6 nmol/ml with the adequate diet in this study. Our value for $\sum J_{FA,i(\alpha\text{-LNA}\rightarrow\text{DHA})}$ of 21.3 nmol/s/g $\times 10^{-4}$ in the n-3 adequate rats is 1.3-fold higher than the 15.8 nmol/s/g $\times 10^{-4}$ in high-DHA diet rats (7). As liver weight in those rats was 10 g (10), their liver secreted α -LNA-derived DHA at a rate of 1.37 μ mol/day, 60% of the 2.19 μ mol/day in the n-3 PUFA-adequate rats of this study. A lower rate is expected because of the inhibitory effect of DHA on liver conversion (3, 4).


Unlabeled total cholesterol in liver was increased by 2-fold (see supplementary Table III) in the n-3 PUFA-deficient rats, suggesting upregulated cholesterol synthe-

sis. This is consistent with evidence that α -LNA feeding in rats produced hypocholesterolemia and repressed the activity and mRNA expression of liver 3-hydroxy-3-methylglutaryl-CoA reductase, an enzyme involved in cholesterol synthesis (59).

Twenty-eight percent of liver radioactivity at 5 min represented oxidation products in both the adequate and deprived rats, compared with 30% in rats fed a high-DHA-containing diet (7). These are lower estimates for the oxidation fraction, because [14 C]CO₂ likely was lost through respiration. α -LNA is reported to be β -oxidized more rapidly than other C18 fatty acids in whole body studies and to be transported more rapidly than DHA by carnitine *O*-palmitoyl transferase-1 into liver mitochondria (28, 60).

In summary, we have estimated coefficients and rates of incorporation of plasma α -LNA into stable liver lipids, as well as coefficients and rates of DHA synthesis from plasma α -LNA, in unanesthetized rats fed an n-3 PUFA-adequate or -deprived diet. Deprivation decreased the n-3 PUFA content of liver while increasing coefficients for the synthesis of DHA and its synthesis intermediates. The estimated liver DHA secretion rates exceed published rates of unesterified DHA consumption by brain by 6- and 10-fold in the diet-adequate and -deprived rats, respectively, and should be sufficient to maintain normal and reduced brain DHA concentrations, respectively, in the two dietary conditions.

Our method provides a basis for comparing the effects of different experimental conditions on in vivo rates of liver metabolism and the synthesis of DHA from plasma α -LNA or other plasma precursors, including EPA (20:5n-3)

(61, 62). This issue is particularly important because of controversy concerning the extent to which dietary α -LNA and EPA ω -3 can substitute for DHA in therapeutic trials in certain human brain disorders (63, 64). Our method might also be used to estimate liver α -LNA metabolism in animal models in which α -LNA conversion to DHA would be expected to be disturbed (e.g., models of type I diabetes, peroxisomal disorders, alcoholism, and dietary changes) (34, 65, 66) as well as during fetal and postnatal development (6, 67) 

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