

Whole-body synthesis-secretion rates of long-chain n-3 PUFAs from circulating unesterified α -linolenic acid in unanesthetized rats

Fei Gao,^{1,*} Dale Kiesewetter,[†] Lisa Chang,^{*} Kaizong Ma,^{*} Jane M. Bell,^{*} Stanley I. Rapoport,^{*} and Miki Igarashi^{*}

Brain Physiology and Metabolism Section,^{*} National Institute on Aging, and Positron Emission Tomography Radiochemistry Group,[†] National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD 20892

Abstract Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), long-chain n-3 PUFAs important for brain and heart function, can be obtained from dietary fish products or by liver synthesis from α -linolenic acid (α -LNA). Their daily human dietary requirements are not clear, and their liver synthesis rates in humans and nonhumans are unknown. We estimated whole-body (presumably liver) synthesis rates in unanesthetized rats by infusing [U - ^{13}C] α -LNA intravenously for 2 h and measuring labeled and unlabeled n-3 PUFA in arterial plasma using negative chemical ionization GC-MS. Newly synthesized esterified [^{13}C]DHA, [^{13}C]EPA, and [^{13}C]docosapentaenoic acid (DPA) appeared in arterial plasma after 60 min of infusion, then their concentrations rose in an S-shaped manner. Esterified concentration \times plasma volume data were fit with a sigmoidal equation, whose peak first derivatives provided synthesis rates of unlabeled EPA, DPA, and DHA equal to 8.40, 6.27, and 9.84 $\mu\text{mol}/\text{day}$, respectively. The DHA synthesis rate exceeded the published daily rat brain DHA consumption rate by 30-fold, suggesting that liver synthesis from α -LNA could maintain brain DHA homeostasis were DHA absent from the diet. **■** This stable isotope infusion method could be used to quantify whole-body DHA synthesis rates in human subjects.—Gao, F., D. Kiesewetter, L. Chang, K. Ma, J. M. Bell, S. I. Rapoport, and M. Igarashi. Whole-body synthesis-secretion rates of long-chain n-3 PUFAs from circulating unesterified α -linolenic acid in unanesthetized rats. *J. Lipid Res.* 2009. 50: 749–758.

Supplementary key words docosahexaenoic acid • stable isotopes • liver • esterified • rat • kinetics

The long-chain n-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), are found in fish and fish products and are considered important for maintaining nervous

system and cardiac integrity (1–3). Controversy exists regarding daily nutritional requirements for EPA and DHA, with expert recommendations ranging from 0.1 to 1.6 g/day (4–7). One reason for this controversy may be that the liver synthesis rate of DHA from α -linolenic acid (α -LNA, 18:3 n-3), which is obtained from dietary plant oils, is uncertain. Estimated whole-body conversion fractions of ingested α -LNA to DHA range from 0.2% to 9%, and some clinical studies have been interpreted to mean that whole-body conversion is insufficient to maintain adequate brain and heart DHA homeostasis (8–13).

The liver is the body's primary organ for synthesizing DHA from its circulating precursor, α -LNA, and it secretes DHA into blood within very low density lipoproteins (VLDLs) (14–18). Other organs, including the brain and kidney, can synthesize DHA because they express enzymes that allow complete synthesis (19, 20). Compared with liver, however, the rate of brain DHA synthesis in the rat is very low (21–23), and the rat heart cannot accomplish a complete DHA synthesis because it lacks a critical enzyme, elongase 2 (20, 24).

We have estimated the liver synthesis rate of DHA from circulating unesterified α -LNA in unanesthetized male rats that had been fed a diet containing DHA as 2.3% of total fatty acids for 15 weeks after weaning. The liver synthesis-secretion rate of DHA was reported as 1.57 $\mu\text{mol}/\text{day}$ from measurements of labeled and unlabeled liver lipid concentrations following a 5 min intravenous infusion of [1 - ^{14}C] α -LNA (14, 15, 22, 23, 25, 26). However, because published data indicate that steady-state conditions for the labeled PUFAs were not established in the liver during the 5 min [1 - ^{14}C] α -LNA infusion (17, 27, 28), and [^{14}C]DHA could not be identified in plasma at the end of the infusion period (26), the value of 1.57 $\mu\text{mol}/\text{day}$ is probably an underestimate.

This research was supported by the Intramural Research Programs of the National Institutes on Aging and of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD.

Manuscript received 27 October 2008 and in revised form 8 December 2008.

*Published, JLR Papers in Press, December 11, 2008.
DOI 10.1194/jlr.D800056-JLR200*

Abbreviations: DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; α -LNA, α -linolenic acid; NCI, negative chemical ionization; PFB, pentafluorobenzyl.

¹To whom correspondence should be addressed.

e-mail: gaof@mail.nih.gov

To overcome these limitations, in the present study, we infused unanesthetized rats intravenously with unesterified [^{13}C]α-LNA for 2 h, which rapidly established a constant plasma α-LNA specific activity. At fixed times during infusion, esterified and unesterified arterial plasma concentrations of [^{13}C]α-LNA, [^{13}C]EPA, [^{13}C]docosapentaenoic acid ([^{13}C]DPA), and [^{13}C]DHA were measured using negative chemical ionization GC-MS (NCI-GC-MS). Plasma concentrations of newly synthesized esterified labeled n-3 PUFAs started to rise in an approximate S-shaped fashion after about 60 min of infusion, suggesting that a steady-state tracer distribution within hepatic compartments was established by then. Whole-body synthesis-secretion rates of EPA, DPA, and DHA were estimated by plotting plasma esterified concentration \times plasma volume data against infusion time for each labeled PUFA, then fitting the individual PUFA data with a sigmoidal function and determining maximum values of the first derivatives (slopes) of the best-fit curves. Plasma turnover rates of the PUFAs were calculated by dividing secretion rates by unlabeled esterified concentrations, from which plasma half-lives were estimated.

MATERIALS AND METHODS

Materials

[^{13}C]α-LNA was purchased from Spectra Stable Isotopes (Columbia, MD). [^{13}C]α-LNA was purified by HPLC (Agilent, Palo Alto, CA) with aSymmetry[®] C18 column (4.6 \times 250 mm, 5 μm , Waters); purity was determined as 98% by HPLC, and the concentration of purified [^{13}C]α-LNA was determined by GC. Diheptadecanoate phosphatidylcholine (Di-17:0 PC), free heptadecanoic acid (17:0), n-3 PUFA standards such as α-LNA, EPA, DPA, and DHA, TLC standards for cholesterol, unesterified FAs, phospholipids, triacylglycerol, cholesteryl esters, pentafluorobenzyl (PFB) bromide, and diisopropylamine were purchased from Sigma-Aldrich (St. Louis, MO). 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 Eunice Kennedy Schriver National Institute of Child Health and Human Development, and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 80-23). Fischer-344 (CDF) male rats (4 months old) were purchased from Charles River Laboratories (Portage, MI) and housed in an animal facility with regulated temperature and humidity and a 12 h light/dark cycle. The rats had free access to water and rodent chow: NIH-31 Auto18-4, which contained soybean oil and fishmeal, and had 4% by weight crude fat, whose FA composition has been reported (22, 26). Saturated and monounsaturated FAs contributed to 20.1% and 22.5%, respectively, of its total FA content, whereas the n-3 PUFAs α-LNA, EPA, and DHA contributed 5.1, 2.0, and 2.3%, respectively, and the n-6 PUFAs linoleic acid and arachidonic acid were 47.9% and 0.02%, respectively. The 9:1 linoleic acid to α-LNA concentration ratio is within the ratio for optimal synthesis of arachidonic acid and DHA, respectively (22). The dietary DHA content was \sim 3-fold

greater than required by mammals on a daily basis (29, 30). The rats were acclimated for 1 week before surgery.

Surgery

Animals were provided food the night prior to surgery. On the day of surgery, they were anesthetized with 1–3% halothane, and polyethylene catheters (PE 50, Intramedic[®], Clay Adams[®]; Becton Dickinson, Sparks, MD) filled with heparinized saline (100 IU/ml) were implanted in the right femoral artery and vein (26). The skin was closed and treated with 1% lidocaine (Hospira, Lake Forest, IL) for pain control. Two milliliters of normal saline was slowly infused intravenously to prevent dehydration. The rats were 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 approximately 20 min, was performed between 10:00 and 12:00 AM. After recovering from anesthesia, the rats were infused with the stable isotope solution to determine secretion rates, during which 2 ml of normal saline was injected subcutaneously to prevent dehydration. In some studies (see below), Evans blue solution was injected intravenously to determine plasma volume.

Stable-isotope tracer infusion

Each rat was infused via the femoral vein catheter with 3 μmol /100 g body weight [^{13}C]α-LNA (31), at a constant rate of 0.021 ml/min. An aliquot of [^{13}C]α-LNA was dissolved in 5 mM HEPES buffer (pH 7.4) containing 50 mg/ml FA-free BSA (32) to a final volume of 2.5 ml. The mixture was sonicated at 40°C for 20 min and mixed by vortexing. A variable-speed pump (No. 22; Harvard Apparatus, South Natick, MA) was used to infuse 2.5 ml of trace solution at a constant rate 0.021 ml/min to establish a steady-state plasma unesterified [^{13}C]α-LNA concentration (33, 34). Arterial blood (130 μl) was collected in centrifuge tubes (polyethylene-heparin lithium fluoride-coated; Beckman) at 0, 0.25, 0.5, 0.75, 1.5, 3.0, 4.0, 5.0, 6.5, 7.5, 10, 20, 30, 60, and 90 min. At 120 min, 500 μl blood was removed, and the rat was euthanized by an overdose of sodium pentobarbital (100 mg/kg iv). The blood samples were centrifuged at 13,000 rpm for 1 min, and plasma was collected and kept at -80°C until use.

Separation of unesterified FAs and esterified FAs from plasma

Esterified FAs in rat plasma were extracted and separated from unesterified FAs using a basic partition system based on their solubility properties. Di-17:0 PC and heptadecanoic acid (17:0) were added to 50 μl plasma as an internal standard before extraction. The plasma was then mixed with 1 ml H_2O , 2 ml 10% KOH-methanol, and 3 ml hexane. The samples were vortexed and then centrifuged. The upper (hexane) phase was transferred to another tube, and then the lower phase was extracted again by 3 ml hexane. The combined upper phase (the first hexane phase) contained esterified FAs (phospholipids, triacylglycerol, and cholesteryl ester), whereas the lower phase (aqueous) contained the unesterified FAs. To extract unesterified FAs, the lower phase was acidified with 1 ml 12 N HCl plus 1 ml H_2O , and extracted twice with 3 ml hexane. The upper phase containing unesterified FAs was collected (the second hexane phase). Both hexane phases were dried with N_2 . The unesterified FAs were derivatized to PFB esters for GC-MS analysis, and the esterified FAs were subjected to hydrolysis followed by PFB derivatization (described below). Separation of unesterified and esterified FAs was checked by TLC (silica gel 60 plates, EM Separation Technologies, Gibbstown, NJ)

using heptane-diethyl ether-glacial acetic acid (60:40:3, v/v/v) (35). The plates were sprayed with 0.03% 6-p-toluidine-2-naphthalene sulfonic acid in 50 mM Tris buffer (pH 7.4), and the lipid bands were visualized under ultraviolet light. The first hexane phase was confirmed to contain esterified FAs and the second, unesterified FAs.

Hydrolysis of esterified FAs and PFB ester derivatization

FA PFB esters were prepared and used for GC-MS analysis (36–38). First, the esterified FAs from plasma (described above) were dissolved in 1 ml of 10% KOH-methanol and heated at 70°C for 1 h to release FAs. After the reaction, the sample was acidified with 12 N HCl, and unesterified FAs were extracted twice with 3 ml hexane and dried under N₂ (37). The unesterified FAs were subjected to PFB esterification by the method of Strife and Murphy (38), which had been shown to produce greater than 95% derivatization. Unesterified FAs were added to 100 µl freshly prepared PFB derivatizing solution (pentafluorobenzylbromide-diisopropylamine-acetonitrile, 10:100:1,000, v/v/v) and shaken

for 15 min at room temperature. Excess reagent was removed under N₂ stream and the PFB esters were redissolved in hexane for GC-MS analysis.

NCI-GC-MS analysis

GC-MS conditions were as described by Pawlosky, Sprecher, and Salem (37). GC-MS analyses were performed using a Finnigan TRACE DSQ mass spectrometer (Thermo Electron, Austin, TX) equipped with TRACE GC. The fatty acyl PFB esters in hexane were injected onto a DB-FFAP capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness; J and W Scientific, Folsom, CA) interfaced directly into the NCI source using methane as reagent gas and helium as carrier gas. The GC column oven temperature was programmed from 80°C to 185°C at 20°C per min, then to 240°C at 10°C per min and held for 30 min. The injector and transfer lines were maintained at 240°C and 220°C, respectively. Nonlabeled and labeled n-3 PUFAs (α-LNA, EPA, DPA, and DHA) were monitored by selected-ion mode of the base peak (M-PFB) (Fig. 1). The *m/z* values for the various isotopically labeled FAs measured are listed

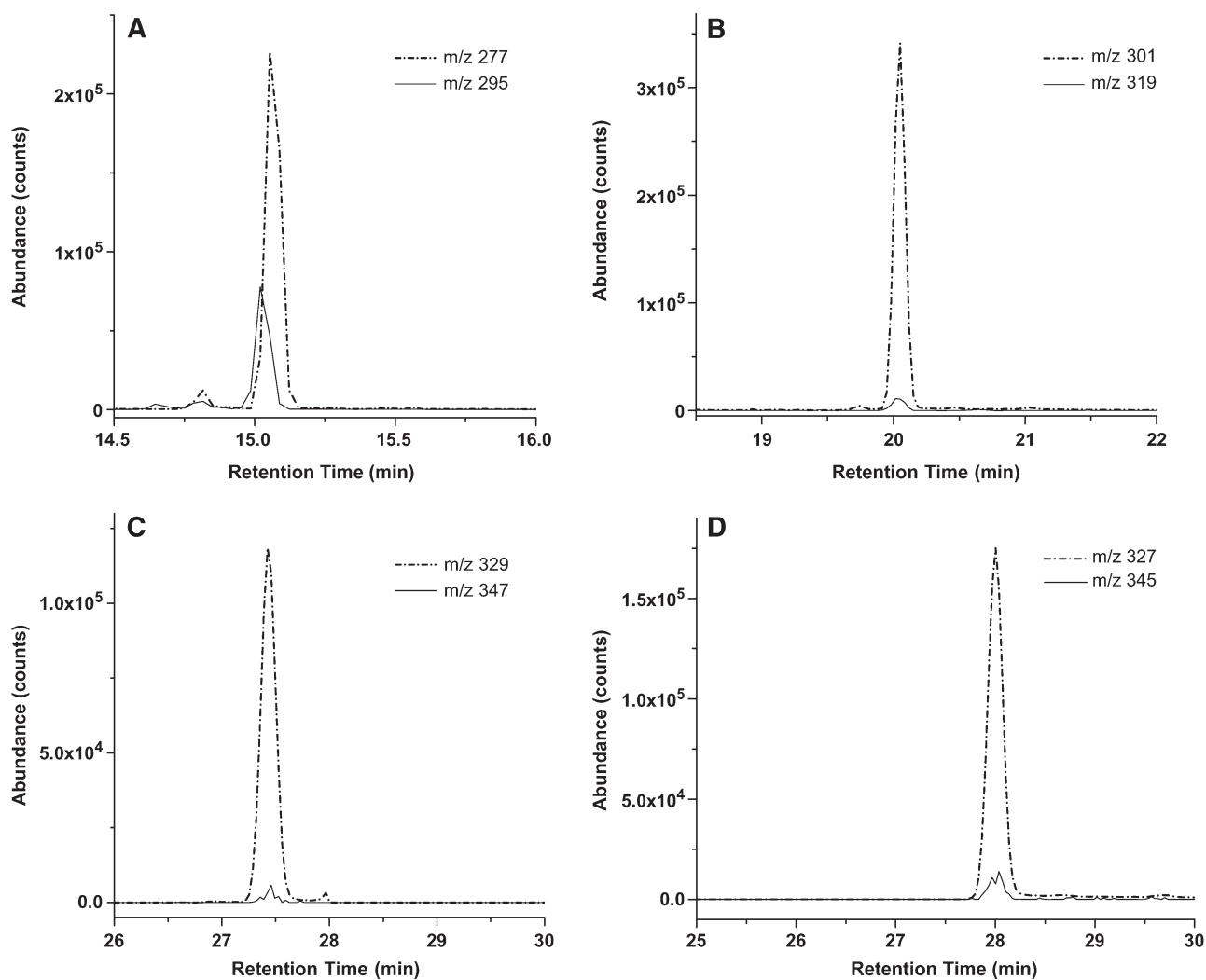


Fig. 1. Selected ion chromatograms of major ¹³C-labeled n-3 PUFA isotopomers versus unlabeled endogenous components in plasma at the end of U-[¹³C]α-linolenic acid ([¹³C]α-LNA) infusion. The *m/z* value of each ion indicated was used to detect the M-pentafluorobenzyl ([M-PFB]⁻) ions of each unlabeled and stable labeled n-3 PUFA, respectively. Depicted are the various isotopomers of (A) α-linolenic acid (α-LNA) (18:3 n-3), (B) eicosapentaenoic acid (EPA) (20:5 n-3), (C) docosapentaenoic acid (DPA) (22:5 n-3), and (D) docosahexaenoic acid (DHA) (22:6 n-3).

TABLE 1. Pentafluorobenzyl ester ions of n-3 PUFAs monitored by negative chemical ionization GC-MS

| n-3 PUFA | Ion-monitored [M-PFB] ⁻ | |
|------------------|------------------------------------|-------------------------|
| | Unlabeled | ¹³ C-labeled |
| | <i>m/z</i> | |
| α-LNA (18:3 n-3) | 277 | 295 |
| EPA (20:5 n-3) | 301 | 321 |
| DPA (22:5 n-3) | 329 | 347 |
| DHA (22:6 n-3) | 327 | 345 |

α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; [M-PFB]⁻, M-pentafluorobenzyl ion. The ion monitored is the fragment resulting from neutral loss of PFB from the ester.

in Table 1. The concentration of each PUFA was quantified by relating its peak area to the area of the internal standard with standard equations (Table 2). Each equation was determined using unesterified n-3 PUFAs and internal standard 17:0 FA at a ratio in which *x* represents the ratio of the peak area of the n-3 PUFA to 17:0 FA acid and *y* represents the ratio of the concentration (mol) of n-3 PUFA to 17:0 FA. The limit of detection of NCI-GC-MS was about 10.0 fg for each n-3 PUFA when the signal-to-noise ratio was 10. This high-sensitivity detection allows detailed studies of tissue essential FA metabolism (36, 37, 39, 40).

Plasma volume

Plasma volume was measured in four rats by the method of Wang (41), as modified by Schreihöfer, Hair, and Stepp (42). Polyethylene catheters were surgically implanted into the right femoral artery and vein of a rat, which then was allowed to recover from anesthesia for 3–4 h, as described above (26). One milligram of Evans blue dye (Sigma) was injected intravenously (200 μl of 0.5% Evans blue in saline) and flushed with 500 μl of saline. After 10 min, 1 ml of blood was withdrawn from the arterial line. The aliquots were centrifuged at 13,000 rpm for 1 min, and 100 μl of plasma was diluted into 1 ml of saline. The absorbance of the diluted sample was measured at 604 nm, and the concentration of the dye was calculated using a standard curve.

Model for n-3 PUFA synthesis-secretion by liver

Figure 2 illustrates possible pathways of diffusion and liver metabolism of intravenously injected [¹³C]α-LNA, designated as α-LNA*. As shown by the solid arrows, α-LNA* dissociates from circulating albumin to enter the liver unesterified α-LNA pool, and then is converted to α-LNA*-CoA within the acyl-CoA* pool or is elongated and desaturated to longer-chain PUFA*-CoA intermediates within that pool. These intermediates, as well as α-LNA*,

are esterified into “stable” lipids (triglycerides, phospholipids, or cholesteryl esters) and packaged within VLDLs* that are secreted into blood. Secreted labeled PUFA*s in the VLDL*s will be recycled over time into the liver via lipoprotein receptors or hydrolyzed by lipoprotein lipases and then recycled (dashed arrows). Unlabeled unesterified circulating α-LNA follows the same pathways. Additionally, unlabeled α-LNA esterified within intestine-derived chylomicrons can contribute to and thereby reduce specific activities of the liver α-LNA and α-LNA-CoA pools (dashed arrows) (16, 26, 28, 43).

Assuming a constant plasma unesterified labeled α-LNA concentration (which was established soon after infusing [U-¹³C]α-LNA) and a steady-state distribution of labeled PUFAs within liver compartments, the rate of change of the quantity of labeled esterified longer-chain PUFA *i* (*i* = EPA, DPA, or DHA) in plasma is the sum of its rates of appearance and loss, as shown in equation 1:

$$V_{\text{plasma}} \frac{dC_{i,es}^*}{dt} = k_{1,i} C_{\alpha-LNA,unes}^* - k_{-1,i} C_{i,es}^* \quad (\text{Eq. 1})$$

V_{plasma} is plasma volume (ml), $C_{i,es}^*$ (nmol/ml) is plasma concentration of esterified radiolabeled *i*, $C_{\alpha-LNA,unes}^*$ (nmol/ml) is plasma unesterified [U-¹³C]α-LNA concentration, *t* is time after infusion has begun (min), $k_{1,i}$ is the steady-state synthesis-secretion coefficient of esterified longer-chain labeled PUFA *i* (nmol/min), and $k_{-1,i}$ is the disappearance rate coefficient (nmol/ml) of esterified longer-chain labeled PUFA *i* (representing hydrolysis to unesterified PUFA *i* or diffusion out of blood). There is no isotope effect, so that the rate coefficients $k_{1,i}$ and $k_{-1,i}$ are valid for unlabeled as well as labeled PUFAs.

As shown in Results, intravenous infusion of unesterified [¹³C]α-LNA at a rate of 0.021 ml/min raised $C_{\alpha-LNA,unes}^*$ to a constant level within 3 min. $V_{\text{plasma}} C_{i,es}^*$ for labeled esterified [¹³C]EPA, [¹³C]DPA, and [¹³C]DHA did not change during the first 30 min of [¹³C]α-LNA infusion, started to rise between 30 min and 60 min, and rose faster in an apparently S-shaped fashion between 60 and 120 min. Equation 2 represents a sigmoidal function fit to experimental $V_{\text{plasma}} C_{i,es}^*$ values as a function of infusion time for each n-3 PUFA in each experiment, using nonlinear least-squares (Origin 7.0 software; OriginLab, Northampton, MA):

$$V_{\text{plasma}} C_{i,es}^* = \frac{A}{1 + \exp[(t - t_0)/B]} + C \quad (\text{Eq. 2})$$

A, *B*, and *C* are best-fit constants obtained by curve fitting, $t_0 = 0$ is time at beginning of infusion.

The first derivative of equation 2 was determined for each esterified PUFA in each rat as a function of time, using Origin 7.0 software. Its maximum value, $S_{\text{max},i}$ nmol/min, should occur when a steady state had been reached in the different liver metabolic compartments with regard to the ¹³C-labeled PUFAs and

TABLE 2. Calibration equations, correlation coefficients, linear ranges, and limits of detection for n-3 PUFAs

| n-3 PUFA | Calibration Equation ^a | Correlation Coefficient | Linear Range | Limit of Detection ^b |
|----------|-----------------------------------|-------------------------|--------------|---------------------------------|
| | | <i>r</i> | | <i>fg</i> |
| α-LNA | $y = 0.4105x + 0.0001$ | 0.9973 | 4 pg–8 ng | 8.0 |
| EPA | $y = 0.7084x - 0.0027$ | 0.9987 | 5 pg–10 ng | 10.0 |
| DPA | $y = 0.5345x - 0.0021$ | 0.9935 | 5 pg–11 ng | 11.0 |
| DHA | $y = 0.9395x - 0.0032$ | 0.9936 | 5 pg–10 ng | 10.0 |

^a Calibration equations were determined by using standard unesterified n-3 PUFAs and internal standards, where *x* represents the peak area ratio of the unlabeled n-3 PUFA to the internal standard, and *y* represents the concentration (mol) ratio of the analyte to the internal standard, respectively.

^b Limit of detection was determined when the ratio of signal to noise was 10.

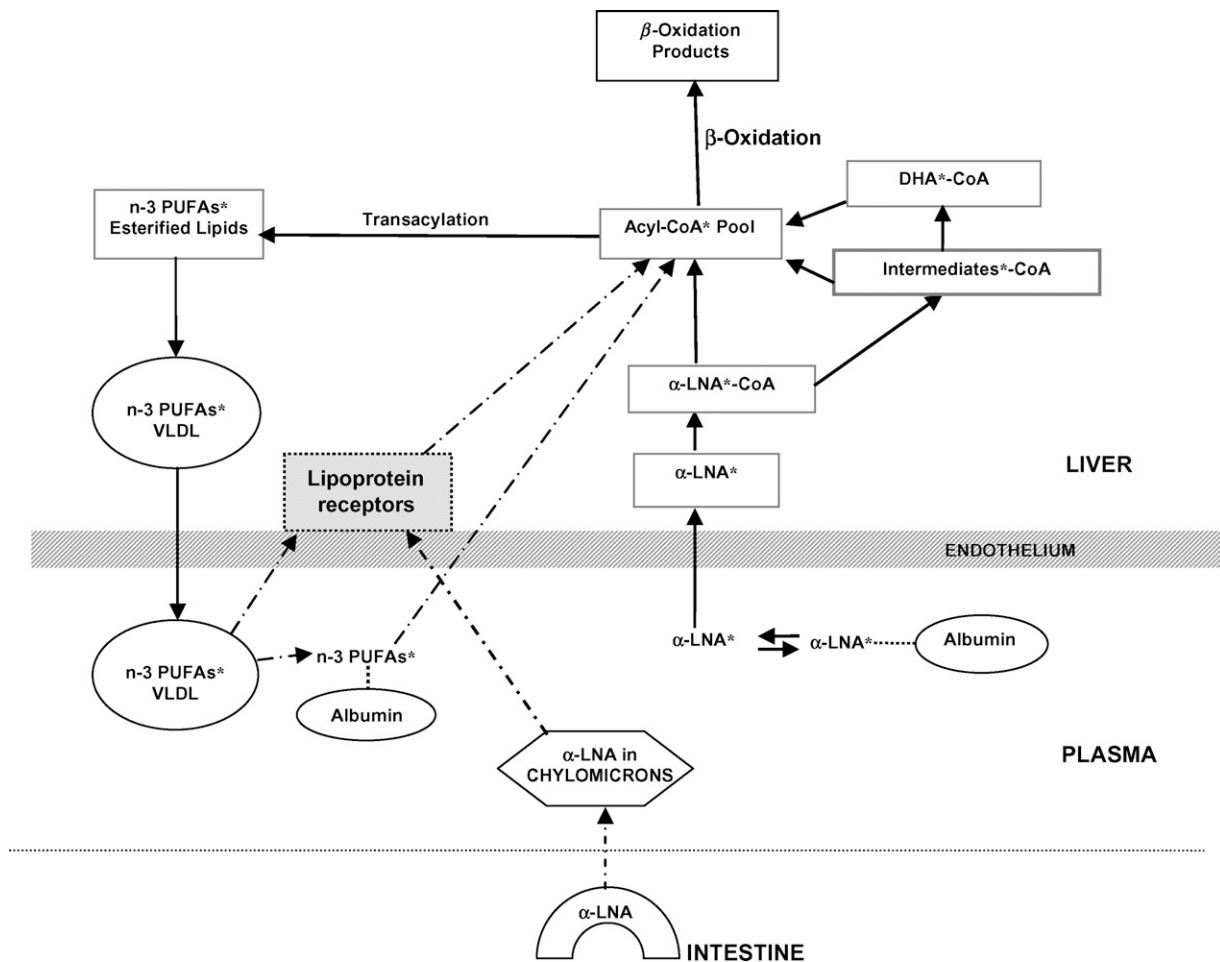


Fig. 2. Suggested pathways of diffusion and liver metabolism of intravenously injected unesterified [^{13}C] α -LNA. * represents ^{13}C -labeled n-3 PUFA. α -LNA* enters the liver α -LNA* pool after dissociating from plasma albumin (the albumin- α -LNA* complex may enter as well), then is converted to α -LNA*-CoA in the liver acyl-CoA* pool, or elongated to PUFA*-CoA intermediates within this pool. α -LNA* then may be transferred to mitochondria for β -oxidation. The intermediates, including DHA*, are esterified into stable lipids (triglycerides, phospholipids, or cholesteryl esters) and packaged within VLDLs, which are secreted into blood. Labeled PUFA*s in VLDLs may, over time, be retaken up in the liver via lipoprotein receptors (dashed arrows), or may be hydrolyzed and then taken up. Unlabeled unesterified circulating α -LNA follows the same pathways. Unlabeled α -LNA esterified within intestine-derived chylomicrons or circulating lipoproteins may contribute to and dilute the specific activities of the liver α -LNA and α -LNA-CoA pools.

their metabolites. $S_{\max,i} < k_{1,i}$ because the analysis ignores the rate of esterified plasma PUFA loss, $-k_{1,i}C_{i,es}^*$, in equation 1.

Thus, the estimated synthesis-secretion rate (nmol/min) J_i of elongated esterified PUFA i approximates:

$$J_i = S_{\max,i} \times \frac{C_{\alpha-LNA,unes}}{C_{\alpha-LNA,unes}^*} \quad (\text{Eq. 3})$$

where $C_{\alpha-LNA,unes}$ is the unesterified unlabeled α -LNA plasma concentration.

Because the plasma concentration $C_{i,es}$ (nmol/ml) of total esterified PUFA i is constant during the study, the turnover rate F_i (min^{-1}) (equation 4) and half-life $t_{1/2,i}$ (min) (equation 5) of esterified plasma PUFA i equal, respectively,

$$F_i = \frac{J_i}{C_{i,es} \times V_{plasma}} \quad (\text{Eq. 4})$$

and

$$t_{1/2,i} = \frac{0.693}{F_i} \quad (\text{Eq. 5})$$

Synthesis-secretion coefficients $k_{1,i}$ and rates J_i and plasma turnover rates and half-lives were calculated for $i = \text{EPA, DPA, and DHA}$. Data are given as mean \pm SD.

RESULTS

Unesterified and esterified n-3 PUFA concentrations in plasma

Table 3 presents concentrations of unlabeled esterified and unesterified FAs in arterial plasma, determined before infusing rats with [^{13}C] α -LNA. The concentration of unesterified α -LNA equaled 13.9 ± 2.6 nmol/ml, and that of esterified α -LNA equaled 26.1 ± 2.3 nmol/ml. Unesterified concentrations of EPA, DPA, and DHA equaled 11.3 ± 5.6 , 17.1 ± 7.9 , and 13.5 ± 5.1 nmol/ml plasma, respectively, whereas their respective esterified concentrations were 87.6 ± 8.4 , 59.4 ± 5.8 , and 210 ± 30 nmol/ml plasma. Unesterified α -LNA concentrations were used to

TABLE 3. Unlabeled unesterified and esterified n-3 PUFA concentrations in arterial plasma

| n-3 PUFA | Unesterified | Esterified |
|---------------|-----------------------|----------------|
| | <i>nmol/ml plasma</i> | |
| α -LNA | 13.9 \pm 2.6 | 26.1 \pm 2.3 |
| EPA | 11.3 \pm 5.6 | 87.6 \pm 8.4 |
| DPA | 17.1 \pm 7.9 | 59.4 \pm 5.8 |
| DHA | 13.5 \pm 5.1 | 210 \pm 30 |

Data are mean \pm SD ($n = 4$).

calculate J_i for each elongated PUFA i (equation 3), which, with the esterified net long-chain PUFA concentrations, were used to calculate half-lives and turnover rates (equations 4 and 5).

Labeled unesterified n-3 PUFA concentrations in plasma

Labeled unesterified α -LNA, EPA, DPA, and DHA concentrations in plasma were determined by GC-MS at 16 time points during the 120 min infusion. A steady-state unesterified plasma [^{13}C] α -LNA concentration was achieved within 3 min after infusion began (Fig. 3) and equaled 2.61 \pm 0.36 nmol/ml at the end of infusion. No other labeled unesterified long-chain n-3 PUFA was detected in plasma at any time during infusion.

Labeled esterified n-3 PUFA concentrations in plasma

Mean plasma concentrations (nmol/ml plasma) of esterified [^{13}C] α -LNA, [^{13}C]EPA, [^{13}C]DPA, and [^{13}C]DHA after 30, 60, 90, and 120 min of [^{13}C] α -LNA infusion are summarized in Table 4. Esterified [^{13}C] α -LNA was detected at 30 min and afterwards, whereas esterified longer-chain n-3 PUFA concentrations were detected at 60 min and afterwards. The plasma concentrations of esterified [^{13}C] α -LNA were higher than those of its labeled n-3 PUFA products,

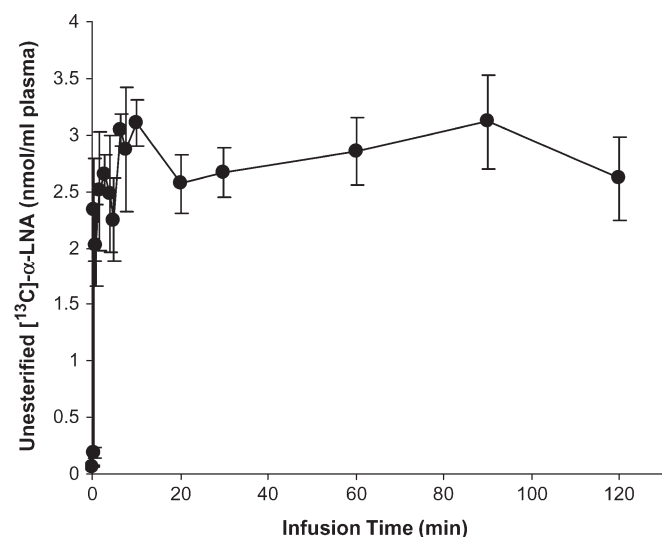


Fig. 3. Plasma unesterified [^{13}C] α -LNA during 2 h of intravenous infusion of 3 $\mu\text{mol}/100\text{g}$ [^{13}C] α -LNA in unanesthetized rats. Data are mean \pm SD ($n = 4$).

TABLE 4. ^{13}C -labeled esterified n-3 PUFA concentrations in arterial plasma during [^{13}C] α -LNA infusion study

| n-3 PUFA ^a | Infusion Time | | | |
|-----------------------|-----------------------|-------------------|-----------------|-----------------|
| | 30 min | 60 min | 90 min | 120 min |
| | <i>nmol/ml plasma</i> | | | |
| α -LNA | 2.15 \pm 0.32 | 4.97 \pm 1.23 | 7.79 \pm 2.04 | 24.0 \pm 2.84 |
| EPA | ND | 1.47 \pm 0.60 | 4.17 \pm 0.72 | 7.46 \pm 0.86 |
| DPA | ND | 0.011 \pm 0.003 | 0.86 \pm 0.22 | 2.71 \pm 0.56 |
| DHA | ND | 0.68 \pm 0.15 | 4.54 \pm 0.99 | 6.89 \pm 0.60 |

^a Indicates the ^{13}C -labeled n-3 PUFA. ND, not detected. Data are mean \pm SD ($n = 4$).

and the concentrations of esterified labeled DHA and EPA were higher than that of labeled DPA.

Plasma volume

Mean plasma volume in four 4-month-old male rats that weighed on average 321 \pm 13 g equaled 26.9 \pm 0.82 ml/kg body weight, in line with a reported volume of 29 \pm 3 ml/kg (44). This value was used to calculate V_{plasma} in the infusion study according to body weight, which was 276.7 \pm 5.8 g.

n-3 PUFA synthesis-secretion coefficients and rates, turnover rates, and half-lives

Figure 4A presents values of $k_{1,i}C_{i,es}^*$ plotted against infusion time for each of four esterified n-3 PUFAs in one rat infusion study. The corresponding best-fit curves represented by equation 2, calculated by Origin 7.0, also are shown. These curves fit the data reasonably well and began to plateau for newly synthesized [^{13}C]EPA, [^{13}C]DPA, and [^{13}C]DHA, but the esterified [^{13}C] α -LNA curve continued to rise during the entire infusion period.

Figure 4B presents slopes (first derivatives) of the best-fit sigmoidal curves shown in Fig. 4A. The maximal values of the slopes, $S_{\text{max},i}$ were estimated for each elongated esterified labeled n-3 PUFA, and means for the four experiments are summarized in Table 5. Because the plasma concentration of [^{13}C] α -LNA did not appear to plateau during the 120 min infusion (Fig. 4A, B), we did not estimate or use $S_{\text{max},\alpha\text{-LNA}}$ for further analysis.

The mean values of $S_{\text{max},i}$ were used to calculate synthesis-secretion rates J_i by equation 3 for EPA, DPA, and DHA (Table 5). J_i equaled 5.83, 4.35, and 6.83 nmol/min for the three PUFAs, respectively, equivalent to 8.40, 6.27, and 9.84 $\mu\text{mol}/\text{day}$. These rates were used to calculate plasma turnover rates F_i (equation 4), equal to 9.04, 9.85, and $4.38 \times 10^{-3} \text{ min}^{-1}$, and plasma half-lives equal to 80.1, 72.1, and 160.3 min, for esterified EPA, DPA, and DHA, respectively (Table 5).

DISCUSSION

In this study, we estimated whole-body synthesis-secretion rates of EPA, DPA, and DHA from circulating unesterified α -LNA in unanesthetized male rats fed a DHA-containing diet. Unesterified U- ^{13}C] α -LNA was infused intravenously for 120 min, and arterial plasma concentrations of esteri-

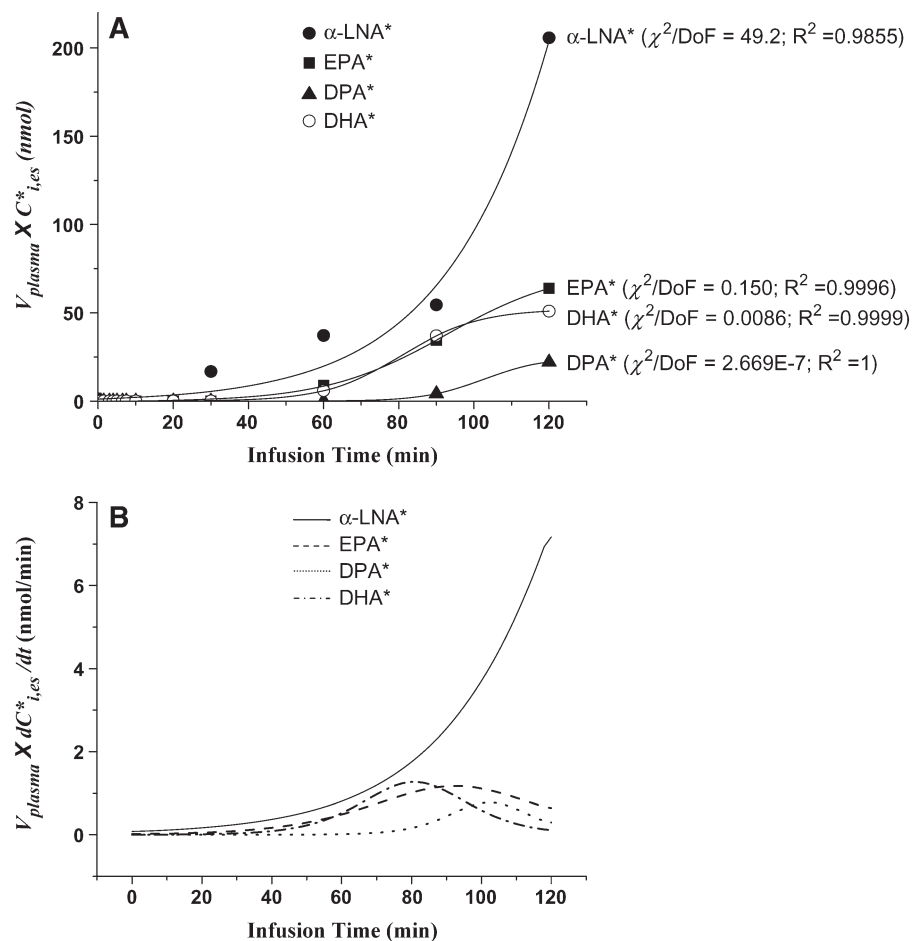


Fig. 4. A: Labeled n-3 PUFA esterified arterial plasma concentrations \times plasma volume plotted against time for experiment in which 3 $\mu\text{mol}/100\text{g}$ [^{13}C] α -LNA was infused intravenously for 120 min in a rat, and best-fit sigmoidal curves estimated by Origin using equation 2. B: Curves representing first derivatives of curves in Fig. 4A. * Represents ^{13}C -labeled n-3 PUFAs; χ^2 , Chi square; DoF, degrees of freedom; R^2 , coefficient of determination.

fied ^{13}C -labeled α -LNA, EPA, DPA, and DHA were measured as a function of infusion time, as were concentrations of the labeled unesterified PUFAs. Esterified [^{13}C] α -LNA appeared in plasma after about 30 min of infusion, but its labeled esterified PUFA elongation products appeared only after about 60 min. They then rose rapidly and later appeared to plateau. Plasma concentration \times plasma volume data for labeled EPA, DPA, and DHA, but not for α -LNA, were fit well by least-squares with a sigmoidal equation (equation 2). The shapes of the curves indicated that labeled esterified n-3 PUFAs had reached steady-state levels within the different (presumably hepatic) metabolic compartments and that steady-state synthesis-secretion rates were approximated by about 60 min of infusion. Unester-

ified labeled EPA, DPA, and DHA could not be identified in arterial plasma at any time during infusion. This is not surprising, because plasma half-lives of unesterified PUFAs are less than 1 min in the rat (45).

Although elongation and desaturation of α -LNA can occur in different body organs (19–24), the fact that we could only identify esterified elongation products in plasma suggests that our whole-body measurements largely, if not exclusively, represented their liver metabolism and secretion within VLDLs (16, 18). For further discussion, the whole-body measurements will be considered to represent liver synthesis-secretion rates of esterified EPA, DPA, and DHA.

Synthesis-secretion rates of esterified EPA, DPA, and DHA derived from circulating unesterified α -LNA, estimated

TABLE 5. Mean parameters for synthesis-secretion of n-3 PUFAs from α -LNA in rat liver

| n-3 PUFA, <i>i</i> | $S_{max,i}$ | J_i | Daily Secretion Rate | F_i | $t_{1/2,i}$ |
|--------------------|-------------------------------|-----------------|---------------------------------|----------------------------------|------------------|
| | $\text{nmol}/\text{min}^{-1}$ | | $\mu\text{mol}/\text{day}^{-1}$ | $\text{min}^{-1} \times 10^{-3}$ | min |
| EPA | 1.03 \pm 0.21 | 5.83 \pm 1.23 | 8.40 \pm 1.77 | 9.04 \pm 2.15 | 80.1 \pm 18.9 |
| DPA | 0.76 \pm 0.10 | 4.35 \pm 0.86 | 6.27 \pm 1.23 | 9.85 \pm 1.78 | 72.1 \pm 13.2 |
| DHA | 1.19 \pm 0.11 | 6.83 \pm 1.29 | 9.84 \pm 1.85 | 4.38 \pm 0.57 | 160.3 \pm 21.0 |

Data are mean \pm SD ($n = 4$).

from the maximal first derivatives $S_{max,i}$ of equation 2 relating $V_{plasma}C_{i,es}^*$ to infusion time (Fig. 4 and Table 5), equaled 8.40, 6.27, and 9.84 $\mu\text{mol}/\text{day}$, respectively. In comparison, in rats fed an identical 2.3% DHA-containing diet and infused intravenously for 5 min with $[1-^{14}\text{C}]\alpha\text{-LNA}$, the DHA synthesis rate was reported as 1.57 $\mu\text{mol}/\text{day}$ (23, 26), one-sixth of the rate in this study. The lower 5 min infusion rate is clearly an underestimate, because this and prior studies indicate that steady-state rat hepatic tracer conditions and secretion rates are not established before 30 min (15, 17, 18, 22, 26–28). This length of time is necessary for the multiple metabolic and transfer steps to reach a steady state with regard to tracer (Fig. 2) (16, 18, 43, 46–48).

As suggested by Fig. 2, some secreted unesterified n-3 PUFAs within VLDL could be recycled over time to the liver via lipoprotein receptors, or could be hydrolyzed in blood by lipoprotein lipases to their unesterified forms and then taken up (16, 43, 46). Thus, the net rate of hepatic secretion of DHA derived from unesterified $\alpha\text{-LNA}$ may approach the sum of the secretion rates given in Table 5 for EPA, DPA, and DHA, 24.5 $\mu\text{mol}/\text{day}$. Furthermore, dilution of liver $\alpha\text{-LNA-CoA}$ specific activity by unlabeled esterified $\alpha\text{-LNA}$ coming from circulating lipoproteins, stable liver lipids (triacylglycerol, phospholipid, cholesteryl ester), or chylomicrons (Fig. 4) (16, 49–52) may be about 50% (14, 15, 53), which implies that the liver DHA synthesis rate is even higher.

The plasma turnover rates and half-lives of the synthesized n-3 PUFAs in unanesthetized rats, summarized in Table 5, can be compared with values obtained using compartmental analysis in humans fed $[^{13}\text{C}]\alpha\text{-LNA}$. These human values for EPA, DPA, and DHA equaled 67, 58, and 20 h, respectively (40), compared with the respective values of 80.1, 72.1, and 160.3 min in this rat study (Table 5). These order of magnitude differences may reflect higher rates of energy and lipid metabolism in rats than in humans (54, 55) and the fact that the rat study considers only turnover of the esterified n-3 PUFAs.

Although hepatic DHA conversion from $\alpha\text{-LNA}$ is considered less “efficient” than direct DHA ingestion for maintaining body DHA stores (10, 40, 56), measuring fractional conversion does not provide a direct rate of DHA synthesis as does the method presented in this paper. Reported fractional conversions of ingested $\alpha\text{-LNA}$ to DHA in humans and nonhuman primates range from 0.2% to 9% (8, 10, 40, 57–60), suggesting roles for multiple uncontrolled factors. In any case, observations that vegetarians, whose plasma DHA levels are substantially less than those of omnivores, have the same rates of net mortality and of mortality from different causes as omnivores (61, 62) suggest that liver conversion is sufficient to maintain body DHA stores in healthy subjects in the absence of dietary EPA and DHA.

Supporting this conclusion with regard to brain is evidence that brain DHA consumption in rats fed the 2.3% DHA-containing diet of this study equaled 0.25–0.29 $\mu\text{mol}/\text{day}$ (23, 25, 63–65), much less than the estimated whole-body conversion rate from unesterified $\alpha\text{-LNA}$ of 9.84–24.5 $\mu\text{mol}/\text{day}$ (see above) and orders of magnitude higher than the brain conversion rate (22). The ratio of the synthesis-secretion rate of DHA from unesterified

$\alpha\text{-LNA}$ to the rate of brain DHA consumption thus may exceed 30. This ratio probably would be elevated by removing DHA from the diet and reducing dietary $\alpha\text{-LNA}$ content, which results in upregulated hepatic conversion of $\alpha\text{-LNA}$ to DHA and increased transcription and activities of elongases 2 and 5, and of $\Delta 5$ and $\Delta 6$ desaturases (15, 53).

The stable-isotope infusion method of this study could be extended to determine rates of whole-body synthesis of EPA, DPA, and DHA from circulating unesterified $\alpha\text{-LNA}$, or of DPA and DHA from circulating EPA, under different dietary and disease conditions in humans as well as animals. Knowing these rates in humans might help to arrive at a consensual recommendation for daily EPA plus DHA consumption, which currently ranges, depending on the expert committee, from 0.1 to 1.6 g/day/2000 kcal (0.05–0.72% kcal) (4–7). Brain and heart function and metabolism also could be related to diet and liver conversion rates, as well as to organ PUFA consumption rates, which can be measured using positron emission tomography (23, 66, 67).

In summary, whole-body synthesis-secretion rates, probably representing liver rates, of esterified EPA, DPA, and DHA from circulating unesterified $\alpha\text{-LNA}$, were quantified by infusing $[^{13}\text{C}]\alpha\text{-LNA}$ intravenously for 2 h in unanesthetized rats fed a DHA-containing diet. The DHA synthesis rate in this paper, 9.84 $\mu\text{mol}/\text{day}$, is at least 30-fold the reported rat brain DHA consumption rate, which suggests that liver synthesis alone could maintain brain DHA homeostasis. The stable-isotope infusion method could be extended to examine long-chain n-3 as well as n-6 PUFA synthesis-secretion rates in human subjects in relation to diet, aging, and disease. ■

The authors thank the National Institutes of Health Fellows Editorial Board and Ms. Kathy Benjamin for editorial assistance, and Dr. Deanna Greenstein for statistical assistance.

REFERENCES

1. Contreras, M. A., and S. I. Rapoport. 2002. Recent studies on interactions between n-3 and n-6 polyunsaturated fatty acids in brain and other tissues. *Curr. Opin. Lipidol.* **13**: 267–272.
2. 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.
3. Uauy, R., and A. D. Dangour. 2006. Nutrition in brain development and aging: role of essential fatty acids. *Nutr. Rev.* **64**: S24–S33.
4. Board, F. N. 2005. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academies Press, Washington, DC.
5. Committee, S. R. 1990. Minister of National Health and Welfare, Ottawa, Canada.
6. Foundation, B. N., editor. 1992. Unsaturated Fatty Acids Nutritional and Physiological Significance: The Report of the British Nutrition Foundation’s Task Force. Chapman and Hall, New York.
7. Simopoulos, A. P. 2000. Human requirement for N-3 polyunsaturated fatty acids. *Poult. Sci.* **79**: 961–970.
8. Burdge, G. C., and P. C. Calder. 2005. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* **45**: 581–597.
9. 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 $[^{13}\text{C}]\alpha\text{-linolenic}$ acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. *Br. J. Nutr.* **90**: 311–321.

10. Burdge, G. C., A. E. Jones, and S. A. Wootton. 2002. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br. J. Nutr.* **88**: 355–363.
11. Kris-Etherton, P. M., W. S. Harris, and L. J. Appel. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation.* **106**: 2747–2757.
12. Kris-Etherton, P. M., and A. M. Hill. 2008. N-3 fatty acids: food or supplements? *J. Am. Diet. Assoc.* **108**: 1125–1130.
13. 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.
14. Igarashi, M., J. C. DeMar, Jr., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. 2007. Docosahexaenoic acid synthesis from alpha-linolenic acid by rat brain is unaffected by dietary n-3 PUFA deprivation. *J. Lipid Res.* **48**: 1150–1158.
15. 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.
16. Lehninger, A. L., D. L. Nelson, and M. M. Cox. 1993. Principles of Biochemistry. 2 ed. Worth, New York.
17. 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.
18. Vance, J. E., and D. E. Vance. 1990. Lipoprotein assembly and secretion by hepatocytes. *Annu. Rev. Nutr.* **10**: 337–356.
19. Matsuzaka, T., H. Shimano, N. Yahagi, M. Amemiya-Kudo, T. Yoshikawa, A. H. Hasty, Y. Tamura, J. Osuga, H. Okazaki, Y. Iizuka, et al. 2002. Dual regulation of mouse delta(5)- and delta(6)-desaturase gene expression by SREBP-1 and PPARalpha. *J. Lipid Res.* **43**: 107–114.
20. 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.
21. 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.
22. 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.
23. Rapoport, S. I., J. S. Rao, and M. Igarashi. 2007. Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. *Prostaglandins Leukot. Essent. Fatty Acids.* **77**: 251–261.
24. Igarashi, M., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. 2008. Rat heart cannot synthesize docosahexaenoic acid from circulating alpha-linolenic acid because it lacks elongase-2. *J. Lipid Res.* **49**: 1735–1745.
25. DeMar, J. C., Jr., H. J. Lee, K. Ma, L. Chang, J. M. Bell, S. I. Rapoport, and R. P. Bazinet. 2006. Brain elongation of linoleic acid is a negligible source of the arachidonate in brain phospholipids of adult rats. *Biochim. Biophys. Acta.* **1761**: 1050–1059.
26. 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.
27. Anderson, G. J., and W. E. Connor. 1988. Uptake of fatty acids by the developing rat brain. *Lipids.* **23**: 286–290.
28. 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.
29. 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.
30. Van Aerde, J. E., and M. T. Clandinin. 1993. Controversy in fatty acid balance. *Can. J. Physiol. Pharmacol.* **71**: 707–712.
31. Sheaff, R. C., H. M. Su, L. A. Keswick, and J. T. Brenna. 1995. Conversion of alpha-linolenic to docosahexaenoate is not depressed by high dietary levels of linoleate in young rats: tracer evidence using high precision mass spectrometry. *J. Lipid Res.* **36**: 998–1008.
32. Murphy, E. J., T. A. Rosenberger, C. B. Patrick, and S. I. Rapoport. 2000. Intravenously injected [1-14C]arachidonic acid targets phospholipids, and [1-14C]palmitic acid targets neutral lipids in hearts of awake rats. *Lipids.* **35**: 891–898.
33. Wolf, R. R., J. E. Evans, C. J. Mullany, and J. F. Burke. 1980. Measurement of plasma free fatty acid turnover and oxidation using [1-13C] palmitic acid. *Biomed. Mass Spectrom.* **7**: 168–171.
34. 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.
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. Lin, Y., and N. Salem, Jr. 2002. A technique for the in vivo study of multiple stable isotope-labeled essential fatty acids. *Prostaglandins Leukot. Essent. Fatty Acids.* **67**: 141–146.
37. Pawlosky, R. J., H. W. Sprecher, and N. Salem, Jr. 1992. High sensitivity negative ion GC-MS method for detection of desaturated and chain-elongated products of deuterated linoleic and linolenic acids. *J. Lipid Res.* **33**: 1711–1717.
38. Strife, R. J., and R. C. Murphy. 1984. Stable isotope labelled 5-lipoxygenase metabolites of arachidonic acid: analysis by negative ion chemical ionization mass spectrometry. *Prostaglandins Leukot. Med.* **13**: 1–8.
39. Lin, Y. H., and N. Salem, Jr. 2005. In vivo conversion of 18- and 20-C essential fatty acids in rats using the multiple simultaneous stable isotope method. *J. Lipid Res.* **46**: 1962–1973.
40. Pawlosky, R. J., J. R. Hibbeln, J. A. Novotny, and N. Salem, Jr. 2001. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. *J. Lipid Res.* **42**: 1257–1265.
41. Wang, L. 1959. Plasma volume, cell volume, total blood volume and F cells factor in the normal and splenectomized Sherman rat. *Am. J. Physiol.* **196**: 188–192.
42. Schreihofer, A. M., C. D. Hair, and D. W. Stepp. 2005. Reduced plasma volume and mesenteric vascular reactivity in obese Zucker rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**: R253–R261.
43. Gibbons, G. F., D. Wiggins, A. M. Brown, and A. M. Hebbachi. 2004. Synthesis and function of hepatic very-low-density lipoprotein. *Biochem. Soc. Trans.* **32**: 59–64.
44. Guide for the Care and Use of Laboratory Animals. 1985. Institutes of Laboratory Animal Resources, National Research Council, National Academy Press, Washington, DC.
45. DeGeorge, J. J., J. G. Noronha, J. M. Bell, P. Robinson, and S. I. Rapoport. 1989. Intravenous injection of [1-14C]arachidonate to examine regional brain lipid metabolism in unanesthetized rats. *J. Neurosci. Res.* **24**: 413–423.
46. Gibbons, G. F. 1990. Assembly and secretion of hepatic very-low-density lipoprotein. *Biochem. J.* **268**: 1–13.
47. Liang, X., L. Wu, T. Hand, and K. Andreasson. 2005. Prostaglandin D2 mediates neuronal protection via the DP1 receptor. *J. Neurochem.* **92**: 477–486.
48. Sprecher, H. 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim. Biophys. Acta.* **1486**: 219–231.
49. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88**: 8342–8346.
50. Huettinger, M., J. R. Corbett, W. J. Schneider, J. T. Willerson, M. S. Brown, and J. L. Goldstein. 1984. Imaging of hepatic low density lipoprotein receptors by radionuclide scintiscanning in vivo. *Proc. Natl. Acad. Sci. USA.* **81**: 7599–7603.
51. Lewis, G. F. 1997. Fatty acid regulation of very low density lipoprotein production. *Curr. Opin. Lipidol.* **8**: 146–153.
52. Wu, X., N. Sakata, J. Dixon, and H. N. Ginsberg. 1994. Exogenous VLDL stimulates apolipoprotein B secretion from HepG2 cells by both pre- and post-translational mechanisms. *J. Lipid Res.* **35**: 1200–1210.
53. Igarashi, M., K. Ma, L. Chang, J. M. Bell, and S. I. Rapoport. 2007. Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain. *J. Lipid Res.* **48**: 2463–2470.
54. Sokoloff, L. 1977. Relation between physiological function and energy metabolism in the central nervous system. *J. Neurochem.* **29**: 13–26.
55. Purdon, A. D., and S. I. Rapoport. 2007. Energy consumption by phospholipid metabolism in mammalian brain. In *Neural Energy Utilization: Handbook of Neurochemistry and Molecular Biology*. G. Gibson and G. Dienel, editors. Springer, New York. 401–427.
56. Brenna, J. T. 2002. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Curr. Opin. Clin. Nutr. Metab. Care.* **5**: 127–132.
57. Burdge, G. C., and S. A. Wootton. 2002. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br. J. Nutr.* **88**: 411–420.
58. Cunnane, S. C., C. R. Menard, S. S. Likhodii, J. T. Brenna, and M. A.

- Crawford. 1999. Carbon recycling into de novo lipogenesis is a major pathway in neonatal metabolism of linoleate and alpha-linolenate. *Prostaglandins Leukot. Essent. Fatty Acids*. **60**: 387–392.
59. Lefkowitz, W., S. Y. Lim, Y. Lin, and N. Salem, Jr. 2005. Where does the developing brain obtain its docosahexaenoic acid? Relative contributions of dietary alpha-linolenic acid, docosahexaenoic acid, and body stores in the developing rat. *Pediatr. Res*. **57**: 157–165.
60. Vermunt, S. H., R. P. Mensink, M. M. Simonis, and G. Hornstra. 2000. Effects of dietary alpha-linolenic acid on the conversion and oxidation of ¹³C-alpha-linolenic acid. *Lipids*. **35**: 137–142.
61. Key, T. J., P. N. Appleby, G. K. Davey, N. E. Allen, E. A. Spencer, and R. C. Travis. 2003. Mortality in British vegetarians: review and preliminary results from EPIC-Oxford. *Am. J. Clin. Nutr.* **78** (Suppl.): 533–538.
62. Rosell, M. S., Z. Lloyd-Wright, P. N. Appleby, T. A. Sanders, N. E. Allen, and T. J. Key. 2005. Long-chain n-3 polyunsaturated fatty acids in plasma in British meat-eating, vegetarian, and vegan men. *Am. J. Clin. Nutr.* **82**: 327–334.
63. 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.
64. 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.
65. Rapoport, S. I., M. C. Chang, and A. A. Spector. 2001. Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. *J. Lipid Res.* **42**: 678–685.
66. Giovacchini, G., A. Lerner, M. T. Toczek, C. Fraser, K. Ma, J. C. DeMar, P. Herscovitch, W. C. Eckelman, S. I. Rapoport, and R. E. Carson. 2004. Brain incorporation of [¹¹C]arachidonic acid, blood volume, and blood flow in healthy aging: a study with partial-volume correction. *J. Nucl. Med.* **45**: 1471–1479.
67. Umhau, J. C., W. Zhou, A. Polozova, J. Demar, A. Bhattacharjee, K. Ma, G. Esposito, S. I. Rapoport, W. Eckelman, P. Hersovitch, R. E. Carson, J. Hibbeln, K. Kurtzdel, M. Channing, B. K. Vuong, and N. Salem. 2009. Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. *J. Lipid Res.* (in press).