

Whole-body synthesis secretion of docosahexaenoic acid from circulating eicosapentaenoic acid in unanesthetized rats

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Abstract Dietary docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3) are considered important for maintaining normal heart and brain function, but little EPA is found in brain, and EPA cannot be elongated to DHA in rat heart due to the absence of elongase-2. Ingested EPA may have to be converted in the liver to DHA for it to be fully effective in brain and heart, but the rate of conversion is not agreed on. This rate was determined in male adult rats fed a standard n-3 PUFA, containing diet by infusing unesterified albumin-bound [¹³C]EPA intravenously for 2 h and measuring esterified [¹³C]labeled PUFAs in arterial plasma lipoproteins, as well as the specific activity of unesterified plasma EPA. Whole-body (presumably hepatic) synthesis secretion rates from circulating unesterified EPA, calculated from peak first derivatives of plasma esterified concentration × volume curves, equaled 2.61 μmol/day for docosapentaenoic acid (22:5n-3) and 5.46 μmol/day for DHA. The DHA synthesis rate was 24-fold greater than the reported brain DHA consumption rate in rats. Thus, dietary EPA could help to maintain brain and heart DHA homeostasis because it is converted at a relatively high rate in the liver to circulating DHA.—Gao, F., D. Kiewewetter, L. Chang, K. Ma, S. I. Rapoport, and M. Igarashi. **Whole-body synthesis secretion of docosahexaenoic acid from circulating eicosapentaenoic acid in unanesthetized rats.** *J. Lipid Res.* 2009. 50: 2463–2470.

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Docosahexaenoic acid (DHA; 22:6n-3) is the major n-3 PUFA in brain and retina (1, 2). Low levels of circulating DHA and reduced dietary intake of fish have been associated with clinical depression (3, 4), Alzheimer's disease

(5–7), bipolar disorder (8), and other human brain disorders. DHA also is considered important for optimum cardiac function (9), but it cannot be synthesized from its n-3 PUFA precursors in the heart due to a lack of elongase-2 (10).

Some but not all clinical studies have indicated that dietary fish and marine oils that contain DHA and eicosapentaenoic acid (EPA), as well as dietary DHA or EPA given alone, can reduce brain or heart disease (7, 11–16). Some of the EPA effects on these organs could be direct, as dietary EPA can lower plasma triacylglycerol (17, 18), is a more potent platelet inhibitor than DHA (19, 20), and its products have strong anti-inflammatory effects (21). Other EPA effects may be indirect, through its conversion to DHA in the liver (22). Studies using compartmental analysis in humans (23) and in rats (24) fed α-linolenic acid (α-LNA; 18:3n-3) or EPA labeled with a stable isotope reported that both α-LNA and EPA were converted to DHA.

To date, the rate of liver conversion of EPA to secreted DHA has not been quantified directly in the intact organism. Knowing this rate in humans might help to estimate daily dietary requirements of EPA and DHA (25–28). To measure this rate in rats for the first time, in this article we employed a published method that was used to determine whole-body DHA synthesis secretion from circulating α-LNA (29) in unanesthetized adult rats on an n-3 PUFA-containing diet. [¹³C]EPA was infused intravenously for 2 h, which allowed the establishment of a steady-state DHA synthesis secretion after about 1 h. Operational equations were used to calculate whole-body synthesis secretion rates of esterified docosapentaenoic acid (DPA) and DHA and

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

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secretion of esterified EPA from circulating unesterified EPA, as well as their turnover rates and half-lives in plasma.

MATERIALS AND METHODS

Materials

[U-¹³C]EPA ethyl ester was generously provided by Dr. Joseph Hibbeln (National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD). It was hydrolyzed in 1 ml 10% KOH/methanol and heated at 70°C for 1 h to release unesterified [U-¹³C]EPA (29). The sample was acidified with 12 N HCl, and unesterified fatty acids were extracted twice with 3 ml hexane and then dried under N₂. The unesterified [U-¹³C]EPA was purified further by HPLC (Agilent, Palo Alto, CA) using a Symmetry® C18 column (4.6 × 250 mm, 5 μm; Waters, Milford, MA); purity was found to be 95% by HPLC. The concentration of purified [U-¹³C]EPA 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 fatty acids, 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).

Animals

This 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/12 h dark cycle. They were acclimated for 1 week before surgery in this facility and had free access to water and rodent chow (NIH-31 Auto18-4). The chow contained soybean oil and fishmeal and had 4% by weight crude fat. Its fatty acid composition has been reported (30). α-LNA, EPA, and DHA contributed 5.1%, 2.0%, and 2.3%, respectively, and the n-6 PUFAs linoleic acid and arachidonic acid contributed 47.9% and 0.02%, respectively, of total fatty acids.

Surgery

Rats were provided food the night prior to surgery. On the day of surgery, a rat was anesthetized using 1–3% halothane. Polyethylene catheters (PE 50, Clay Adams®; Becton Dickinson, Sparks, MD) filled with heparinized saline (100 IU/ml) were surgically implanted in the right femoral artery and vein (31), after which the skin was closed and treated with 1% lidocaine (Hospira, Lake Forest, IL) for pain prevention. Two milliliters of normal saline was slowly infused intravenously to prevent dehydration. 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 was performed between 10 AM and 12 PM, lasting approximately 20 min. After recovering from anesthesia, the rat was infused with a stable isotope solution as described below.

Stable isotope tracer infusion and blood collection

Following the procedure of Gao et al. (29), a rat was infused via the femoral vein catheter with 3 μmol/100 g body weight

[U-¹³C]EPA at a constant rate of 0.021 ml/min. An aliquot of [U-¹³C]EPA was dissolved in 5 mM HEPES buffer (pH 7.4) containing 50 mg/ml fatty acid-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 tracer solution at a constant rate 0.021 ml/min to establish a steady-state plasma unesterified [U-¹³C]EPA concentration (29). During the 2 h, 2 ml of normal saline was injected subcutaneously to prevent dehydration. 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, 5.0, 8.0, 10, 20, 30, 60, 75, 90, and 105 min. At 120 min, 500 μl blood was removed, and the rat was euthanized by an overdose of sodium pentobarbital (100 mg/kg intravenously). The blood samples were centrifuged at 13,000 rpm for 1 min, and plasma was collected and kept at –80°C until use.

Plasma lipid extraction and PFB derivatization

Plasma lipid extraction and PFB derivatization procedures have been described (29). Briefly, appropriate amounts of internal standards (di-17:0 PC and free 17:0) were added to plasma, and then KOH solution was added. Stable lipids (phospholipids, triacylglycerol, and cholesteryl esters) containing esterified fatty acids were extracted with hexane twice. After transferring the hexane phase to another tube, the remaining lower phase was acidified with HCl, and unesterified fatty acids were extracted with hexane twice. The extracted esterified fatty acids in stable lipids were hydrolyzed (10% KOH in methanol). Plasma unesterified fatty acids and fatty acids that had been esterified in stable lipids were derivatized to PFB esters for GC-MS analysis (29, 33).

GC-MS analysis

The fatty acid PFB esters from the plasma samples were analyzed by GC-MS as previously described (29). Nonlabeled and labeled n-3 PUFAs (α-LNA, EPA, DPA, and DHA) were monitored by selected ion mode of the base peak (M-PFB). The concentration of each n-3 PUFA was quantified by relating its peak area to the area of the internal standard using standard equations.

TLC separation of plasma neutral lipid subclasses

Plasma concentrations of [U-¹³C]EPA, [¹³C]DPA, and [¹³C]DHA were determined in esterified stable lipids (phospholipids, triacylglycerol, and cholesteryl esters). Total lipids at the end of infusion (120 min) were extracted from plasma by the Folch method (34). The extracts were separated into neutral lipid subclasses by TLC on silica gel 60 plates (EM Separation Technologies, Gibbstown, NJ) using heptane-diethyl ether-glacial acetic acid (60:40:3, v/v/v) (35). Authentic standards of triacylglycerol, phospholipids, cholesterol, cholesteryl ester, and unesterified fatty acids were run on the plates for identification. 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 UV light. The bands of phospholipids, triacylglycerol, and cholesteryl esters were scraped, and then the silica gel was transferred to a tube. An appropriate amount of internal standard (di-17:0-PC) was added to the tube, and then 1 ml of 10% KOH-methanol was added and the tube was heated for 1 h at 70°C to release free fatty acids from the lipids. After release, the sample was acidified with 12 N HCl, and unesterified fatty acids were extracted with hexane and dried under N₂. The unesterified fatty acids were subjected to PFB derivatization for GC-MS analysis as described above.

Calculations

Following the model of Gao et al. (29), circulating unesterified [U-¹³C]EPA dissociates from circulating albumin to enter

the liver unesterified EPA pool. Here, it is converted to [U-¹³C] EPA-CoA or is elongated and desaturated to longer-chain [¹³C] DPA-CoA or [¹³C]DHA-CoA. The PUFAs from these acyl-CoA intermediates are esterified within the liver into stable lipids (triglycerides, phospholipids, or cholesteryl esters) that are packaged within VLDLs, which eventually are secreted into the blood. The labeled PUFAs in the VLDLs will be recycled over time into the liver via lipoprotein receptors, will be hydrolyzed by lipoprotein lipases within plasma and then recycled, or will be lost to other organs (31, 36, 37).

At a constant plasma unesterified labeled EPA concentration (established by infusing [U-¹³C]EPA at a constant rate) (Fig. 1, below), the rate of change of the quantity of labeled esterified n-3 PUFA *i* (*i* = EPA, DPA, or DHA) in plasma is the sum of its rates of appearance and loss:

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

V_{plasma} is plasma volume (ml), $C_{i,es}^*$ (nmol/ml) is plasma concentration of esterified stable isotope labeled *i*, $C_{\text{EPA,unes}}^*$ (nmol/ml) is plasma unesterified [U-¹³C]EPA concentration, *t* is time after infusion has begun (min), $k_{1,i}$ is the steady-state synthesis secretion coefficient of esterified labeled PUFA *i* (nmol/min), and $k_{-1,i}$ is the disappearance rate coefficient (nmol/ml) of the esterified 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.

The following equation (29) was fit to data for each plasma esterified PUFA *i* as a function to time,

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

where *A*, *B*, and *C* are best-fit constants and $t_0 = 0$ min 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, occurs when a steady state is approximated in the different liver metabolic compartments with regard to the labeled PUFAs and their metabolites and when loss from plasma is minimal. The estimated synthesis secretion rate (nmol/min) J_i of esterified PUFA *i* then equals,

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

where $C_{\text{EPA,unes}}$ is the unesterified total EPA plasma concentration (there is no statistically significant effect of infusing the label; Table 1, below).

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}) and half-life $t_{1/2,i}$ (min) of esterified plasma PUFA *i* equal, respectively,

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

and

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

Synthesis secretion rates, J_i , and plasma turnover rates and half-lives were calculated for *i* = EPA, DPA, and DHA by equations 2–5. Data are given as mean \pm SD. Student's *t*-tests were used to determine the significance of difference between means, taken as $P < 0.05$.

RESULTS

Plasma endogenous n-3 PUFA concentrations

Concentrations of unlabeled esterified and unesterified fatty acids in arterial plasma were determined before and after the 2 h [U-¹³C]EPA infusion (Table 1). These concentrations agree with reported concentrations in adult male rats fed the identical diet (29, 30) and showed no significant differences between before and after infusion. This is consistent with the absence of an isotope effect and with steady-state cold concentration conditions throughout the 2 h infusion period.

Labeled n-3 PUFA concentrations in plasma

After the start of [U-¹³C]EPA infusion, a constant unesterified plasma concentration was achieved within 5 min, as illustrated in Fig. 1. No other labeled unesterified fatty acid was detected in plasma at any time during infusion.

In the initial 60 min of infusion, labeled esterified [U-¹³C]EPA, [¹³C]DPA, and [¹³C]DHA could not be detected in arterial plasma. Each was detected at 60 min, after which its concentration increased rapidly, suggesting that steady-state synthesis secretion had been reached in the liver (Table 2). After 60 min, the plasma concentration of esterified [U-¹³C]EPA was always higher than the concentrations of its two labeled elongation products.

Concentrations of esterified [¹³C]n-3 PUFAs in neutral lipid classes

Table 3 summarizes mean esterified concentrations of [U-¹³C]EPA, [¹³C]DPA, and [¹³C]DHA in plasma triacylglycerols, phospholipids, and cholesterol esters at the end of the 120 min infusion period. Esterified [U-¹³C]EPA and esterified [¹³C]DPA were mainly in the triacylglycerol fraction, whereas esterified [¹³C]DHA was divided about equally between the triacylglycerol and phospholipid fractions. Labeled esterified concentrations in cholesteryl ester were negligible.

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

Synthesis secretion rates, turnover rates, and half-lives of esterified EPA, DPA, and DHA from circulating unesterified EPA were calculated from the experimental measurements, using equations 1–4. For equation 1, plasma volume was taken as 26.9 ml/kg body weight for each rat, based on prior measurements under the same experimental conditions (29). Mean rat weight equaled 299.5 ± 7.3 g.

Figure 2A presents the product $V_{\text{plasma}} \times C_{i,es}^*$ plotted against infusion time in one rat infusion study, for each of the three labeled esterified PUFAs *i*. The best-fit curves in the figure were calculated by equation 2 using least-

TABLE 1. Unlabeled unesterified and esterified n-3 PUFA concentrations in arterial plasma before and after infusion [$U\text{-}^{13}\text{C}$]EPA infusion

n-3 PUFA	Unesterified Fatty Acids		Esterified Fatty Acids	
	Before Infusion	After Infusion	Before Infusion	After Infusion
<i>nmol/ml plasma</i>				
α -LNA	13.3 \pm 3.6	14.4 \pm 4.4	13.7 \pm 3.3	14.1 \pm 4.4
EPA	13.7 \pm 4.3	14.2 \pm 3.3	74.3 \pm 10.4	84.4 \pm 28.9
DPA	16.6 \pm 6.5	18.2 \pm 5.2	47.7 \pm 5.8	54.5 \pm 23.5
DHA	24.8 \pm 7.4	26.3 \pm 6.5	190 \pm 14	204 \pm 55

Data are means \pm SD ($n = 6$). No mean after infusion differed significantly from mean prior to infusion ($P > 0.05$).

squares, by Origin 7.0. The slopes (first derivatives of these curves) as a function of time, also calculated using Origin 7.0, are given in Fig. 2B. Their maximal values, $S_{\max,i}$, were estimated for each n-3 PUFA in each study; mean maximal values for the six experiments are summarized in Table 4. The maximum slopes for the EPA, DPA, and DHA curves then were used to calculate their synthesis secretion rates J_i by equation 3, when taking $C_{\text{EPA,unes}}^*$ from individual data points as illustrated in Fig. 1 and $C_{\text{EPA,unes}}$ or total EPA as constant from Table 1. J_i equaled 1.81 and 3.79 nmol/min for DPA and DHA, respectively, equivalent to 2.61 and 5.46 $\mu\text{mol/day}$. J_i was much higher for EPA, equal to 6.55 nmol/min or 9.43 $\mu\text{mol/day}$.

Plasma turnover rates F_i , calculated by equation 4 using values of $C_{i,es}$ from Table 1, equaled 10.4, 4.71, and 2.47 $\times 10^{-3} \text{ min}^{-1}$ for EPA, DPA, and DHA, respectively, corresponding to half-lives (equation 5) of 69.7, 149, and 284 min (Table 4).

DISCUSSION

Whole-body synthesis secretion rates of esterified EPA, DPA, and DHA from circulating unesterified EPA, thus presumably hepatic rates, were determined in unanesthetized male rats fed a standard n-3 PUFA containing diet. [$U\text{-}^{13}\text{C}$]EPA was infused intravenously for 2 h, and rates of

appearance of its esterified products, presumably within VLDLs (36–39), were measured in arterial plasma. A sigmoidal equation was fit by least squares to plasma esterified concentration \times plasma volume data for the [^{13}C] labeled esterified PUFAs, and peak first derivatives $S_{\max,i}$ were obtained to calculate these rates. Esterified labeled PUFAs were identified in plasma after about 60 min of [$U\text{-}^{13}\text{C}$]EPA infusion, suggesting that steady-state liver metabolism and secretion of these tracers had been established at this time. Unesterified labeled DPA and DHA could not be identified in arterial plasma at any time during infusion, suggesting that any tracer that was hydrolyzed from plasma lipids rapidly disappeared. This is consistent with evidence that plasma half-lives of unesterified PUFAs are <1 min in the unanesthetized rat (40).

Synthesis secretion rates of esterified DPA and DHA derived from circulating unesterified EPA equaled 2.61 and 5.46 $\mu\text{mol/day}$, respectively. These rates are much higher than rates estimated by a 5 min intravenous infusion of [$1\text{-}^{14}\text{C}$]EPA, based on the appearance of labeled [^{14}C]DPA and [^{14}C]DHA in the liver, which likely reflects differences between nonsteady state and steady state tracer conditions (M. Igarashi et al., unpublished observations). Similar differences apply to calculated DHA secretion rates from labeled α -LNA (41, 42). The higher DPA than DHA synthesis rate with the 5 min intravenous infusion of [$1\text{-}^{14}\text{C}$]EPA (M. Igarashi et al., unpublished observations) also suggests that once DPA is formed from EPA within the liver, it is directed to further elongation more strongly than to immediate secretion within VLDLs. A similar selectivity may account for why esterified DHA derived from circulating α -LNA is synthesized and secreted at a higher rate than is esterified EPA (see below) (29). Selectivity may be related to comparative rates of β -oxidation within the liver of newly formed EPA, DPA, and DHA (43, 44).

The brain incorporates unesterified DHA from plasma at a rate of 0.23 $\mu\text{mol/day}$ in rats on the same diet (45).

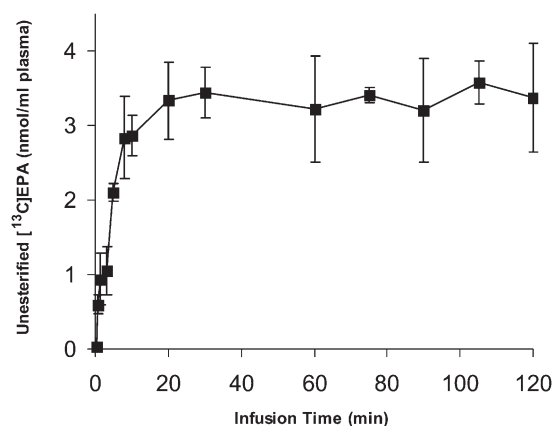


Fig. 1. Time course of arterial plasma unesterified [$U\text{-}^{13}\text{C}$]EPA during intravenous [$U\text{-}^{13}\text{C}$]EPA infusion. Unanesthetized rats were infused with 3 $\mu\text{mol}/100\text{g}$ [$U\text{-}^{13}\text{C}$]EPA during 2 h, and unesterified [$U\text{-}^{13}\text{C}$]EPA concentrations were analyzed in plasma at different time points. Data are mean \pm SD ($n = 6$).

TABLE 2. [^{13}C]labeled esterified n-3 PUFA concentrations in arterial plasma during [$U\text{-}^{13}\text{C}$] EPA infusion

n-3 PUFA	Infusion Time (min)				
	60	75	90	105	120
	<i>nmol/ml plasma</i>				
EPA	1.39 \pm 0.21	2.57 \pm 0.51	4.54 \pm 0.66	8.12 \pm 2.02	9.73 \pm 2.29
DPA	0.36 \pm 0.17	0.94 \pm 0.24	1.55 \pm 0.53	2.38 \pm 0.98	2.68 \pm 0.81
DHA	0.80 \pm 0.29	2.17 \pm 0.69	3.39 \pm 0.93	4.69 \pm 0.53	5.82 \pm 0.56

Data are means \pm SD ($n = 6$).

TABLE 3. Concentrations of esterified [^{13}C] labeled n-3 PUFAs in stable plasma lipids at 120 min after [^{13}C]EPA infusion

n-3 PUFA	Triacylglycerol	Phospholipids	Cholesteryl Ester
	<i>nmol/ml plasma</i>		
EPA	8.86 \pm 2.03	0.58 \pm 0.21	0.18 \pm 0.12
DPA	2.04 \pm 0.51	0.48 \pm 0.21	ND
DHA	3.00 \pm 0.59	2.51 \pm 0.30	0.10 \pm 0.05

Data are means \pm SD ($n = 6$). ND, not determined.

This incorporation rate equals the rate of DHA loss by brain metabolism, since DHA cannot be synthesized de novo, is not elongated from any α -LNA or EPA that is taken up by brain from plasma (M. Igarashi et al., unpublished observations) (30, 39, 45–49). Thus, the DHA synthesis secretion rate from EPA is 5.46/0.23 = 24 times the brain DHA consumption rate. Since liver-synthesized DHA can get into brain and retina after being hydrolyzed from circulating lipoproteins (29, 50), hepatic synthesis has the potential of supplying the brain's DHA. Additionally, the rat heart cannot synthesize either α -LNA or EPA to DHA, since it lacks elongase 2 (M. Igarashi et al., unpublished observations) (10), so that in the absence of dietary DHA, liver synthesis is the only source of heart DHA. These observations indicate that EPA could exert a central or cardiac action via the DHA formed from it in the liver.

Under identical conditions when [^{13}C] α -LNA was infused intravenously for 2 h, whole-body rates of DPA and

DHA synthesis secretion from unesterified α -LNA equaled 6.27 and 9.84 $\mu\text{mol/day}$, respectively, and EPA synthesis from unesterified α -LNA equaled 8.40 $\mu\text{mol/day}$ (29). Therefore, the net DHA synthesis secretion rate from circulating unesterified α -LNA plus EPA equals 5.56 + 9.84 = 15.3 $\mu\text{mol/day}$, 15.3/0.23 = 66 times the brain DHA consumption rate. In view of this high ratio, dietary EPA and α -LNA could supply sufficient circulating DHA to maintain brain and heart DHA homeostasis in the rat.

The diet in this study contained 4.8 $\mu\text{mol/g}$ α -LNA, 1.9 $\mu\text{mol/g}$ EPA, and 2.2 $\mu\text{mol/g}$ DHA (30). Assuming a daily ingestion rate of 15 g diet/day (51), a rat consumed 72 $\mu\text{mol/day}$ α -LNA, 29 $\mu\text{mol/day}$ EPA, and 33 $\mu\text{mol/day}$ DHA. Ignoring recycling of labeled esterified EPA and DPA, 5.46/(29 + 8.4) = 15% of dietary EPA was converted to DHA per day, since dietary α -LNA was converted EPA (8.40 $\mu\text{mol/day}$). With regard to α -LNA, the fractional conversion to DHA ignoring recycling would equal 9.84/72 = 14%. Conversion rates and fractions would be elevated if DHA were removed from the diet due to increased expression of liver conversion enzymes (41, 52, 53).

Rats are better converters of DHA from its precursors than are humans, since activities of liver elongation and desaturation enzymes are higher in rats than in humans (53, 54). The liver synthesis rates of DHA from α -LNA and EPA are uncertain in humans. In humans, dietary α -LNA and/or EPA is reported to be converted to DHA at fractional rates of 0–11%, a very wide range, using methods involving collection of heavy isotope-labeled exhaled CO_2 or compartmental isotopic analysis (23, 55–57). It would be of interest to estimate these rates with our direct heavy isotope infusion method in humans under different dietary and experimental conditions. Knowing these rates might help to arrive at a consensual recommendation for daily EPA plus DHA consumption, which currently ranges, depending on the expert committee, from 0.1–1.6 g/day/2000 kcal (0.05–0.72% kcal) (25–28).

In humans, dietary supplementation with α -LNA or EPA does not markedly alter plasma DHA levels (58–60), and the same phenomenon was noted in rats (61). These observations have led to the conclusion that DHA must be ingested directly to increase the DHA content of brain and other organs and thus whole-body n-3 PUFA status (58). Nevertheless, significant differences in plasma and brain DHA composition, and in brain DHA consumption rates, were reported in relation to differences in dietary n-3 PUFA content and composition in other rat studies (45, 53, 62). In view of the rat liver's ability to convert α -LNA or EPA to DHA, a constant DHA concentration following precursor supplementation may mean that body organs consume DHA at higher rates with supplementation, and/or that liver metabolism is downregulated to maintain plasma DHA within a homeostatic range (cf. equation 1).

The calculated plasma half-lives of esterified EPA, DPA, and DHA equaled 69.7, 149, and 284 min, respectively, consistent with respective half-lives of 80, 118, and 200 min that were calculated by infusing unesterified [^{13}C] α -LNA under comparable dietary conditions (29). In contrast, half-lives of EPA, DPA, and DHA in humans were

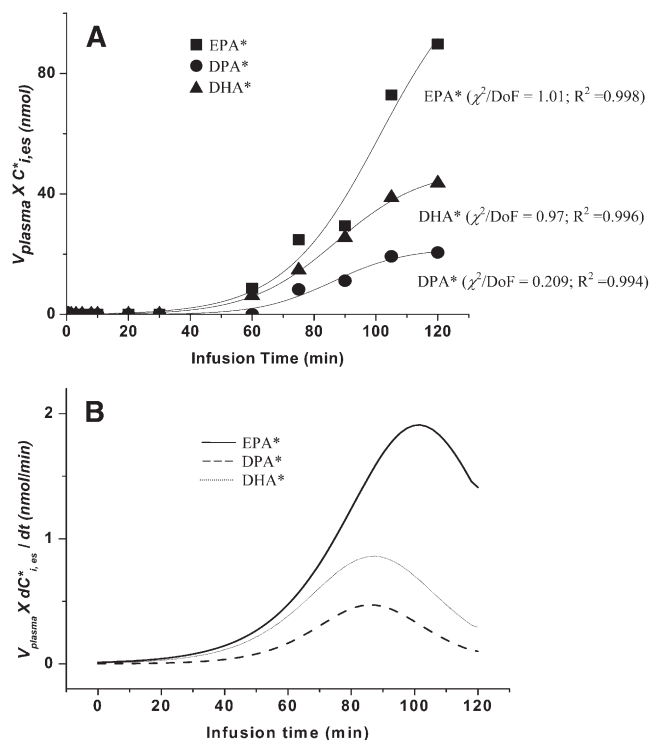


Fig. 2. A: Arterial plasma concentrations of esterified [^{13}C]labeled n-3 PUFAs \times plasma volume plotted against time for an experiment in which 3 $\mu\text{mol}/100\text{g}$ [^{13}C]EPA was infused intravenously for 120 min in one rat. Best-fit sigmoidal curves were estimated using equation 2. B: Curves representing first derivatives of curves for EPA, DPA, and DHA in Fig. 2A. Asterisk represents [^{13}C]labeled n-3 PUFAs; DoF, degrees of freedom.

TABLE 4. Synthesis secretion parameters obtained with [U-¹³C]EPA infusion

n-3 PUFA, <i>i</i>	$S_{max,i}$ $nmol\ min^{-1}$	f_i $nmol\ min^{-1}$	Daily Secretion Rate $\mu mol\ day^{-1}$	F_i $min^{-1} \times 10^{-3}$	$t_{1/2,i}$ min
EPA	1.53 ± 0.48	6.55 ± 1.31	9.43 ± 1.89	10.4 ± 2.3	69.7 ± 18.1
DPA	0.43 ± 0.09	1.81 ± 0.59	2.61 ± 0.86	4.71 ± 0.67	149 ± 18
DHA	0.90 ± 0.10	3.79 ± 0.48	5.46 ± 0.69	2.47 ± 0.33	284 ± 35

Data are means ± SD (*n* = 6).


calculated as 67, 58, and 20 h, respectively, using compartmental analysis after feeding [¹³C]α-LNA (23). The shorter half-lives in rats may reflect 2.5-fold higher rates of energy and lipid metabolism in rats than humans (63, 64) or the fact that we considered turnover only of esterified plasma PUFAs.

The differences in rat plasma half-lives of esterified EPA, DPA, and DHA may reflect differences in their distribution in neutral lipid classes (Tables 3 and 4). In rat plasma, the half-life of VLDL-TG is about 50 min (65), while that of VLDL-DHA equals 13.7 h (66). Another possible explanation for the different half-lives is that EPA undergoes more rapid β-oxidation than DHA within body organs, including liver and muscle (44). It has a higher affinity than DHA for carnitine palmitoyltransferase-1, which transfers acyl groups from the acyl-CoA pool into mitochondria for β-oxidation (43, 67).

In our previous study with [U-¹³C]α-LNA infusion, esterified [¹³C]α-LNA increased monotonically in arterial plasma after 60 min of infusion, which was roughly the case for esterified [¹³C]EPA in this study (Fig. 2A). Both studies are consistent with steady state hepatic synthesis-secretion requiring about an hour to be established in the rat.

Most secreted esterified [¹³C]EPA and [¹³C]DPA during [U-¹³C]EPA infusion was in circulating triacylglycerol, whereas esterified [¹³C]DHA was about equally divided between triacylglycerol and phospholipids (Table 3). In comparison, following a 5 min intravenous infusion of [1-¹⁴C]EPA in unanesthetized rats, 75% of [1-¹⁴C]EPA in the liver was in triacylglycerol, whereas 45% of hepatic [¹⁴C]DHA was in phospholipid (M. Igarashi, unpublished observations). Unlike circulating triacylglycerol and phospholipid, cholesteryl ester was minimally labeled (Table 3) during [U-¹³C]EPA infusion. Together, the observations confirm that the liver channels plasma-derived unesterified n-3 PUFAs and their elongation products into different stable lipids (68), which are assembled in VLDLs and secreted into blood (36–39).

In summary, whole-body (presumably liver) synthesis secretion rates of esterified DPA and DHA from circulating unesterified EPA were quantified by infusing [U-¹³C] EPA intravenously for 2 h in unanesthetized rats fed an n-3 PUFA-containing diet. The estimated DHA synthesis rate from EPA was 5.46 μmol/day, 24 times the brain DHA consumption rate, 0.23 μmol/day (45, 69). To the extent that similar conversion occurs in humans, some reported effects of dietary EPA on the heart and brain in humans (7, 11–14) could be mediated by the DHA that is formed from it. More generally, the heavy isotopic infusion method could be used to quantify liver synthesis secretion of DHA

under different experimental conditions in rodents and humans in relation to health and disease. 

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