

Quantifying conversion of linoleic to arachidonic and other n-6 polyunsaturated fatty acids in unanesthetized rats

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Abstract Isotope feeding studies report a wide range of conversion fractions of dietary shorter-chain polyunsaturated fatty acids (PUFAs) to long-chain PUFAs, which limits assessing nutritional requirements and organ effects of arachidonic (AA, 20:4n-6) and docosahexaenoic (DHA, 22:6n-3) acids. In this study, whole-body (largely liver) steady-state conversion coefficients and rates of circulating unesterified linoleic acid (LA, 18:2n-6) to esterified AA and other elongated n-6 PUFAs were quantified directly using operational equations, in unanesthetized adult rats on a high-DHA but AA-free diet, using 2 h of intravenous [¹³C]LA infusion. Unesterified LA was converted to esterified LA in plasma at a greater rate than to esterified γ -linolenic (γ -LNA, 18:3n-6), eicosatrienoic acid (ETA, 20:3n-6), or AA. The steady-state whole-body synthesis-secretion (conversion) coefficient k_i^* to AA equaled $5.4 \times 10^{-3} \text{ min}^{-1}$, while the conversion rate (coefficient \times concentration) equaled 16.1 $\mu\text{mol/day}$. This rate exceeds the reported brain AA consumption rate by 27-fold. As brain and heart cannot synthesize significant AA from circulating LA, liver synthesis is necessary to maintain their homeostatic AA concentrations in the absence of dietary AA. The heavy-isotope intravenous infusion method could be used to quantify steady-state liver synthesis-secretion of AA from LA under different conditions in rodents and in humans.—Gao, F., D. Kiesewetter, L. Chang, S. I. Rapoport, and M. Igarashi. **Quantifying conversion of linoleic to arachidonic and other n-6 polyunsaturated fatty acids in unanesthetized rats.** *J Lipid Res.* 2010. 51: 2940–2946.

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Linoleic acid (LA, 18:2n-6), a dietary essential n-6 polyunsaturated fatty acid (PUFA), is a precursor of arachi-

donic acid (AA, 20:4n-6) (1). Conversion of LA to AA occurs by sequential $\Delta 6$ desaturation, elongation, and $\Delta 5$ desaturation reactions, passing through γ -linolenic acid (γ -LNA, 18:3n-6) and eicosatrienoic acid (ETA, 20:3n-6) intermediates (2). AA, which largely is esterified at the stereospecifically numbered (*sn*)-2 position of membrane phospholipids, is abundant in brain, heart, liver, testes, and kidney (3). As it and its eicosanoid metabolites have multiple actions within different organs (4, 5), the AA concentration must be maintained within homeostatic limits and in balance with the concentration of the long chain n-3 PUFA, docosahexaenoic acid (DHA, 22:6n-3), for optimal organ function (6).

Omnivores can obtain AA and DHA from fish and meat sources, but vegetarians and vegans must synthesize these PUFAs from their shorter chain plant-derived precursors in the diet, including LA and α -linolenic acid (α -LNA), respectively. Nevertheless, there is no general agreement whether AA or DHA is required in the diet and, if so, to what extent, as whole-body conversion estimates from their precursors range from 1 to 10% (7–10).

One way to address this issue is to assess the *in vivo* ability of the body (mainly liver) to synthesize and secrete esterified AA and DHA from their respective precursors under different dietary conditions (7). Synthesis can occur following diffusion of the circulating unesterified precursor into the liver, activation to its acyl-CoA intermediate, and further activation and esterification into “stable lipids” (phospholipids, triacylglycerol, or cholesteryl ester) within very low density lipoproteins (VLDLs), which later are secreted into blood (11–15). Over time, the esterified

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Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatrienoic acid; GC, gas chromatography; LA, linoleic acid; α -LNA, α -linolenic acid; γ -LNA, γ -linolenic acid; MS, mass spectrometry; PFB, pentafluorobenzyl; *sn*, stereospecifically numbered.

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PUFAs in the VLDLs may be recycled back into the liver via lipoprotein receptors, hydrolyzed by lipoprotein lipases within plasma and adipose tissue, and then recycled, or they may be lost to other organs and undergo β -oxidation (13, 16, 17).

The brain, kidney, and testes, as well as liver, can synthesize AA and DHA from their shorter-chain precursors, since they, like the liver, express the enzymes for a complete synthesis (18–20). However, they do so at much lower rates, because their enzyme activities are at lower levels (21–24). Rat heart and brain have low capacities of synthesizing DHA and possibly AA from circulating unesterified α -LNA or LA, respectively, due to their low expression of synthesizing enzymes (23, 25–27).

It is not clear whether the liver has a higher efficiency of conversion of α -LNA to DHA than of LA to AA, but data suggest this to be the case (28–32). Exact rates of synthesis in the intact organism are not known, yet knowing them would help to estimate daily dietary requirements of α -LNA and LA compared with their elongated products and would provide a firmer basis for making nutritional recommendations (8, 9).

To address this issue, we developed a heavy-isotope intravenous infusion method to determine rates of whole-body, steady-state DHA synthesis from circulating unesterified α -LNA and eicosapentaenoic acid (EPA, 20:5n-3) in the unanesthetized rat (11, 12); this method might be extended for human studies. Briefly, the method involves infusing the isotopically labeled precursor intravenously at a constant rate, sampling arterial plasma concentrations of unesterified and esterified labeled precursor and elongation products as a function of time, and then applying operational equations to calculate steady-state synthesis-secretion coefficients and rates of the esterified products when taking plasma volume into account. With this method, we reported that whole-body steady-state rate of conversion of unesterified α -LNA to esterified DHA, in rats fed a high DHA-containing diet, equaled 9.84 $\mu\text{mol}/\text{day}$.

To compare this rate to the rate of AA synthesis from LA in rats fed the same diet, in the present study we infused [U - ^{13}C]LA intravenously for 2 h and determined rates of appearance of esterified AA in arterial plasma, as well as of other n-6 PUFA elongation products, γ -LNA and ETA. We applied our operational equations to estimate steady-state synthesis coefficients and rates, and plasma turnovers and half-lives (11, 12).

MATERIALS AND METHODS

Materials

[U - ^{13}C]LA was purchased from Cambridge Isotope Laboratories (Andover, MA), and was purified by high-performance liquid chromatography (HPLC) (Agilent, Palo Alto, CA) with a Symmetry[®] C18 column (9.2 \times 250 mm, 5 μm , Agilent) before use. Purity was determined to be >95% by HPLC, and the concentration of purified [U - ^{13}C]LA was determined by gas chromatography (GC). Diheptadecanoate phosphatidylcholine (Di-17:0 PC), free heptadecanoic acid (17:0), n-6 PUFA standards (LA, γ -LNA, ETA, and AA), 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 Sigma-Aldrich.

Animals

This protocol was approved by the Animal Care and Use Committee of the Eunice Kennedy Shriver 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). Adult male Fischer-344 (CDF) rats (4 months old) were purchased from Charles River Laboratories (Portage, MI) and housed in a facility with regulated temperature, humidity, and a 12 h light/12 h dark cycle. They were acclimated for one 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; it had 4% by weight crude fat. Its fatty acid composition has been reported (27). Of n-3 PUFAs, α -LNA, EPA, and DHA contributed 5.1%, 2.0%, and 2.3% of total fatty acid, respectively, whereas n-6 PUFAs LA and AA contributed 47.9% and 0.02%, respectively. The surgery and infusion procedures have been described in detail (11). The rats were provided food the night before surgery. During the surgery, recovery from anesthesia (3–4 h), and the 2 h infusion period, they did not have access to food.

A rat was anesthetized with 1–3% halothane, and polyethylene catheters (PE 50, Clay Adams, Becton Dickinson, Sparks, MD) filled with heparinized isotonic saline (100 IU/ml) were surgically implanted in the right femoral artery and vein. After it had recovered from anesthesia, the rat was infused via the femoral vein catheter with 3 $\mu\text{mol}/100$ g body weight [U - ^{13}C]LA, dissolved in 5 mM 4-(2-hydroxy-methyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4) containing 50 mg/ml fatty acid-free bovine serum albumin, at a constant rate of 0.021 ml/min. During the 2 h, body temperature was maintained at 36–38°C using a feedback-heating element (YSI Indicating Temperature Controller, Yellow Springs Instruments, Yellow Springs, OH), and 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, 0.5, 1.0, 2.0, 3.0, 5.0, 8.0, 10.0, 20, 30, 60, and 90 min of infusion. 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.

Lipid extraction and PFB derivatization

Plasma lipid extraction and pentafluorobenzyl (PFB) derivatization procedures have been reported (11). 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 ester) containing esterified fatty acids were extracted with hexane twice. 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) at 70°C to release their free fatty acids, which were extracted with hexane and dried under N_2 . A freshly made PFB derivatizing reagent (PFB: diisopropylamine: acetonitrile, 10:100:1000) was added to the sample residue of hexane extraction and shaken for 15 min at room temperature. The sample was evaporated again to dryness under N_2 and redissolved in 100 μl of hexane.

GC/MS analysis of fatty acid PFB esters

The fatty acid PFB esters from the plasma samples were analyzed by GC/MS as described (11). Nonlabeled and labeled n-6 PUFAs were monitored by selected ion mode (SIM) of the base

peak (M-PFB). The concentration of each n-6 PUFA was quantified by relating its peak area to the area of the internal standard, using an experimentally determined response factor for each fatty acid.

Calculations

Steady-state conversion rates of circulating unesterified LA to esterified AA and other elongated n-6 PUFAs were calculated by the method of Gao et al. (11, 12). At steady-state secretion, the rate of change of labeled esterified n-6 PUFA i ($i = \text{LA}, \gamma\text{-LNA}, \text{ETA}, \text{or AA}$) in plasma is the sum of its rates of appearance and loss according to the following differential equation with constant coefficients,

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

where V_{plasma} is plasma volume (ml); $C_{i,es}^*$ (nmol/ml) is plasma concentration of esterified stable isotope labeled i ; $C_{LA,unes}^*$ (nmol/ml) is plasma unesterified [$U\text{-}^{13}\text{C}$]LA concentration; t is time (min); $k_{1,i}$ is the synthesis-secretion rate coefficient (ml/min) of conversion of unesterified labeled LA to esterified labeled PUFA i [which relates the esterified plasma PUFA concentration to the unesterified LA concentration times plasma volume]; and $k_{-1,i}$ is the disappearance rate coefficient (ml/min) of esterified labeled PUFA i from plasma. There is no isotope effect, so that $k_{1,i}$ and $k_{-1,i}$ are valid for unlabeled as well as labeled PUFAs.

As reported (11, 12), esterified labeled PUFAs during intravenous labeled precursor infusion start to appear in rat plasma after a 30–60 min delay, after which their concentrations rapidly rise but later tend to rise more slowly due to the loss from plasma (see Fig. 2). To estimate their steady-state rates of secretion, we fit the following sigmoidal equation using nonlinear least squares (Origin 7.0 software, Originlab, Northampton, MA) to esterified concentration \times plasma volume data plotted against time, for each esterified n-6 PUFA i (see Fig. 2),

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

where A, B, C are best-fit constants, and $t_0 = 0$ is time (min) at the beginning of infusion and t is time of infusion.

The first derivative of equation 2 was determined for each esterified PUFA i in each rat as a function of time. Its maximum value, $S_{\text{max},i}$ nmol/min, occurs when steady-state liver secretion is best approximated and was taken to represent the left-hand side of equation 1. A steady-state synthesis-secretion coefficient, k_i^* (min^{-1}), equal to $k_{1,i}/V_{\text{plasma}}$, was calculated as,

$$k_i^* = \frac{S_{\text{max},i}}{C_{LA,unes}^* \times V_{\text{plasma}}} \quad (\text{Eq. 3})$$

k_i^* is proportional to the net rate of the elongation and desaturation reactions, and unlike $k_{1,i}$, is independent of plasma volume; thus, it is a marker for evaluating net efficiency of synthesis.

The synthesis-secretion rate (nmol/min) J_i of unlabeled esterified PUFA i from unlabeled unesterified LA equals,

$$J_i = S_{\text{max},i} \times \frac{C_{LA,unes}}{C_{LA,unes}^*} \quad (\text{Eq. 4})$$

where $C_{LA,unes}$ is the unesterified total (labeled and unlabeled) plasma LA concentration.

TABLE 1. Unlabeled concentrations and concentration ratios of unesterified and esterified PUFAs in arterial plasma before [$U\text{-}^{13}\text{C}$]LA infusion

n-6 PUFA	Unesterified PUFA	Esterified PUFA	Ratio of unesterified to esterified PUFA
	nmol/ml plasma	nmol/ml plasma	
LA (18:2 n-6)	219 \pm 25	961 \pm 80	0.23 \pm 0.02
γ -LNA (18:3 n-6)	3.4 \pm 1.6	25.3 \pm 4.3	0.14 \pm 0.04
ETA (20:3 n-6)	8.7 \pm 4.2	60.6 \pm 9.8	0.20 \pm 0.06
AA (20:4 n-6)	27.8 \pm 6.9	809 \pm 51	0.034 \pm 0.011
DTA (22:4 n-6)	ND	16.1 \pm 5.9	–
DPA (22:5 n-6)	ND	10.8 \pm 3.2	–

Data are mean \pm SD ($n = 5$). AA, arachidonic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; ETA, eicosatrienoic acid; LA, linoleic acid; γ -LNA, γ -linolenic acid; ND, not detected.

Because plasma concentration $C_{i,es}$ (nmol/ml) of esterified PUFA i was constant during the study, turnover F_i (min^{-1}) and half-life $t_{1/2,i}$ (min) of esterified plasma PUFA i due explicitly to conversion from unesterified LA equal, respectively,

$$F_i = \frac{J_i}{C_{i,es} \times V_{\text{plasma}}} \quad (\text{Eq. 5})$$

and

$$t_{1/2,i} = \frac{0.693}{F_i} \quad (\text{Eq. 6})$$

Steady-state synthesis-secretion rates J_i and plasma turnover and half-lives were calculated for $i = \text{LA}, \gamma\text{-LNA}, \text{ETA}, \text{and AA}$ by equations 1–6.

Data are given as means \pm SD.

RESULTS

Plasma n-6 PUFA concentrations

Concentrations and ratios of unlabeled esterified and unesterified n-6 PUFAs in arterial plasma were determined before the 2 h [$U\text{-}^{13}\text{C}$]LA infusion (Table 1) and agree

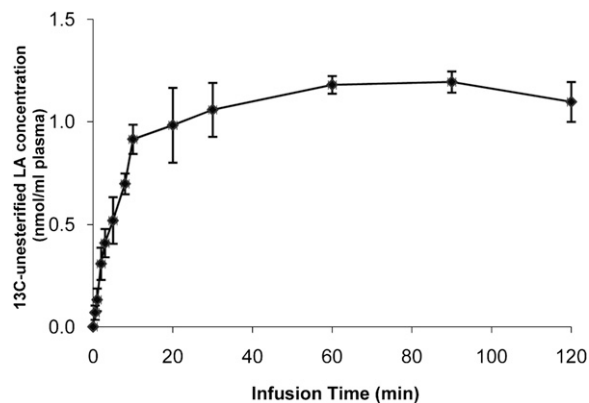


Fig. 1. Arterial plasma unesterified [$U\text{-}^{13}\text{C}$]LA concentration during intravenous [$U\text{-}^{13}\text{C}$]LA infusion. Unanesthetized rats were infused with 3 $\mu\text{mol}/100$ g [$U\text{-}^{13}\text{C}$]LA during 2 h, and unesterified [$U\text{-}^{13}\text{C}$]LA concentrations were analyzed in plasma at different times. Data are means \pm SD ($n = 5$). Abbreviation: LA, linoleic acid.

TABLE 2. [¹³C]labeled esterified n-6 PUFA concentrations in arterial plasma during intravenous infusion of [U-¹³C]LA

n-6 PUFA ^a	Infusion Time			
	30 min	60 min	90 min	120 min
	<i>Concentration (nmol/ml plasma)</i>			
LA (18:2 n-6)	5.66 ± 1.00	7.05 ± 0.55	8.87 ± 1.03	11.1 ± 1.8
γ-LNA (18:3 n-6)	ND	0.60 ± 0.07	0.84 ± 0.13	1.13 ± 0.26
ETA (20:3 n-6)	ND	0.08 ± 0.06	0.36 ± 0.05	0.45 ± 0.10
AA (20:4 n-6)	ND	0.08 ± 0.02	0.23 ± 0.04	0.30 ± 0.04

Data are mean ± SD (n = 5). AA, arachidonic acid; ETA, eicosatrienoic acid; LA, linoleic acid; γ-LNA, γ-linolenic acid; ND, not detected.

^aIndicates the ¹³C-labeled n-6 PUFA.

with reported values in adult male rats fed the same diet (27). The concentrations of unesterified LA, γ-LNA, ETA, and AA equaled 219 ± 25, 3.4 ± 1.6, 8.7 ± 4.2, and 27.8 ± 6.9 nmol/ml plasma, respectively, whereas their respective esterified concentrations equaled 961 ± 80, 25.3 ± 4.3, 60.6 ± 9.8, and 809 ± 51 nmol/ml plasma. Mean unesterified to esterified concentration ratios equaled 0.23, 0.14, 0.20,

and 0.034 for LA, γ-LNA, ETA, and AA, respectively (Table 1). The unesterified LA concentration was used to calculate J_i for each esterified PUFA i by equation 4, and J_i was used to calculate respective plasma half-lives and turnovers by equations 5 and 6.

[¹³C]labeled unesterified n-6 PUFA concentrations in plasma

A constant unesterified arterial plasma concentration was achieved within 10 min after the start of intravenous [U-¹³C]LA infusion (Fig. 1). No other labeled unesterified fatty acid was detected in plasma during the 2 h infusion. The mean concentration of unesterified [U-¹³C]LA, $C_{LA,unes}^*$, equaled 1.10 ± 0.10 nmol/ml.

Esterified [¹³C]LA was detected in plasma 30 min after infusion had started. Esterified longer-chain n-6 PUFAs could be detected at 30–60 min, after which their concentrations increased rapidly with time but then began to plateau. At all times, esterified plasma [¹³C]LA was higher than the concentration of each of its three esterified labeled elongation products (Table 2).

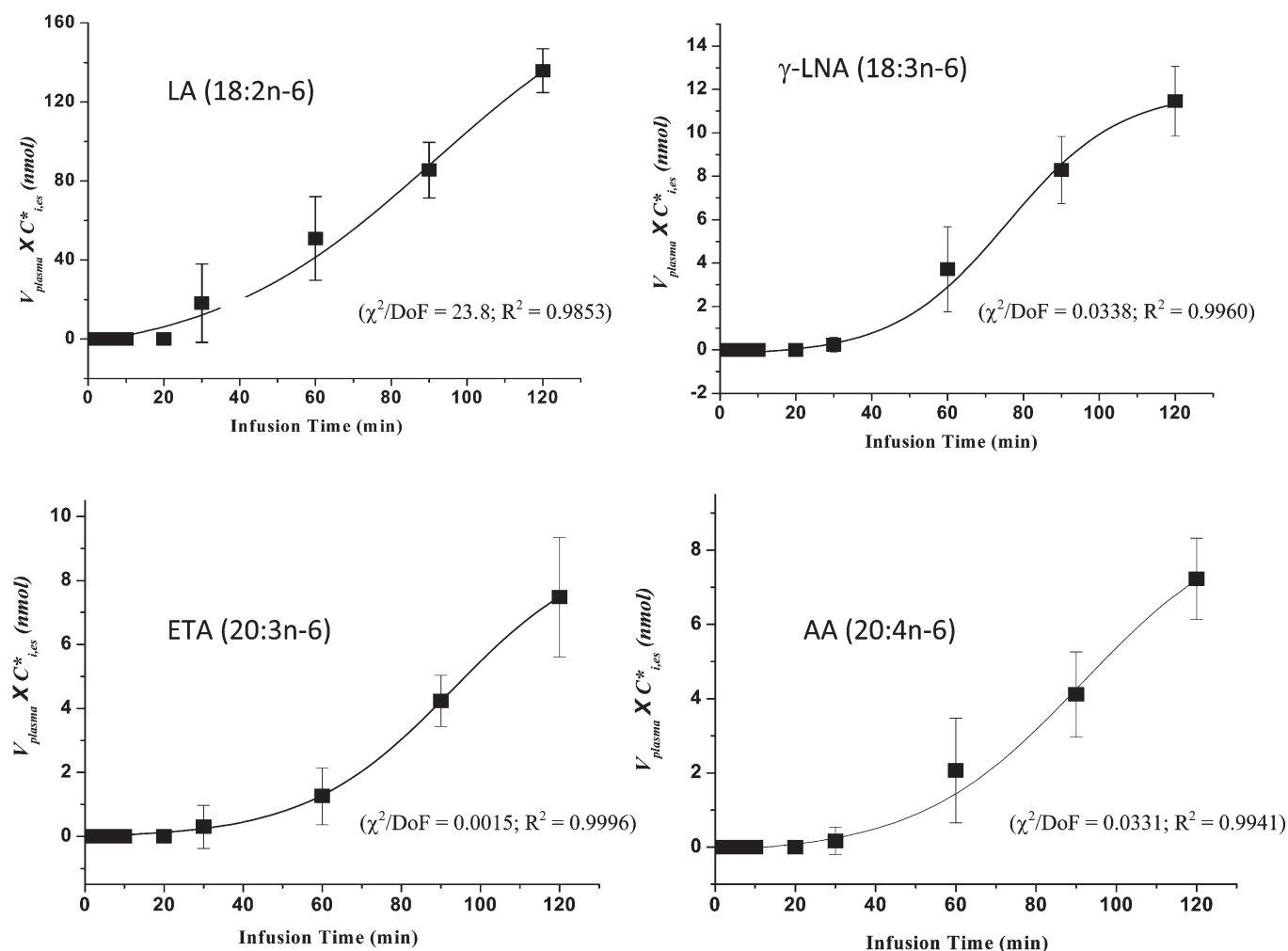


Fig. 2. Sigmoidal curves were determined using Origin software (see “Materials and Methods”) by fitting equation 2 to plasma concentration × volume data plotted against time of unesterified labeled PUFAs. Best-fit parameters: χ^2 , Chi square; DoF, degrees of freedom; R^2 , coefficient of determination. Results summarize five experiments. Abbreviations: AA, arachidonic acid; ETA, eicosatrienoic acid; LA, linoleic acid; γ-LNA, γ-linolenic acid.

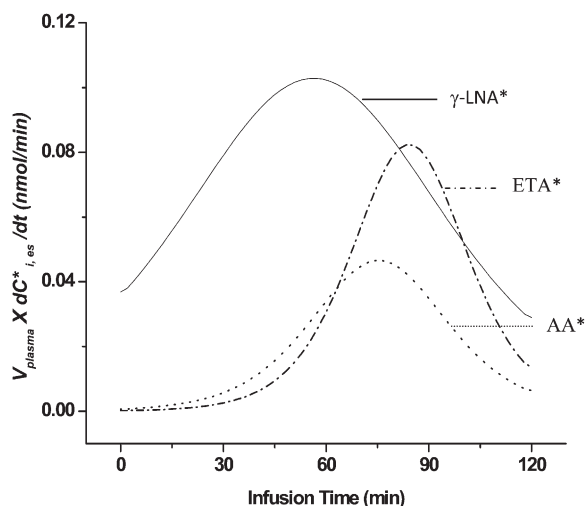


Fig. 3. First derivatives of plasma concentration \times volume curves (see Fig. 2) for labeled (*) γ -LNA, ETA, and AA from one rat experiment. Means of derived peaks $S_{max,i}$ are summarized for five experiments in Table 3. Abbreviations: AA, arachidonic acid; ETA, eicosatrienoic acid; γ -LNA, γ -linolenic acid.

N-6 PUFA synthesis-secretion coefficients and rates, turnovers, and half-lives

Synthesis-secretion coefficients and rates, turnovers, and half-lives of esterified LA, γ -LNA, ETA, and AA were calculated from experimental measurements using equations 1–6. V_{plasma} was taken as 26.9 ml/kg body weight, based on prior measurements under the same experimental conditions (11). Mean body weight equaled 305.3 ± 1.8 g.

Figure 2 presents plots of $V_{plasma} \times C_{i,es}$ against infusion time for each of the four labeled esterified n-6 PUFAs. Nonlinear least-squares was used with Origin 7.0 software to determine best-fit curves of equation 2 for each n-6 PUFA, and best-fit parameters are noted with each curve fit. Esterified [13 C] γ -LNA, [13 C]ETA and [13 C]AA started to appear in arterial plasma at about 30 min. After 60 min, their concentrations rose rapidly and then more slowly. The concentration \times plasma volume curve for esterified [13 C]LA continued to rise approximately linearly after about 30 min, more rapidly than the elongated products.

Rates of change (first derivatives) of concentration \times plasma volume curves were plotted as a function of time for the elongated products, and their maximal values $S_{max,i}$ were determined (**Fig. 3**). Because the curve for esterified [13 C]LA did not start to level off during the 120 min infusion (Fig. 2), we estimated $S_{max,LA}$ from the slope of its linear plot

in the figure. Mean values of $S_{max,i}$ from five experiments are summarized in **Table 3**.

Values of $S_{max,i}$ as determined from the first derivatives of the labeled esterified LA, γ -LNA, ETA, and AA curves were used to calculate synthesis-secretion coefficients k_i^* by equation 3 and rates J_i by equation 4, when taking $C_{LA, unes}^*$ at 2 h (Table 3). Mean values of k_i^* for the five infusion studies equaled 127, 13.4, 10.8, and $5.4 \times 10^{-3} \text{ min}^{-1}$ for LA, γ -LNA, ETA and AA, respectively. Corresponding values of J_i equaled 277, 29.4, 20.7, and 11.2 nmol/min, equivalent to 400, 42.4, 29.8, and 16.1 $\mu\text{mol/day}$. Of net esterified PUFAs derived from unesterified plasma LA, 81% was LA, whereas 19% represented LA elongation products.

Plasma turnover F_i due to synthesis from circulating unesterified LA, calculated by equation 5 using values of $C_{i,es}$ from Table 1, equaled to 34.1, 138, 43.5, and $1.70 \times 10^{-3} \text{ min}^{-1}$ for LA, γ -LNA, ETA, and AA, respectively, corresponding to half-lives (equation 6) of 21.5, 5.4, 17.2, and 427 min.

DISCUSSION

Whole-body (largely liver) steady-state synthesis-secretion coefficients and rates of conversion of circulating unesterified LA to esterified n-6 PUFAs were estimated by infusing unesterified [13 C]LA intravenously for 2 h in unanesthetized 4–5-month-old male rats. The rats had been fed a diet enriched in DHA (2.3% of total fatty acid), with minimal AA (0.02%) and with LA as 47.9% of the total fatty acid concentration. Esterified [13 C]LA and longer-chain labeled n-6 PUFA products appeared in plasma after about 30 min. Their concentrations increased rapidly after 60 min, but later started to plateau due to loss from the vascular compartment. Unesterified labeled γ -LNA, ETA, and AA could not be detected in plasma during [13 C]LA infusion, suggesting that any free labeled longer-chain n-6 PUFA that had been hydrolyzed from plasma VLDLs disappeared rapidly from blood, consistent with plasma half-lives of <1 min for circulating unesterified fatty acids in the unanesthetized rat (33). Of the esterified PUFAs converted from LA, 81% was esterified LA, whereas 19% consisted of its elongated n-6 products. Only 3.4% became esterified AA, indicating a relative inefficiency of conversion.

As reported for conversion of α -LNA to DHA (11), a significant delay of about 30 min occurred before labeled esterified n-6 PUFAs, including AA, appeared in blood during infusion of [13 C]LA. This delay, and additional

TABLE 3. Mean parameters for synthesis-secretion of n-6 PUFAs from LA in rat liver

n-6 PUFA, i	$S_{max,i}$ nmol min^{-1}	k_i^* $\text{min}^{-1} \times 10^{-3}$	J_i nmol min^{-1}	Daily Secretion		
				Rate $\mu\text{mol day}^{-1}$	F_i $\text{min}^{-1} \times 10^{-3}$	$t_{1/2,i}$ min
LA (18:2 n-6)	1.13 ± 0.37	127 ± 48	277 ± 71	400 ± 102	34.1 ± 10.1	21.5 ± 6.4
γ -LNA (18:3 n-6)	0.12 ± 0.04	13.4 ± 5.8	29.4 ± 12.7	42.4 ± 18.3	138 ± 44	5.4 ± 1.8
ETA (20:3 n-6)	0.10 ± 0.02	10.8 ± 2.0	20.7 ± 3.2	29.8 ± 4.6	43.5 ± 15.4	17.2 ± 5.6
AA (20:4 n-6)	0.05 ± 0.01	5.4 ± 0.8	11.2 ± 2.3	16.1 ± 3.4	1.70 ± 0.44	427 ± 116

Data are mean \pm SD ($n = 5$). AA, arachidonic acid; ETA, eicosatrienoic acid; LA, linoleic acid; γ -LNA, γ -linolenic acid.

time to reach a peak secretion rate (considered to approximate the steady-state rate), likely reflected the time needed for the labeled PUFAs to be elongated and desaturated within different liver compartments, esterified within triacylglycerols, phospholipids, and cholesteryl esters in those compartments, and then packaged and secreted within VLDLs (11, 13, 34, 35). Because of these "lag times," it was not possible to simply apply the steady-state differential equation 1 to the concentration \times plasma volume data over the period of study.

However, by applying the sigmoidal equation 2 to the data, we could represent the substantial delay, the slow then rapid rise in concentration, and the later tendency to plateau as material disappeared from plasma to calculate useful kinetic parameters. By taking the peak first derivative of the best-fit curves (e.g. Fig. 3), we calculated an $S_{max,i}$ to estimate the maximum rate of synthesis-conversion. This maximum, when referenced to the constant input function of unesterified [$U^{13}C$]LA, provided steady-state synthesis coefficients and rates.

The synthesis-secretion coefficient k_i^* (equation 3) for conversion of unesterified LA to esterified AA equaled $5.4 \times 10^{-3} \text{ min}^{-1}$ (Table 3), 8.7% of the conversion coefficient of unesterified α -LNA to esterified DHA in rats fed the same high-DHA low-AA diet, which was $62 \times 10^{-3} \text{ min}^{-1}$ (11). Thus, liver synthesis-secretion is more selective for PUFAs of the n-3 than n-6 series, consistent with previous observations (28–32). In this regard, mRNA and protein levels of liver synthetic enzymes were upregulated, as were synthesis coefficients, for unesterified α -LNA conversion to DHA in rats on a diet with low compared to high α -LNA, neither containing DHA (23), whereas enzyme expression was not changed in rats fed a diet with low-LA compared with high-LA, both free of AA (M. Igarashi et al., unpublished observations). This indicates more control of liver enzyme transcription by circulating n-3 than n-6 PUFAs and perhaps an evolutionary need for such differential control (6, 36–38).

Steady-state synthesis-secretion rates J_i of longer chain PUFAs from their 18-carbon precursors are calculated by multiplying $S_{max,i}$ by the inverse of plasma specific activity of the unesterified precursor (equation 4). Mean plasma unesterified LA and α -LNA concentrations were 219 nmol/ml and 13.9 nmol/ml, respectively, whereas the synthesis-secretion rate of esterified AA from LA was 16.1 $\mu\text{mol/day}$, only 1.6 times the rate of esterified DHA synthesis from α -LNA, 9.84 $\mu\text{mol/day}$. Thus, a balanced synthesis-secretion of AA and DHA results from a relatively low conversion coefficient k_i^* of AA from LA with a high plasma unesterified LA concentration $C_{plasma,unes}$, but a comparatively high conversion coefficient DHA from α -LNA with a low unesterified plasma α -LNA concentration.


Hepatic regulation of plasma AA and DHA availability is critical for brain and heart function. The high concentrations of AA and DHA that are maintained within membrane phospholipids of these organs are controlled largely by their plasma concentrations, as circulating unesterified LA and α -LNA precursors are almost completely β -oxidized after entering brain (26, 27), and the rat heart has a low

capacity to synthesize the elongation products from the precursors (25).

Some secreted esterified n-6 PUFAs within VLDLs will be returned over time to the liver via lipoprotein receptors or be hydrolyzed in blood or adipose tissue by lipoprotein lipases to their unesterified forms and then be taken up again (13, 16, 34). Thus, the net rate of hepatic secretion of AA derived using [$U^{13}C$]LA infusion may approach the sum of the secretion rates in Table 3 for γ -LNA, ETA, and AA, 88.3 $\mu\text{mol/day}$, which is 3.6-fold higher than the calculated net rate of secretion of longer chain n-3 PUFAs from α -LNA under the same conditions (11).

The calculated turnovers in this study (Table 3) reflect the contributions of conversion from unesterified circulating LA, but exclude contributions from diet or synthesis from other precursors. Thus they are lower bounds, while the respective calculated half-lives $t_{1/2}$ are overestimates. The turnovers, furthermore, correlated roughly with the ratios of the unesterified to esterified PUFA concentrations (Table 1), particularly for AA. While this issue should be explored further, it may be related to differential selectivities of the esterified PUFAs for circulating or organ esterases, or to a lesser tendency of AA than of the other PUFAs for β -oxidation in tissue mitochondria (39–45).

The brain incorporates unesterified AA from plasma at a rate of 0.30–0.60 $\mu\text{mol/day}$ in unanesthetized rats not consuming AA (46, 47). This rate approximates the rate of AA metabolic loss from brain, as AA cannot be synthesized de novo and is converted minimally (<0.2%) from the LA that enters from plasma (26). Thus, the AA synthesis-secretion rate from circulating shorter term precursors is $\geq 16.1/0.60 = 27$ times the brain AA consumption rate. As newly synthesized AA can get into brain after being hydrolyzed from circulating lipoproteins (11, 14, 48), hepatic synthesis from circulating LA has the potential of maintaining AA homeostasis in brain and other organs.

In summary, whole-body (largely liver) synthesis-secretion coefficients and rates of esterified n-6 PUFAs from circulating unesterified LA were quantified by infusing [$U^{13}C$]LA intravenously for 2 h in unanesthetized rats fed a DHA-enriched, AA-deficient diet. In the future, the heavy isotopic infusion method and model might be used to quantify liver synthesis-secretion of AA in relation to health, disease, and diet in humans as well as rodents. 

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