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Kinetics of eicosapentaenoic acid in brain, heart and liver of conscious rats fed a high *n*-3 PUFA containing diet

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Abstract

Eicosapentaenoic acid (EPA, 20:5 n -3), a precursor of docosahexaenoic acid (DHA), may benefit cardiovascular and brain health. Quantifying EPA's *in vivo* kinetics might elucidate these effects. [1-¹⁴C] EPA was infused i.v. for 5 min in unanesthetized male rats fed a standard EPA–DHA diet. Plasma and microwaved tissue were analyzed. Kinetic parameters were calculated using our compartmental model. At 5 min, 31–48% of labeled EPA in brain and heart was oxidized, 7% in liver. EPA incorporation rates from brain and liver precursor EPA–CoA pools into lipids, mainly phospholipids, were 36 and 2529 nmol/s/g $\times 10^{-4}$, insignificant for heart. Deacylation–reacylation half-lives were 22 h and 38–128 min. Conversion rates to DHA equaled 0.65 and 25.1 nmol/s/g $\times 10^{-4}$, respectively. The low brain concentration and incorporation rate and high oxidation of EPA suggest that, if EPA has a beneficial effect in brain, it might result from its suppression of peripheral inflammation and hepatic conversion to bioactive DHA.

Keywords

Eicosapentaenoic acid; Turnover; Kinetics; Metabolism; Brain; Rat docosahexaenoic

1. Introduction

Eicosapentaenoic acid (EPA, 20:5 n -3) is an n -3 polyunsaturated fatty acid (PUFA) enriched in dietary fish oil and fishmeal. It can be synthesized in mammalian liver through desaturation and elongation steps from ingested α -linolenic acid (18:3 n -3, ALA), which is present in certain vegetable oils [1,2]. In tissue, EPA can be enzymatically converted to lipid mediators such as prostaglandins, leukotrienes, and resolvins [3,4], some of which are antiinflammatory. EPA has been investigated for treating human neurological and cardiovascular diseases [5–8], and may be preferable to its elongation product, docosahexaenoic acid (DHA, 22:6 n -3) for treating depression [9]. However, its concentration is low in brain and heart of rodents and humans compared to DHA [10,11], putting the mechanism of therapeutic efficacy into question.

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Long-chain PUFAs are found in plasma when bound to albumin, or when esterified within phospholipids, triacylglycerol or cholesteryl esters of lipoproteins [12]. Unesterified plasma PUFAs are the primary forms that measurably enter the brain in unanesthetized rodents, although endothelial lipase may facilitate entry of some PUFAs from circulating lipoproteins in the cerebral capillary bed [13]. Both unesterified and esterified PUFAs are rapidly taken up by heart and liver [14–16]. The permeability of unesterified plasma EPA at the blood–brain barrier is similar to that of DHA and arachidonic acid (ARA, 20:4*n*-6) [17]. However, unlike these PUFAs, EPA like ALA is heavily metabolized after entering rodent brain by β -oxidization or other pathways [18–20], making it difficult to understand why it would have a central effect. One possibility is that it acts by suppressing peripheral neuroinflammation, which is thought pathogenic in depression [21,22]. Another is that it is first converted in the liver to DHA, which is the major *n*-3 PUFA taken up and accumulated in brain [23].

To test this latter possibility, we used an *in vivo* pulse labeling method to estimate the rates of brain consumption of plasma derived EPA, as well as its rates of conversion to docosapentaenoic acid (22:5*n*-3) and DHA in brain, heart and liver. This method involves infusing [1-¹⁴C]EPA i.v. for 5 min into a conscious rat, measuring labeled and unlabeled arterial plasma EPA concentrations, then subjecting the rat to high-energy microwaving to prevent postmortem lipid changes, and measuring labeled and/or unlabeled EPA, DPA and DHA in brain, heart and liver. Rates and coefficients of EPA incorporation into each organ, of EPA turnover and half-life due to deacylation–reacylation (recycling) in tissue lipids, and of EPA elongation to DPA and DHA, are calculated [24,25].

Since only unesterified long chain PUFAs measurably enter brain, whereas both esterified and esterified PUFAs enter heart and liver [14–16,26], this method provides exact rates of net EPA incorporation into brain. Furthermore, by determining the specific activity of the precursor EPA–CoA pool in the tissue, relative to steady-state specific activity of EPA in plasma, the method also provides exact values for turnover and half-life of EPA in phospholipids, triacylglycerol or cholesteryl ester in each of the organs. Synthesis rates of DPA and DHA, also calculated with the method, may be underestimates, as steady-state conditions are not established by this pulse-labeling approach. Nevertheless, the method provides a first estimate of *in vivo* synthesis rates in rodents [27,28], and has provided estimates of synthesis rates of elongated *n*-3 PUFAs from intravenously infused ALA [27,28].

These estimates agree with estimates obtained by infusing heavy isotope precursor for 2 h and measuring product appearing in plasma over time (Taha et al., unpublished observations).

2. Materials and methods

2.1. Materials

[1-¹⁴C]EPA in ethanol was purchased from Moravek Biochemicals (Brea, CA, USA). The specific activity was 54 mCi/mmol and the purity was >96% as determined by high performance liquid chromatography (HPLC) and scintillation counting. Di-heptadecanoate phosphatidylcholine (di-17:0 PC), free heptadecanoic acid (17:0), and thin-layer

chromatography (TLC) standards (cholesterol, triacylglycerols, cholesteryl esters, and individual phospholipids) were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Standards for general fatty acid methyl esters (FAMES) for gas chromatography (GC) and HPLC were purchased from NuChek Prep (Elysian, MN, USA). Silica gel 60 plates were purchased from EM Separation Technologies (Gibbstown, NJ, USA). 6-p-Toluidine-2-naphthalene sulfonic acid was from Acros Organics (Geel, Belgium). Fatty acyl-CoAs were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents were HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ, USA) or EMD Chemicals (Gibbstown, NJ, USA). Other chemicals and reagents were purchased from Sigma-Aldrich or Fisher Scientific.

2.2. Animals

The 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 No. 80-23). Fischer-344 (CDF) male rats (4-months old) were purchased from Charles River Laboratories (Portage, MI, USA), and were acclimated for 1 week in our animal facility, with regulated temperature, humidity, and a 12 h light/12 h dark cycle. The rats had free access to water and a high *n*-3 PUFA rodent chow formulation NIH-31 18-4, which contained 4% (wt/wt) crude fat (Zeigler Bros., Gardners, PA, USA). The diet contained (as % of total fatty acid) 20.1% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% α -linolenic, 0.02% ARA, 2.0% eicosapentaenoic, and 2.3% DHA [29], thus was low in ARA and high in EPA and DHA content. For ALA, EPA and DHA, absolute concentrations were 5.9, 2.4 and 2.9 $\mu\text{mol/g}$ diet, respectively. The LA concentration was 59.0 $\mu\text{mol/g}$ (1690 mg/kg), 1.4-fold higher than the LA amount recommended by Bourre to maintain ARA homeostasis in the rat [30].

2.3. Surgery

For radiotracer infusion, polyethylene catheters (PE 50, IntramedicTM, Clay AdamsTM, Becton Dickinson, Sparks, MD, USA) filled with heparinized saline (100 IU/ml) were surgically implanted into the right femoral artery and vein of a rat that was under 1–3% halothane anesthesia (Shirley Aldred & Co, England). After implantation, the skin was closed with staples and treated with 1% lidocaine (Hospira, Lake Forest, IL, USA) for pain control. The rat then was loosely wrapped in a fast-setting plaster cast taped to a wooden block, and allowed to recover from anesthesia for 3–4 h. Body temperature was maintained at 36–38 °C using a feedback-heating element (Indicating Temperature Controller, Yellow Springs Instruments, Yellow Springs, OH, USA).

2.4. Radiotracer infusion

After 3–4 h of recovery from anesthesia, a rat was infused *via* the femoral vein catheter with 300 $\mu\text{Ci/kg}$ [$1\text{-}^{14}\text{C}$]EPA [11,27,31,32]. An adequate amount of [$1\text{-}^{14}\text{C}$]EPA in ethanol was dried under nitrogen gas, and the residue was dissolved in HEPES buffer (pH 7.4) containing 50 mg/ml fatty acid-free bovine serum albumin. The mixture was sonicated at 40 °C for 20 min and mixed by vortexing. The solution (1.3 ml/rat) was infused during 5 min at a rate of $0.223(1 + e^{-0.032t})$ ml/min (t in min) using a computer-controlled variable speed pump (No.

22; Harvard Apparatus, South Natick, MA, USA) to rapidly establish constant plasma radioactivity [11,32,33]. Arterial blood (180 μ l at each time point) was collected in centrifuge tubes (poly-ethylene–heparin lithium fluoride-coated (Beckman Coulter™, Fullerton, CA, USA)) at 0, 0.25, 0.5, 0.75, 1.5, 3, 4, and 5 min after starting infusion. At 5 min, the rat was euthanized with an overdose of sodium pentobarbital (Ovation Pharmaceuticals, Deerfield, IL, USA) (100 mg/kg i.v.). The head and torso were subjected immediately to high-energy focused beam microwave irradiation (5.5 kW, 4.8 s) (Model S6F, Cober Electronics, Stamford, CT, USA). The brain, heart and liver were removed, weighed, frozen in methylbutane cooled by dry ice, and stored at -80°C until assayed. Arterial blood samples were centrifuged at 10,000g for 1 min, and plasma was collected and stored at -80°C until assay.

2.5. Separation and analysis of stable lipids in plasma and brain

Approximately 0.8 g brain, 1.0 g liver, 0.4 g heart or 150 μ l plasma was used for lipid extraction by the Folch procedure [34]. Aqueous extraction phases were washed once with an equal volume of chloroform to remove residual lipid. Aqueous and total lipid radioactivity from samples was counted (see below). Total lipid extracts were separated into neutral lipid subclasses by TLC on silica gel 60 plates 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 for neutral lipid separation were run on the plates to identify individual lipids. The plates were dried, and sprayed with 0.03% 6-p-toluidine-2-naphthalene sulfonic acid in 50 mM Tris–HCl buffer (pH 7.4) (w/v). The lipid bands were visualized under ultraviolet light. The bands then were scraped, and the silica gel was used to directly quantify radioactivity by scintillation counting, or to prepare fatty acid methyl esters (FAMES) (described below).

2.6. Quantification of radioactivity

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

2.7. Fatty acid methyl ester (FAME) preparation

Unesterified and esterified fatty acids in total phospholipids, triacylglycerol, and cholesteryl ester, which had been separated by TLC, were methylated with 1% H_2SO_4 –methanol for 3 h at 70°C [36,37]. Before methylation, appropriate quantities of di-17:0 PC (for phospholipids, triacylglycerol and cholesteryl esters) or 17:0 fatty acid (for free fatty acids) were added as internal standards. Prepared FAMES were analyzed by GC and HPLC as described below.

2.8. GC analysis

Fatty acid concentrations (nmol/g tissue wet wt and nmol/ml plasma) in samples were determined using a GC (6890N, Agilent Technologies, Palo Alto, CA, USA) equipped with an SP™-2330 fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness)

(Supelco, Bellefonte, PA, USA) and a flame ionization detector [37]. Concentrations were calculated by proportional comparison of peak areas to the area of the 17:0 internal standard.

2.9. HPLC analysis

To determine radioactive composition (percent of fatty acid radioactivity) of fatty acids in brain, heart, liver and plasma lipids, FAMES were quantified by HPLC by the method of Avelano et al. [38] with the following modifications. Total lipid extraction of plasma was used to prepare FAMES. Total lipids of liver, brain, and heart were alkalized with KOH solution, and extracted twice with *n*-hexane to extract esterified lipids, and the hexane phase was dried and methylated as described above. FAMES were dissolved in acetonitrile, and the solution was fractionated by reversed phase column-HPLC using a pump (System GOLD 126, Beckman–Coulter) outfitted with a UV detector (UV/VIS-151, Gilson, Middleton, Wisconsin, USA) and an on-line continuous scintillation counter β -RAM detector (β -RAM Model 2, IN/US Systems, FL, USA) mixing in liquid scintillation cocktail (INFLOW™ 2:1, IN/US Systems). The reversed-phase column, Luna 5 μ C18 (2) (5 μ m particle size, 4.6 $\times$ 250 mm), was obtained from Phenomenex (Torrance, CA, USA). For the brain FAME sample, HPLC eluate was collected every 30 s and subjected to liquid scintillation counting to obtain a radioactivity profile. Chromatography was performed using a linear gradient system of water and acetonitrile. The acetonitrile was held at 85% for 30 min, increased to 100% over 10 min, and held at 100% for 20 min. The flow rate was 1.0 ml/min. The UV detector was set at 205 nm.

Four samples from each tissue were equally pooled to analyze HPLC profiles of their FAMES. The analysis was carried out in duplicate. The percentages of radioactivity in [1-¹⁴C]EPA and other [¹⁴C]fatty acids in samples were determined from these HPLC profiles.

2.10. Analysis of long chain acyl-CoAs

Long chain acyl-CoAs were extracted from brain using an affinity chromatography method with slight modification [39]. 2 ml of 25 mM KH₂PO₄ and an appropriate amount of heptadecanoyl-CoA (17:0-CoA) as an internal standard were added to approximately 0.8 g brain, 0.8 g liver, or 0.4 g heart. Then the sample was sonicated for 30 s on ice with a probe sonicator (Model W-225, Misonix, Farmingdale, NY, USA), after adding 2 ml of 2-propanol. Then, 0.25 ml saturated (NH₄)₂SO₄ was added to precipitate protein, and 4 ml of acetonitrile was added after which the sample was mixed vigorously for 5 min by centrifuging at 3000 rpm. The supernatant was diluted with 1.25 volumes of 25 mM KH₂PO₄. The solution was passed 3 times through an oligonucleotide purification cartridge (ABI Masterpiece™, OPC®, Applied Biosystems, Foster City, CA, USA), and the cartridge was washed with 10 ml of 25 mM KH₂PO₄. Acyl-CoA species were eluted with 0.4 ml of 2-propanol/1 mM glacial acetic acid (75:25 v/v).

Extracted acyl-CoAs were separated on a reversed phase HPLC column (Symmetry, 5 μ m particle size, 4.6 mm $\times$ 250 mm, Waters Corporation, Milford, MA, USA), using a pump coupled with a UV/VIS detector (System Gold, Model 168, Beckman). Chromatography was performed using a linear gradient system of 75 mM KH₂PO₄ and acetonitrile. At the

start, acetonitrile was 44% and held for 1 min, then increased to 49% over 25 min, increased to 68% over 10 min, held at 68% for 4 min, returned to 44% over 6 min, and held for 6 min (52 min total run time). The flow rate was 1.0 ml/min. UV detection was set at 260 nm for integration of concentrations and at 280 nm for identifying acyl-CoA species (260/280=4:1) [39]. Peaks were identified from retention times of acyl-CoA standards. The standards for EPA and DPA were prepared from the free fatty acid and free CoA by an enzymatic method [40]. Endogenous acyl-CoA concentrations (nmol/g tissue) were calculated by direct proportional comparison with the peak area of the 17:0-CoA internal standard. Under the HPLC system, 14:0-CoA, EPA-CoA, and ALA-CoA were co-eluted as a single peak [11,39]. This peak was collected and saponified with 2% KOH/EtOH (wt/v) at 100 °C for 45 min, acidified with HCl, and then fatty acids were extracted with n-hexane. The unesterified fatty acids were converted to FAMES and separated on HPLC to measure radioactivity. The concentrations of the FAMES derived from acyl-CoA species also were determined by GC. Thus, 14:0, EPA and ALA in the original acyl-CoA peak were determined by proportional comparison of their GC peak areas to each other.

2.11. Calculations

Equations for determining *in vivo* kinetics of EPA in brain, heart and liver, following a 5-min intravenous infusion of radiolabeled EPA to produce steady-state plasma radioactivity, have been described elsewhere [10,12,24,25,32]. Briefly, with regard to brain, following the 5-min [1-¹⁴C]EPA infusion, incorporation coefficients (ml/s/g tissue), representing transfer of unesterified [1-¹⁴C]EPA from plasma into lipid *i* (e.g., total lipids, phospholipids, triacylglycerol, cholesteryl esters), were calculated as follows:

$$k_{i(\text{EPA})}^* = \frac{c_{\text{tissue},i(\text{EPA})}^*(T)}{\int_0^T c_{\text{plasma}(\text{EPA})}^* dt} \quad (1)$$

where $c_{\text{tissue},i(\text{EPA})}^*$ (nCi/g tissue) is EPA radioactivity in *i* at time *T* (5 min) after starting tracer infusion, *t* is time after starting infusion, and c_{plasma}^* (nCi/ml plasma) is plasma radioactivity of unesterified EPA.

Incorporation–elongation coefficients (ml/s/g tissue), representing conversion of EPA to *j*=DPA or DHA, and subsequent incorporation of *j* into stable lipid *i*, were calculated as follows:

$$k_{i(\text{EPA} \rightarrow j)}^* = \frac{c_{\text{tissue},i(j)}^*(T)}{\int_0^T c_{\text{plasma}(\text{EPA})}^* dt} \quad (2)$$

where $c_{\text{tissue},i(j)}^*$ (nCi/g tissue) is radioactivity of the *n*-3 PUFA *j* in lipid *i* at *T*=5 min.

In Eq. (2), $k_{i(\text{EPA} \rightarrow j)}^*$ can be considered a “lumped” coefficient, much like the “lumped constant” that is used to relate the regional rate of brain glucose consumption to plasma concentration of injected radioactive 2-deoxy-D-glucose in the *in vivo* glucose imaging

method of Sokoloff [41]. $k_{i(\text{EPA} \rightarrow j)}^*$ relates the rate of synthesis of j =DPA or DHA to the plasma unesterified unlabeled EPA concentration (“input function”), and represents the complex series of steps that EPA follows before it is esterified in the form of elongated DPA or DHA in brain lipid i . These steps include diffusion of unesterified plasma EPA into the tissue unesterified pool, enzymatic reactions that include activation of EPA to EPA–CoA by an acyl-CoA synthetase with the consumption of 2 ATP’s, elongation of EPA–CoA to longer chain DPA–CoA or DHA–CoA, involving 5 elongase and 6 desaturase, and transfer of this acyl moiety into phospholipid, triacylglycerol or cholesteryl ester by an acyltransferase [10,12,42]. Each of these steps may be modified independently [43,44], including upregulation of liver elongation and desaturation by dietary n -3 PUFA deprivation [28].

Rates of incorporation of unlabeled unesterified EPA from plasma into lipid i , $J_{in,i(\text{EPA})}$, and rates of synthesis of j from EPA followed by its incorporation into i , $J_{in,i(\text{EPA} \rightarrow j)}$, (nmol/s/g tissue), were calculated as

$$J_{in,i(\text{EPA})} = k_{i(\text{EPA})}^* c_{\text{plasma}(\text{EPA})} \quad (3a)$$

$$J_{in,i(\text{EPA} \rightarrow j)} = k_{i(\text{EPA} \rightarrow j)}^* c_{\text{plasma}(\text{EPA})} \quad (3b)$$

where c_{plasma} is the plasma concentration (nmol/ml) of unlabeled unesterified EPA.

Since shorter-chain circulating ALA is negligibly elongated to EPA or DHA after entering brain, due to its rapid oxidation [45], we can ignore this term and calculate the “metabolic (M)” turnover (representing net EPA loss due to metabolism and equal to reincorporation from plasma unesterified EPA) within lipid i as [24],

$$\text{Turnover}(M)_{i(\text{EPA})} = J_{in,i(\text{EPA})} / c_{\text{tissue},i(\text{EPA})} \quad (4a)$$

and the corresponding half-life as

$$\text{Half-life} = 0.693 / \text{Turnover}(M)_{i(\text{EPA})} \quad (4b)$$

For the brain, the above two terms represent the exact turnover and half-life of EPA, within lipid i , since uptake of esterified EPA from plasma is minimal [12,14,33,46,47]. For heart and liver, calculated uptake and turnover are underestimates, as esterified EPA within circulating lipoproteins also is taken up and can contribute to the tissue EPA–CoA pool [15,16,48].

A ‘dilution factor’ $\lambda_{\text{EPA-CoA}}$ of the tissue EPA–CoA pool represents the unesterified EPA flux from plasma into the pool, divided by the sum of all fluxes into this pool (e.g., unesterified plasma EPA, EPA from esterified plasma EPA that enters the tissue, EPA released by deacylation from tissue lipids i [42,49], and EPA synthesized in brain from its precursor, 20:4 n -3 [2]) [12,25]). $\lambda_{\text{EPA-CoA}}$ is determined as the steady-state ratio of tissue EPA–CoA specific activity to specific activity of unesterified EPA in plasma

$$\lambda_{\text{EPA-CoA}} = \frac{c_{\text{tissue(EPA-CoA)}}^* / C_{\text{tissue(EPA-CoA)}}}{C_{\text{plasma(EPA)}}^* / c_{\text{plasma(EPA)}}} \quad (5)$$

The rate of incorporation (nmol/s/g) of unlabeled EPA from the tissue precursor EPA–CoA pool into tissue lipid i equals

$$J_{\text{FA},i(\text{EPA})} = J_{\text{in},i(\text{EPA})} / \lambda_{\text{EPA-CoA}} \quad (6)$$

The rate of conversion of EPA from the *precursor* EPA–CoA pool to elongated DPA and DHA followed by their incorporation into i equals (nmol/s/g),

$$J_{\text{FA},i(\text{EPA} \rightarrow j)} = J_{\text{in},i(\text{EPA} \rightarrow j)} / \lambda_{\text{EPA-CoA}} \quad (7)$$

Turnover $F_{\text{FA},i(\text{EPA})}$ of EPA, due to deacylation–reacylation (recycling) [42,49] within tissue lipid i equals

$$F_{\text{FA},i(\text{EPA})} = \frac{J_{\text{FA},i(\text{EPA})}}{c_{\text{brain},i(\text{EPA})}} \quad (8)$$

The corresponding recycling half-life of EPA in i equals

$$t_{1/2,i(\text{EPA})} = 0.693 / F_{\text{FA},i(\text{EPA})} \quad (9)$$

Turnover due to deacylation–reacylation in tissue lipid i is related to turnover due to metabolic loss as [24]

$$F_{\text{FA},i(\text{EPA})} = \text{Turnover}(M)_{i(\text{EPA})} / \lambda_{\text{EPA-CoA}} \quad (10)$$

$\lambda_{\text{EPA-CoA}}$ in this equation corrects for the possible underestimate of Turnover(M) $_{i(\text{EPA})}$ in liver and heart due to uptake of plasma esterified EPA (see above).

2.12. Statistics

Means $\pm$ SD are given.

3. Results

3.1. EPA in plasma and tissues

Unesterified plasma EPA concentrations before and after [1- ^{14}C] EPA infusion were 6.9 ± 2.3 nmol/ml and 17.0 ± 3.9 nmol/ml, respectively, compared with 15 ± 7 nmol/ml for DHA (Table 1 and Supplementary Table 1). Concentrations of other unesterified fatty acids and of triacylglycerol, phospholipids and cholesteryl ester in plasma, given in Supplementary Table 1, agree with published values in rats on the same diet [11].

Individual unesterified plasma EPA concentrations in each rat before infusion were used to calculate individual EPA incorporation coefficients and rates into tissue lipid i ($J_{in,i(\text{EPA})}$) using Eq. (3a) (Table 3 below), and rates of synthesis from EPA of elongated $n-3$ PUFAs j (e.g., DPA or DHA) ($J_{in,i(\text{EPA}\rightarrow j)}$) using Eq. (4) (Table 3 below). Individual plasma unesterified EPA concentrations after infusion were used to calculate the dilution factor of the tissue EPA-CoA pool $\lambda_{\text{EPA-CoA}}$, by Eq. (5) (Table 4 below).

Mean total esterified EPA concentrations in brain, heart and liver were 235, 179, and 1120 nmol/g, respectively (Table 1), compared with the respective DHA concentrations of 15,376 (65 fold), 6809 (38 fold) and 6709 (5.4 fold) nmol/g (Supplementary Tables 2–4). Concentrations of other fatty acids in these organs are given in Supplementary Tables 2–4. EPA concentrations from individual rats were used to calculate turnovers of EPA due to deacylation-deacylation ($F_{\text{FA},i(\text{EPA})}$) by Eq. (8) (Table 5, below) and to metabolic recycling (Turnover(M) $_{i(\text{EPA})}$) (Eq. 4a) (Table 6 below).

3.2. Plasma radioactivity

Fig. 1 summarizes mean radioactivity in the total lipid and aqueous phases of plasma at different times during the 5-min infusion of [1- ^{14}C]EPA. A steady-state lipid radioactivity was achieved within 1 min of infusion. For the 5-min infusion period, the integral of radioactivity in plasma total lipids equaled $184,256\pm 16,326$ nCi s/ml. Individual values of integrated radioactivity were used to calculate individual values of $k_{i(\text{EPA})}^*$ by Eq. (1) and of $k_{i(\text{EPA}\rightarrow j)}^*$ by Eq. (2).

In plasma, 90.2% of radioactivity was in the total lipid phase and 9.8% was in the aqueous phase after 5 min of infusion (Fig. 2A). In lipid classes, 95.7% of net radioactivity was in the unesterified fatty acid compartment, and only 4.3% was detected in esterified lipids (triacylglycerol, phospholipids, cholesterol, cholesteryl esters) (Fig. 2B). Furthermore, 96.7% of radioactivity in plasma total fatty acids was radiolabeled EPA (Fig. 3A and Table 2). Other radioactive metabolites of infused [1- ^{14}C]EPA were not detected in plasma at 5 min post-infusion.

3.3. Brain radioactivity

In brain, after the 5-min infusion, 52.9% of radioactivity (42.0 ± 4.8 nCi/g) was in the total lipid phase and 47.1% (37.5 ± 3.1 nCi/g) in the aqueous phase (Fig. 2C). In lipid sub-classes, 76.1% (32.0 ± 3.5 nCi/g) was in phospholipids, 13.6% (5.71 ± 2.06 nCi/g) in triacylglycerol (Fig. 2D). Of total esterified fatty acids, 1.2% was newly synthesized radioactive DHA, and 67.2% was radioactive EPA (Fig. 3B and Table 2). Radioactivity of EPA, DPA and DHA was calculated from the net radioactivity of total esterified fatty acids (Fig. 2) and fractional radioactivity (Table 2). Calculated mean radioactivities of EPA, DPA and DHA were 28.3, 9.61 and 0.509 nCi/g brain, respectively (Table 3, data column 1).

3.4. Heart radioactivity

In the heart, 68.8% of radioactivity (450 ± 94 nCi/g) was in the total lipid phase and 31.2% (205 ± 21 nCi/g) was in the aqueous phase after the 5-min infusion (Fig. 2E). In lipid sub-classes, 23.8% (107 ± 33 nCi/g) was in phospholipids, and 72.9% (328 ± 73 nCi/g) was in triacylglycerol (Fig. 2F). In total esterified fatty acids, 88.9% of radioactivity was as EPA, 5.4% as DPA. Newly synthesized radioactive DHA was not detected (Fig. 3C and Table 2). Calculated mean radioactivities of EPA and DPA were 390 nCi/g and 24.9 nCi/g, respectively (Table 3).

3.5. Liver radioactivity

In the liver, 92.8% of radioactivity (1997 ± 202 nCi/g) was in the total lipid phase and 7.2% (156 ± 36 nCi/g) was in the aqueous phase at 5 min post-infusion (Fig. 2G). In lipid subclasses, 74.6% (1490 ± 170 nCi/g) was in triacylglycerol, and 21.8% (436 ± 69 nCi/g) was in phospholipids (Fig. 2H).

In liver total lipids, [^{14}C]DPA and [^{14}C]DHA could be detected after the 5-min infusion (Fig. 3D). In total esterified lipids, 67.7% of radioactivity was as EPA (Fig. 3D and Table 2), 0.67% as DHA, and 23.3% as DPA. Mean radioactivity of esterified EPA, DPA and DHA in liver esterified lipids was 1352, 465, and 13.4 nCi/g, respectively (Table 3).

3.6. Unlabeled EPA–CoA and associated radioactivity in liver, brain and heart

Unlabeled tissue EPA–CoA concentrations and radioactivities were analyzed using HPLC (Table 4). The unlabeled concentration was 0.0807 nmol/g in brain and 0.271 nmol/g in liver, while radioactivity was 1.10 nCi/g in brain and 2.51 nCi/g in liver. We could not detect EPA–CoA in the heart, consistent with evidence of absent Elongase 2 and lack of measurable synthesis of DHA from ALA [10]. Individual EPA–CoA concentrations and radioactivities were used to calculate the dilution factor $\lambda_{\text{EPA-CoA}}$ using Eq. (5), which was 0.337 and 0.212 for brain and liver, respectively (Table 4).

3.7. EPA incorporation coefficients and rates

Incorporation coefficients $k_{i(\text{EPA})}^*$ and incorporation (synthesis) coefficients $k_{i(\text{EPA} \rightarrow j)}^*$ of plasma unesterified EPA into tissue lipids i and into DPA and DHA were calculated using Eq. (1), with individual experimental values of integrated plasma radioactivity as the input function (Fig. 1) and of radioactivity of EPA and radiolabeled DPA and DHA in the tissue

lipids (Table 3). These coefficients together with the unesterified unlabeled plasma EPA concentrations prior to [$1\text{-}^{14}\text{C}$]EPA infusion (Table 2) then were used to calculate incorporation coefficients and incorporation–conversion rates, $J_{in,i(EPA)}$ and $J_{in,i(EPA)\rightarrow j}$ of plasma unesterified EPA (Table 3) and derived DPA and DHA using Eqs. (3a) and (3b). $J_{in,i(EPA)}$ into brain, heart and liver lipids equaled 10.5 , 143 , and $509 \text{ nmol/s/g} \times 10^{-4}$, respectively. As noted in Section 2, the heart and liver values do not represent net plasma EPA incorporation rates, since esterified EPA also was taken up by these organs. The EPA incorporation–conversion rate to DHA $J_{in,i(EPA)\rightarrow DHA}$ in brain and liver equaled $0.190 \text{ nmol/s/g} \times 10^{-4}$ and $5.05 \text{ nmol/s/g} \times 10^{-4}$ (27 fold higher in liver), respectively.

Incorporation coefficients $k_{i(EPA)}^*$ and $k_{i(EPA\rightarrow j)}^*$ and incorporation rates $J_{in,i(EPA)}$ and $J_{in,i(EPA)\rightarrow j}$ into triacylglycerol and phospholipid into brain, heart and liver also were calculated (Supplementary Table 5).

The dilution factor, $\lambda_{EPA-CoA}$, the ratio of steady-state tissue EPA–CoA specific activity to plasma unesterified EPA specific activity at the end infusion, was calculated using Eq. (3a) and equaled 0.337 ± 0.128 and 0.212 ± 0.041 in brain and liver, respectively (Table 4).

Tissue EPA–CoA is the precursor pool for transfer of EPA into mitochondria *via* carnitine palmitoyl-transferase, or for EPA acylation into lipids *via* an transacylase. Incorporation rates $J_{FA,i(EPA)}$ of EPA from EPA–CoA into tissue lipid i were calculated using Eqs. (6) and (7). $J_{FA,i(EPA)}$ equaled $36.2 \text{ nmol/s/g} \times 10^{-4}$ and $2529 \text{ nmol/s/g} \times 10^{-4}$ (70 fold greater) for brain and liver, respectively. In addition, incorporation (synthesis) rates $J_{FA,i(EPA)\rightarrow DHA}$ into DHA in brain and liver were $0.651 \text{ nmol/s/g} \times 10^{-4}$ and $25.1 \text{ nmol/s/g} \times 10^{-4}$ (39 fold), respectively. These rates are exact, because their calculation takes into account the dilution factor $\lambda_{EPA-CoA}$ of the EPA–CoA pool.

3.8. Turnovers and half-lives for recycling and metabolic loss

As noted in Section 2. 11. two relevant sets of incorporation rates, turnovers and half-lives can be calculated from the data of this paper. One set involves replacement by plasma unesterified EPA of the brain EPA that has been metabolized and lost to other products. The other set follows EPA incorporation from the tissue EPA–CoA pool, and reflects deacylation–reacylation (recycling) within tissue lipids via the EPA–CoA pool [12,24,42,49]. Coefficients related to deacylation–reacylation are exact for each of the three organs, as they take into account the dilution factor $\lambda_{EPA-CoA}$ [24].

For kinetic parameters related to metabolic consumption [24], turnovers and half-lives were calculated using Eqs. (4a) and (4b), respectively. Values are given in Table 6 for total esterified lipids and phospholipids in brain, and for total esterified, triacylglycerol and phospholipids in liver and heart. For the brain, EPA turnover in total esterified lipids was $162 \times 10^{-4} \text{ h}^{-1}$ and half-life was 46.8 h (1.95 days), while total phospholipid parameters were, respectively, $132.5 \times 10^{-4} \text{ h}^{-1}$. and 59.9 h (2.5 days). The EPA metabolic half-life in total lipids equaled 278.5 min (4.6 h) in the liver and 159.7 min (2.7 h) in the heart (Table 6).

For deacylation–reacylation, EPA turnover $F_{FA,i(EPA)}$ was calculated using Eq. (7) with individual values of $J_{FA,i(EPA)}$ and EPA concentrations, in total esterified lipids in rat liver and brain (Table 5). Corresponding half-lives were calculated using Eq. (8) from individual

EPA turnovers (Table 6). Mean deacylation–reacylation half-lives in total lipids were 16.6 h in brain and 62 min in liver. $J_{FA,i(EPA)}$, $J_{FA,i(EPA) \rightarrow DHA}$ and $F_{FA,i(EPA)}$ and half-lives in phospholipids and triacylglycerol in brain and liver are given in Supplementary Table 6.

4. Discussion

This study provides experimental kinetic data that allow us to quantify *in vivo* whole-body EPA metabolism in male adult rats fed a standard rodent diet with a high *n*-3 PUFA content. Using our 5-min infusion method and model, we determined EPA incorporation coefficients and rates, turnovers and half-lives, and rates of synthesis (conversion) of DPA and DHA from EPA in brain, heart and liver. The analysis provided complete kinetic parameters for the brain, since only unesterified long chain fatty acids are taken up measurably by brain, whereas both unesterified and esterified long chain fatty acids are taken up by liver and heart [12,14–16,33,46–48]. Thus, calculated $J_{in,i(EPA)}$ and metabolic turnover $J_{in,i(EPA)}/C_{tissue,i(EPA)}$ are underestimates, and metabolic half-lives are overestimates, for these two organs. In each of the three organs, $J_{FA,i(EPA)}$ and deacylation–reacylation turnover and half-life calculated using $J_{FA,i(EPA)}$ are complete because they are corrected for dilution of the tissue EPA–CoA precursor pool by use of $\lambda_{EPA-CoA}$ [12,24].

As noted in the introduction, EPA has been suggested to be critical for maintaining brain and heart functional integrity, in addition to DHA, and some clinical trials involving dietary EPA supplementation have supported this hypothesis [5–9,50]. Nevertheless, a recent study reported that a greater effect on depression of dietary EPA compared with DHA lost statistical significance on meta-analysis after correction for likely publication bias [9].

In this regard, the present study suggests that EPA would be effective only after being elongated by the liver to DHA for subsequent brain uptake, thus not more effective than DHA. Turnover and half-life of EPA and DHA due to deacylation–reacylation in brain are quantitatively comparable, but the amount of EPA turning over is much less than the amount of DHA because its esterified concentration is much less as well. Furthermore, even though some brain EPA might be converted to bioactive products after being taken up and released from brain phospholipid [51,52], their amounts also would be much less than those of DHA-derived products because of their differences in cold concentration. A low cold brain concentration likely reflects the fact that at least 50% of radiolabeled EPA is found in the aqueous compartment after a 5 min infusion, whereas most radiolabeled DHA is incorporated into phospholipid [53].

EPA and DHA concentrations in plasma and the three organs in this study are comparable to reported concentrations [10,11,18]. Brain and heart EPA concentrations, 235 and 179 nmol/g tissue, respectively, were 65 and 38 fold less than respective DHA concentrations (Table 1), although the rats were fed a high *n*-3 PUFA diet containing 2.0% EPA, and unesterified plasma EPA concentration was half that of DHA. As the conversion rate of dietary ALA to EPA also is very low in rat brain and not measurable in rat heart compared to liver, heart and brain EPA must largely be derived from the diet or from liver synthesis [10,18,28,31,32].

Following the 5-min infusion of [$1\text{-}^{14}\text{C}$]EPA, the distribution of labeled plasma-derived EPA indicated a high level of oxidation in brain and heart but not in liver. In brain, 47.1% of radioactivity was in the aqueous phase compared to 52.9% for the lipid phase [54], similar to prior observations [18,19,55]. In heart, 31% of radioactivity was aqueous while 68.8% was in the lipid phase, but in the liver the respective percentages were 7.2% and 92.8%. The high lipid incorporation fraction in liver is consistent with evidence that long-chain unesterified PUFAs taken up from plasma by liver are rapidly elongated, esterified and secreted within circulating lipoproteins, rather being oxidized [28,56,57]. Phospholipids were the major lipid component into which plasma-derived labeled EPA was esterified in brain, while triacylglycerols were labeled preferentially in heart and liver (Fig. 2).

The incorporation coefficient $k_{i(\text{EPA})}$ represents transfer of unesterified plasma EPA into tissue lipids i by serial steps of diffusion, activation to EPA-CoA by an acyl-CoA synthetase and subsequent esterification by an acyltransferase [12]. Transfer is very rapid, and about 90% of tracer taken up by brain is esterified within a 1 min [33]. $k_{i(\text{EPA})}$ in $\text{ml/s/g} \times 10^{-4}$ followed the order: 1.55 (brain) < 21.5 (heart) < 73.5 (liver) (Table 3). The high value for heart and higher value for liver, compared to brain, likely reflect roles of fatty acid transport proteins in these organs [58], oxidation of fatty acids for energy by the heart [59], and preferential packaging and secretion within lipoproteins by the liver (see above) [28,56,57].

The EPA incorporation–conversion rate to DHA $J_{in,i(\text{EPA} \rightarrow \text{DHA})}$ in brain and liver equaled 0.190 and 5.05 $\text{nmol/s/g} \times 10^{-4}$ (27 fold), respectively. The net rate for the 1.5 g rat brain is equivalent to 2.46 nmol/day while the rate for an 11.5 g rat liver equals 502 nmol/day . The hepatic conversion rate may be higher in animals fed a diet with a lower $n\text{-3}$ PUFA content, due to upregulation of elongation and desaturase enzymes; such diet-dependent upregulation does not occur in brain [32,57]. Thus, even with the high $n\text{-3}$ PUFA diet, the rate of conversion in brain of EPA to DHA is too low to compensate for DHA consumption (226–248 nmol/day/brain) [37,60]. Since brain synthesis from ALA also is negligible [11], most brain DHA comes from plasma due to dietary intake and/or to liver synthesis and secretion from ALA or EPA.

$J_{in,i(\text{EPA} \rightarrow \text{DPA})}$ and $J_{in,i(\text{EPA} \rightarrow \text{DPA})}$ may be approximate because a tissue steady-state was not reached in this study. However, the error may not be large, since the DHA synthesis rate from circulating ALA, obtained by infusing heavy isotopic ALA, when measured under conditions in which a steady-state was approximated (2-h infusion), provided values close to the values in this study (Taha et al., unpublished observations).

EPA half-lives for metabolic loss were 46.8 h (1.95 days), 278 min (4.64 h) and 160 min (2.88 h) for brain, heart and liver, respectively (Table 6), attesting to its rapid loss and replenishment from plasma EPA [24]. The brain half-life of 1.95 days is less than the 5-day half-life calculated following intracerebroventricular infusion of [^{14}C]EPA and sampling of multiple animals over time [18]. In any case, both values are much shorter than calculated DHA half-lives of 31–33 days [37,61], consistent with a higher rate of metabolic breakdown of esterified EPA after it is hydrolyzed than of DHA [18].

$J_{FA,i(EPA)}$ the rate of esterification of EPA into lipid i from the tissue precursor EPA–CoA pool, equaled $27.8 \text{ nmol/s/g} \times 10^{-4}$ for brain phospholipid and 1864 and $574 \text{ nmol/s/g} \times 10^{-4}$ for liver triacylglycerol and phospholipid, respectively (Supplementary Table 6), but could not be measured for the heart. These differences correspond to an EPA deacylation–reacylation turnover of 0.0496 h^{-1} and a half-life of 21.8 h in brain phospholipids, and half-lives of 37.7 and 138 min in liver triacylglycerol and total phospholipid, respectively (Supplementary Table 6) (turnover and half-life in total lipids were 0.057 h^{-1} and 16.6 h^{-1} for brain and 0.0139 min^{-1} and 62 min for liver (Table 5)). In comparison, deacylation–reacylation turnover and half-life of DHA in brain phospholipids are 0.053 h^{-1} and 13 h [62]. Thus brain EPA deacylation–reacylation turnover and half-life are approximately equivalent to respective parameters for DHA, although the esterified EPA concentration is much lower.

Comparable deacylation–reacylation turnover but a lower concentration of esterified EPA than of DHA in rat brain suggests lower rates of formation of bioactive EPA than of DHA metabolites. This has been reported in rats for epoxylated and hydroxylated metabolites produced by cytochrome p450 enzymes [63]. EPA's reported superior clinical efficacy compared with DHA in depression [9], if true, may arise because of specific action of its bioactive metabolites [13] or its direct effect at cerebral capillaries, rather than secondary to its liver conversion to DHA [13,64,65].

In conclusion, we have used a 5-min infusion method to characterize whole body kinetics of EPA. Incorporation coefficients and rates of unesterified plasma EPA in are in the order: brain $\ll$ heart $<$ liver, with much more oxidation of EPA to aqueous metabolites in brain and heart compared to liver. High oxidation, which also has been noted for ALA in brain, likely is related to preferences for mitochondrial oxidation *via* carnitine palmitoyl transferase than for acyltransferase pathways of shorter chain compared with longer chain PUFAs [54,66]. Furthermore, 5-min conversion rates of EPA to DHA in the three organs are in the order liver $\gg$ brain, whereas heart conversion was not measurable. Within brain, the esterified EPA concentration is much less than the esterified DHA concentration and so are concentrations of EPA epoxylated and hydroxylated metabolites [63], whereas turnovers due to deacylation–reacylation are comparable. An indirect effect on peripheral inflammation, which is implicated in depression and other mood disorders [9], a direct effect on capillary integrity, high actions of EPA metabolites, or EPA conversion by the liver to bioactive DHA, are alternative mechanisms of action [21,22,28,67,68].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ARA	arachidonic acid
DHA	docosahexaenoic acid (22:6 <i>n</i> -3)
DPA	docosapentaenoic acid (22:5 <i>n</i> -3)
EPA	eicosapentaenoic acid (20:5 <i>n</i> -3)
FAME	fatty acid methyl ester
GC	gas chromatography
LA	linoleic acid (18:2 <i>n</i> -6)
ALA	α -linolenic acid (18:3 <i>n</i> -3)
PUFA	polyunsaturated fatty acid
HPLC	high performance liquid chromatography
TLC	thin layer chromatography
PC	phosphatidylcholine

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.plefa.2013.09.004>.

Fig. 1. Radioactivity in total lipid (●) and aqueous (○) phases from plasma in rats during i.v. infusion of [1-¹⁴C]EPA. Values are mean±SD ($n=4$ for both groups).

Fig. 2. Radioactivity of aqueous and total lipid phases (A, C, E, G), in neutral lipid classes (B, D, F, H) of plasma (A and B), brain (C and D), heart (E and F) and liver (G and H), in rats after a 5-min i.v. infusion of [1-¹⁴C]EPA. Abbreviations: TG, triacylglycerol; PL, phospholipid; CE, cholesteryl esters; Chol, cholesterol. Values are mean±SD ($n=4$).

Fig. 3.
HPLC chromatograms of fatty acid methyl esters in total lipids of plasma (A) and total esterified lipids of brain (B), heart (C) and liver (D) of rats. Four samples for each group were equally pooled to analyze HPLC profiles of FAMES.

Table 1Unesterified and esterified *n*-3 PUFAs in plasma, brain, heart and liver of unanesthetized rats.

	Plasma (nmol/ml)	Brain (nmol/g tissue)	Heart (nmol/g tissue)	Liver (nmol/g tissue)
Before the infusion				
Unesterified EPA	6.9±2.3			
Unesterified DPA	9.0±3.7			
Unesterified DHA	15±7			
Total esterified EPA	88±25			
Total esterified DPA	47±16			
Total esterified DHA	190±44			
After the infusion				
Unesterified EPA	17±4			
Total esterified EPA		235±16	179±19	1120±194

Data are mean±SD (*n*=4).

Table 2

Percent radioactivity in total lipids of plasma, and percent total radioactivity of esterified fatty acids in brain, heart and liver of rats, after 5-min [1-¹⁴C]EPA infusion.

Fatty acid	Plasma	Brain	Heart	Liver
Radioactivity (%)				
EPA (20:5 n -3)	96.7	67.2	88.9	67.7
DPA (22:5 n -3)	ND	22.9	5.4	23.3
DHA (22:6 n -3)	ND	1.2	ND	0.67
24:5	ND	0.57	ND	0.31
20:3	ND	3.5	0.06	0.27
18:0	ND	0.21	ND	0.07
Others	3.3	4.4	5.7	7.7

Four samples for each organ were equally pooled to analyze HPLC profiles of fatty acid methyl esters.

Table 3

Radioactivity, incorporation coefficients and incorporation rates of plasma unesterified EPA into brain, heart and liver total esterified lipids of unanesthetized rats.

	Radioactivity (nCi/g tissue)	Incorporation coefficients, $k_{i(EPA)}$ or $k_{i(EPA \rightarrow j)}$ (ml/s/g $\times 10^{-4}$)	Incorporation rates, $J_{in,i(EPA)}$ or $J_{in,i(EPA \rightarrow j)}$ (nmol/s/g $\times 10^{-4}$)
Brain			
Total esterified lipids			
EPA	28.8 $\pm$ 3.3	1.55 $\pm$ 0.26	10.5 $\pm$ 3.5
DPA	9.61 $\pm$ 1.11	0.526 $\pm$ 0.088	3.58 $\pm$ 1.18
DHA	0.509 $\pm$ 0.059	0.0279 $\pm$ 0.0047	0.190 $\pm$ 0.063
Heart			
Total esterified lipids			
EPA	390 $\pm$ 81	21.5 $\pm$ 5.9	143 $\pm$ 47
DPA	24.9 $\pm$ 5.2	1.37 $\pm$ 0.37	9.14 $\pm$ 2.99
DHA	ND		
Liver			
Total esterified lipids			
EPA	1352 $\pm$ 137	73.5 $\pm$ 5.4	509 $\pm$ 177
DPA	465 $\pm$ 47	25.3 $\pm$ 1.9	175 $\pm$ 61
DHA	13.4 $\pm$ 1.4	0.729 $\pm$ 0.054	5.05 $\pm$ 1.76

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

Table 4

EPA–CoA concentration, radioactivity and calculated dilution factor ($\lambda_{EPA-CoA}$) in brain, heart and liver of unanesthetized rats following 5 min of [1- 14 C]EPA infusion.

Tissue	EPA–CoA concentration (nmol/g tissue)	Radioactivity of EPA–CoA (nCi/g tissue)	$\lambda_{EPA-CoA}$
Brain	0.0807±0.0228	1.10±0.14	0.337±0.128
Heart	ND	NM	NC
Liver	0.271±0.080	2.51±0.75	0.212±0.041

Values are mean±SD ($n=4$).

ND, not detected; NM, not measured; NC, not calculated.

Table 5

Incorporation rates of EPA from tissue EPA–CoA ($J_{FA,i(EPA)}$ or $J_{FA,i(EPA \rightarrow j)}$), turnovers ($F_{FA,i(EPA)}$) and deacylation–reacylation EPA half-lives (recycling) in brain and liver total lipids of unanesthetized rats.

	Incorporation rate, $J_{FA,i(EPA)}$ or $J_{FA,i(EPA \rightarrow j)}$	Turnover, $F_{FA,i(EPA)}$	Half-life
Brain	nmol/s/g $\times 10^{-4}$	$\text{h}^{-1} \times 10^{-4}$	h
Total esterified lipids			
EPA	36.2 $\pm$ 21.3	567 $\pm$ 362	16.6 $\pm$ 9.9
DPA	12.3 $\pm$ 7.2		
DHA	0.651 $\pm$ 0.383		
Liver	nmol/s/g $\times 10^{-4}$	$\text{min}^{-1} \times 10^{-4}$	min
Total esterified lipids			
EPA	2529 $\pm$ 1114	139 $\pm$ 67	62.0 $\pm$ 34.5
DPA	870 $\pm$ 383		
DHA	25.1 $\pm$ 11.1		

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

Table 6

Metabolic turnover ($J_{\text{in}}/c_{\text{plasma}}$) and half-lives of EPA (0.693/metabolic turnover) in brain, liver and heart.

	Turnover	Half-life
Brain	($\text{h}^{-1} \times 10^{-4}$)	(h)
Total esterified		
EPA	162.1±55.1	46.8±16.1
Total phospholipids		
EPA	132.5±62.3	59.9±22.1
Liver	($\text{min}^{-1} \times 10^{-4}$)	(min)
Total esterified		
EPA	27.4±9.0	278.5±106.7
Triacylglycerol		
EPA	44.4±14.1	170.2±61.0
Total phospholipids		
EPA	13.7±6.2	610.1±329.3
Heart	($\text{min}^{-1} \times 10^{-4}$)	(min)
Total esterified		
EPA	49.5±20.3	159.7±64.2
Triacylglycerol		
EPA	280.8±113.3	29.2±15.8
Total phospholipids		
EPA	16.2±9.0	517.0±229.0

Values are mean±SD ($n=4$).