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QUANTITATIVE CONTRIBUTIONS OF DIET AND LIVER SYNTHESIS TO DOCOSAHEXAENOIC ACID HOMEOSTASIS

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Abstract

Dietary requirements for maintaining brain and heart docosahexaenoic acid (DHA, 22:6n-3) homeostasis are not agreed on, in part because rates of liver DHA synthesis from circulating α -linolenic acid (α -LNA, 18:2n-3) have not been quantified. These rates can be estimated *in vivo* using intravenous radiotracer- or heavy isotope-labeled α -LNA infusion. In adult unanesthetized male rats, such infusion shows that liver synthesis-secretion rates of DHA from α -LNA markedly exceed brain and heart DHA synthesis rates and brain DHA consumption rate, and that liver but not heart or brain synthesis is upregulated as dietary n-3 PUFA content is reduced. These differences in rate reflect much higher expression of DHA-synthesizing enzymes in liver, and upregulation of liver but not heart or brain enzyme expression by reduced dietary n-3 PUFA content. A noninvasive intravenous [^{13}C] α -LNA infusion method that produces steady-state liver tracer metabolism gives exact liver DHA synthesis-secretion rates and could be extended for human studies.

Keywords

secretion; synthesis; docosahexaenoic acid; α -linolenic acid; kinetics; liver; brain; heart; rat; PUFA; n-3

1. INTRODUCTION

Docosahexaenoic acid (DHA, 22:6n-3), abundant in fish and fish products, is critical for maintaining nervous system, cardiac, and general body organ function [1-3]. DHA cannot be synthesized *de novo* from 2-carbon fragments in vertebrate tissue. However, DHA can be converted from its shorter chain nutritionally essential polyunsaturated fatty acid (PUFA) precursor, α -linolenic acid (α -LNA, 18:3n-3) [4,5], which is found in plant foods [6].

Controversy exists about human dietary requirements of the long chain PUFAs, DHA + eicosapentaenoic acid (EPA 20:5n-3), as expert recommendations range from 0.1 g/day to 1.6 g/day [7-12]. Since both liver synthesis from α -LNA and ingestion of dietary DHA/EPA can contribute to whole body DHA content and homeostasis, this controversy might be resolved by quantifying the liver's ability to synthesize and secrete DHA from circulating α -LNA under different dietary or pathological conditions. Reported whole-body synthesis (conversion)

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fractions of ingested α -LNA to DHA range from 0.2 to 9% in humans [6,13-16], suggesting that new methods are required to more exactly quantify DHA synthesis in relation to organ DHA consumption. This paper reviews such methods as applied to the rat, and suggests approaches for noninvasive human studies with one of them.

2. ORGAN SYNTHESIS OF DHA FROM α -LNA STUDIED IN THE RAT

We studied unanesthetized male rats that had been fed diets with differing n-3 PUFA content and composition for 15 weeks after weaning (at 21 days). In the initial method, $[1-^{14}\text{C}]\alpha$ -LNA was infused intravenously for 5 min, radioactive and unlabeled plasma concentrations of unesterified α -LNA were measured, and the liver, brain or heart removed after being subjected to high energy microwaving to stop metabolism. Specific activities (radioactivity/unlabeled concentration) of DHA in organ phospholipid, triacylglycerol, and cholesteryl ester, as well as in the α -LNA-CoA pool (precursor of α -LNA elongation and desaturation), were determined [17-19].

With the 5-min $[1-^{14}\text{C}]\alpha$ -LNA infusion, coefficients of organ conversion of α -LNA to DHA, $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^*$ (ml/sec/g), are calculated as,

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

where $c_{\text{tissue},i(\text{DHA})}^*(T)$ (nCi/g) is the labeled DHA concentration in stable lipid i at time T of sampling (5 min), t is time after beginning tracer infusion, and $c_{\text{plasma}(\alpha\text{-LNA})}^*$ (nCi/ml) is plasma radioactivity due to α -LNA*. The rate of DHA synthesis by the organ is the product of the synthesis coefficient (Eq. 1) and the unlabeled unesterified plasma α -LNA concentration, $c_{\text{plasma}(\alpha\text{-LNA})}$ (nCi/g),

$$J_{\text{in},i(\alpha\text{-LNA}\rightarrow\text{DHA})} = k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}^* c_{\text{plasma}(\alpha\text{-LNA})} \quad (\text{Eq. 2})$$

Table 1 summarizes brain and liver synthesis (conversion) coefficients in rats fed each of the following three diets for 15 weeks: (i) a “supplemented” diet high in EPA and DHA (DHA was 2.3% of total fatty acids), (ii) a n-3 PUFA “adequate” diet containing no DHA or EPA but 4.6 % α -LNA (percent of total fatty acids), and (iii) a n-3 PUFA “deficient” diet containing no DHA and only 0.2% α -LNA [18-25]. The “adequate” diet is considered to maintain normal body function and lipid-DHA homeostasis rats [20,21].

Synthesis (conversion) coefficients (Eq. 1) were much less in brain than in liver in rats fed each of the three diets (Table 1). In brain they were higher for phospholipid than for triacylglycerol, whereas the opposite was true for the liver, consistent with a human study [14]. While the coefficients were not significantly related to diet in brain, they increased in liver as rats were moved from the DHA-supplemented to the n-3 PUFA adequate to the n-3 PUFA deficient diet. Differences in the coefficients were shown to reflect differences in mRNA, protein and activity levels between the two organs in the $\Delta 5$ and $\Delta 6$ desaturases, elongases 2 and 5 and acyl-CoA oxidase that mediate conversion of α -LNA to DHA [23,26,27]. Furthermore, upregulation of liver but not brain synthesis-conversion coefficients as dietary n-3 PUFA content declined corresponded to upregulation of the liver but not the brain conversion enzymes.

Table 2 presents conversion coefficients of unesterified circulating α -LNA to longer chain n-3 PUFAs by the heart in unanesthetized rats fed the n-3 PUFA adequate or deficient diet for 15

weeks, calculated by Eq. 1 following a 5-min intravenous infusion of [$1\text{-}^{14}\text{C}$] α -LNA [5]. While coefficients were measurable for 20:4n-3, 20:5n-3 (EPA), 22:5n-3 (DPAn-3), and 20:3n-3, they could not be determined for DHA, since [^{14}C]DHA was not detected in the heart after the infusion. This agrees with evidence that cultured rat myocytes converted EPA to 22:5n-3 but minimally, if at all, to DHA [28]. Conversion coefficients for the PUFAs in Table 2 did not differ significantly between rats on the n-3 PUFA deficient compared with n-3 PUFA adequate diet, consistent with evidence that expression of conversion enzymes did not differ significantly between the dietary groups. Thus, the rat heart has a very low (if any) capacity to synthesize DHA, and its elongation-desaturation of α -LNA to longer chain n-3 PUFAs (Table 2) cannot be upregulated by reducing circulating α -LNA. Its inability to measurably synthesize DHA from α -LNA was ascribed to an absence of elongase 2 [5], but this interpretation was shown to be erroneous because of misidentification at the time of publication of the elongase 2 gene accession number (Michael James, personal communication).

Table 3 summarizes liver, brain and plasma concentrations and conversion-synthesis rates in rats fed one of the three diets for 15 weeks post-weaning [17-19,23-25,29,30]. The first two data columns show that unesterified DHA and α -LNA concentrations in brain ($\mu\text{mol/g}$) and in plasma (nmol/ml) decreased in rats on the n-3 PUFA adequate compared with the high DHA diet, and decreased further in rats on the n-3 PUFA deficient compared with adequate diet. Rates of brain DHA consumption, obtained by methods described elsewhere [29,31,32], markedly exceeded the finite but low rate of brain DHA synthesis from α -LNA in rats on each diet. In contrast, the rate of liver DHA synthesis (Eq. 2), the product of the synthesis coefficient (Eq. 1) and the unlabeled unesterified plasma α -LNA concentration, markedly exceeded rates of both brain DHA synthesis and DHA consumption. Thus, in rats on a DHA-free “adequate” diet, the liver is almost the entire source of brain and heart DHA, since the conversion capacity of both organs is comparatively insignificant [5].

Liver DHA synthesis rates estimated from 5-min [$1\text{-}^{14}\text{C}$] α -LNA infusion studies (Table 2), while exceeding brain DHA synthesis and consumption rates, nevertheless are underestimates, since steady-state tracer conditions for synthesis and secretion are not established in the liver during the 5-min infusion [19,33-35]. Indeed, at the end of the [$1\text{-}^{14}\text{C}$] α -LNA infusion, neither unesterified nor esterified [^{14}C]DHA was identified in plasma [24].

To overcome this limitation, we developed a second method and model to determine steady-state liver synthesis and secretion of DHA from circulating unesterified α -LNA [34,35]. We infused intravenously [$\text{U-}^{13}\text{C}$] α -LNA bound to serum albumin in unanesthetized rats for 2 hours. We measured plasma concentrations of unesterified [$\text{U-}^{13}\text{C}$] α -LNA (input function), and of its labeled elongation products EPA, DPAn-3, and DHA, esterified within circulating very low density lipoprotein as a function of time. We also determined plasma volume using a dye dilution technique. Isotopic concentrations were measured using negative chemical ionization gas chromatography mass spectrometry (NCI-GC/MS), and data were analyzed by equations given elsewhere [34,35].

As illustrated in Figure 1, plasma concentration $\times$ plasma volume of esterified labeled newly synthesized elongation products of unesterified [$\text{U-}^{13}\text{C}$] α -LNA started to rise after about 30 min of constant intravenous infusion, approached linearity after about 60 min, then started to level off as these products disappeared from plasma. Whole-body synthesis-secretion rates of esterified EPA, DPA, and DHA from unesterified α -LNA were estimated by fitting esterified concentration $\times$ plasma volume data as a function of time with a sigmoidal function [34], then taking the first derivative of the function to obtain its peak value. Turnover within plasma was calculated by dividing individual esterified PUFA secretion rates by their respective unlabeled esterified plasma concentration.

Table 4 presents calculated whole body synthesis-secretion rates of EPA, DPA and DHA from circulating unesterified α -LNA in rats infused with [U- ^{13}C] α -LNA, which were fed the 2.3% DHA-containing diet. The DHA synthesis rate equaled 9.84 $\mu\text{mol/day}$, 6.3 times the hepatic rate obtained by the 5-min [1- ^{14}C] α -LNA infusion, but 43 times the rate of brain DHA consumption (Table 2). Plasma esterified PUFA half-lives ranged from 80 to 160 min. In contrast, compartmental analysis provided plasma half-lives of esterified EPA, DPA and DHA equal to 67, 58 and 22 hours, respectively, in humans fed [^{13}C] α -LNA [36].

3. DISCUSSION

In the adult rat, neither the brain nor heart is capable of synthesizing sufficient DHA from circulating unesterified α -LNA to maintain DHA homeostasis. Both organs depend on liver synthesis when DHA/EPA is absent from the diet. Even with a high dietary DHA content, the liver's synthesis-secretion rate of DHA is 43 times the brain's consumption rate. This ratio would be increased in rats fed the DHA-free n-3 PUFA adequate diet, as liver conversion coefficients, estimated with [1- ^{14}C] α -LNA infusion, are upregulated when this diet replaces the DHA-supplemented diet (Table 1).

Differences in synthesis rates among liver, heart and brain in rats fed any of the three diets discussed in this paper reflect differences in expression levels of $\Delta 5$ and $\Delta 6$ desaturase, elongase 2 and 5 and acyl-CoA oxidase. As dietary DHA is removed, and then dietary α -LNA is reduced, liver DHA synthesis coefficients increase (Table 1) due to upregulation of the liver conversion enzymes, whereas neither synthesis coefficients nor enzyme expression changes significantly in heart or brain [5]. Since the DHA-free n-3 PUFA adequate diet containing 4.6% α -LNA can maintain body organ integrity and n-3 PUFA homeostasis [20,37], at least in the rat, liver synthesis from circulating unesterified α -LNA is sufficient to maintain organ DHA homeostasis in rats fed this diet. As the heavy isotope infusion technique is minimally invasive, and heavy isotope infusion has been used in clinical studies, it should be possible to use it in human subjects to estimate whole body (presumably liver) synthesis rates of DHA from circulating α -LNA in relation to diet and other relevant conditions.

While the rat brain appears unable to upregulate its limited DHA synthetic capacity when dietary n-3 PUFA content is reduced (Table 1), the brain has homeostatic mechanisms that counter or retard the pathological effects of reduced α -LNA intake. The DHA half-life in brain is prolonged from 33 to 90 days in rats on the n-3 PUFA “deficient” compared with “adequate” diet [32], through transcriptional downregulation of genes coding for two DHA-metabolizing enzymes, DHA-selective Ca^{2+} -independent phospholipase A_2 (iPLA $_2$) and cyclooxygenase (COX)-1 [38-40]. However, this downregulation is accompanied by increased brain expression of n-6 PUFA metabolizing enzymes, Ca^{2+} -dependent cytosolic cPLA $_2$, secretory sPLA $_2$, and COX-2. These n-6 PUFA enzymes also are upregulated in animal models of neuroinflammation and excitotoxicity [41,42], which suggests that their upregulation by dietary n-3 PUFA deprivation can worsen in these conditions.

Our data as well as that of others [19,20,34,37] indicate that the adult rat liver is capable of synthesizing sufficient DHA from circulating α -LNA in rats on an “adequate” α -LNA containing diet to maintain a normal brain DHA content in the absence of dietary EPA or DHA. We do not know, however, the extent to which this conclusion applies to humans. The issue could be addressed directly by measuring brain DHA consumption with positron emission tomography, as we have done in young healthy adults [43], as well as by measuring DHA conversion from circulating α -LNA using intravenous infusion of [U- ^{13}C] α -LNA [34]. In this regard, although total plasma DHA concentrations were lower in vegetarians (29 mg/L) than in omnivores (50 mg/L) [44], mortality due to different causes and mortality in general did not differ significantly between vegetarians and omnivores [45].

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Abbreviations

DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
α -LNA	α -linolenic acid
PUFA	polyunsaturated fatty acid

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Figure 1.

Labeled esterified n-3 PUFA arterial plasma concentrations $\times$ plasma volume in an unanesthetized rat infused with $3 \mu\text{mol}/100 \text{ g}$ [^{13}C] α -LNA intravenously for 120 min, fit with a sigmoidal function. * represents [^{13}C]labeled n-3 PUFAs. From [34]

Table 1

Brain and liver synthesis (conversion) coefficients of DHA from circulating unesterified α -LNA (Eq. 1), in rats fed different n-3 PUFA containing diets for 15 weeks. Unanesthetized rats were infused intravenously with $[1-^{14}\text{C}]\alpha$ -LNA for 5 minutes; coefficients were calculated using Eq. 1. From [17-19,23,30].

Diet	Brain	Liver
	$k_{i(\alpha\text{-LNA} \rightarrow \text{DHA})}^* \text{ ml / s / g} \times 10^{-4} (i = \text{PL}, \text{TG})$	
High DHA, fishmeal containing NIH-31-18-4 diet (2.3% FA)	0.0055, 0.00040	0.03, 0.1
High α -LNA diet (4.6% FA); no DHA	0.0063, 0.00077	0.053, 0.219
Low α -LNA diet (0.2% FA); no DHA	0.0051, 0.00089	0.444, 1.45

FA = fatty acid; PL, phospholipid; TG, triacylglycerol

Table 2

Heart synthesis (conversion) coefficients of unesterified α -LNA to different elongated n-3 PUFAs in rats fed an n-3 PUFA adequate or deficient diet for 15 weeks. Unanesthetized rats were infused intravenously with [$1\text{-}^{14}\text{C}$] α -LNA for 5 minutes; coefficients were calculated using Eq. 1. From [5].

PUFA, i	N-3 PUFA adequate (4.6% α -LNA)	N-3 PUFA deficient (0.2% α -LNA)
	$k_{\alpha\text{-LNA} \rightarrow j}^* \text{ ml / s / g} \times 10^{-4}$	
20:4n-3	0.026 ± 0.007	0.024 ± 0.005
20:5n-3	0.042 ± 0.012	0.041 ± 0.008
22:5n-3	0.018 ± 0.005	0.019 ± 0.004
20:3n-3	0.020 ± 0.006	0.018 ± 0.003

Values are means $\pm$ SD (n = 10 and 7 for diet adequate and deficient groups, respectively).

Table 3

DHA synthesis rates from α -LNA by liver but not by brain exceed brain DHA consumption rates with each of 3 diets (5-min i.v. [$1\text{-}^{14}\text{C}$] α -LNA infusion). From [17-19,23,29,30].

Diet	$C_{\text{brain}}(\text{DHA})$ [$C_{\text{brain}}(\alpha\text{-LNA})$]	$C_{\text{plasma}}(\text{DHA})$ [$C_{\text{plasma}}(\alpha\text{-LNA})$]	Estimated rate of DHA consumption by 1.5 g brain, J_{in}	Estimated rate of DHA formation from α -LNA by 1.5 g brain, $\mu\text{mol/day}$	Estimated rate of DHA secretion by 11.5 g liver $\mu\text{mol/day}$
	$\mu\text{mol/g}$	nmol/ml	$\mu\text{mol/day}$	$\mu\text{mol/day}$	$\mu\text{mol/day}$
High DHA, fishmeal containing NIH-31-18-4 diet (2.3% FA)	$17.6 \pm 0.3^{\#}$ [0.010 ± 0.002]	26 ± 12 [41 ± 13]	0.23	0.002	1.57
High α -LNA diet (4.6% FA); no DHA	11.4 ± 0.8 [0.16 ± 0.003]	6.5 ± 2.6 [27 ± 6]	0.29	0.0016	2.19
Low α -LNA diet (0.2% FA); no DHA	7.14 ± 0.24 [ND]	0.23 ± 0.10 [1.0 ± 0.45]	0.06	0.000006	0.82

Mean $\pm$ SD; FA = fatty acid; ND, not detected; C_{brain} , brain concentration; C_{plasma} , plasma concentration; J_{in} , brain incorporation (consumption) rate (Eq. 2).

Table 4

Mean hepatic steady-state synthesis rates of esterified EPA, DPA and DHA from circulating unesterified α -LNA, and their plasma half-lives, in unanesthetized rats infused intravenously with [U- ^{13}C] α -LNA for 2 hours. From [34]

n-3 PUFA	Daily secretion rate from α -LNA	Plasma half-life
	$\mu\text{mol/day}$	min
EPA, 20:5n-3	8.40 ± 1.77	80.1 ± 18.9
DPA, 22:5n-3	6.27 ± 1.23	72.1 ± 13.2
DHA, 22:6n-3	9.84 ± 1.85	160.3 ± 21.0

Data are mean $\pm$ S.D. ($n = 4-6$)