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CAN THE RAT LIVER MAINTAIN NORMAL BRAIN DHA METABOLISM IN THE ABSENCE OF DIETARY DHA?^{2,3}

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Abstract

Background—Docosahexaenoic acid (DHA) is required for normal brain function. The concentration of DHA in the brain depends on both diet and liver metabolism.

Objective—To determine rat brain DHA concentration and consumption in relation to dietary n-3 (omega-3) polyunsaturated fatty acid (PUFA) content and liver secretion of DHA derived from circulating α -linolenic acid (α -LNA).

Design—Following weaning, male rats were fed for 15 weeks either: (1) a diet with a high DHA and α -LNA content, (2) an n-3 PUFA “adequate” diet containing 4.6% α -LNA but no DHA, or (3) an n-3 PUFA “deficient” diet containing 0.2% α -LNA and no DHA. Brain DHA consumption rates were measured following intravenous infusion in unanesthetized rats of [$1\text{-}^{14}\text{C}$]DHA, whereas liver and brain DHA synthesis rates were measured by infusing [$1\text{-}^{14}\text{C}$] α -LNA.

Results—Brain DHA concentrations equaled 17.6 $\mu\text{m/g}$, 11.4 $\mu\text{m/g}$ and 7.14 $\mu\text{m/g}$ in rats on diets 1, 2 and 3, respectively. With each diet, the rate of brain DHA synthesis from α -LNA was much less than the brain DHA consumption rate, whereas the liver synthesis-secretion rate was 5-10 fold higher. Higher elongase 2 and 5 and desaturase $\Delta 5$ and $\Delta 6$ activities in liver than in brain accounted for the higher liver DHA synthesis rates; these enzymes were transcriptionally upregulated in liver but not in brain of rats fed the deficient diet.

Conclusions—While DHA is essential to normal brain function, this need might be covered by dietary α -LNA when liver metabolic conversion machinery is intact and the diet has a high α -LNA content.

Keywords

docosahexaenoic acid; liver; brain; rat; n-3; omega-3; PUFA; imaging; metabolism; diet; synthesis; α -linolenic acid

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Introduction

Studies of animals fed diets with different proportions of polyunsaturated fatty acids (PUFAs) have identified dietary requirements for maintaining optimal brain function and PUFA composition (1,2), and have shown that metabolic, functional and behavioral changes can arise from long-term n-3 PUFA dietary deprivation. Clinical studies also have indicated that a low dietary consumption of n-3 PUFAs or a low plasma docosahexaenoic acid (DHA, 22:6n-3) concentration is correlated with a number of brain diseases, and with cognitive or behavioral defects during early development and aging (3-6). Dietary n-3 PUFA supplementation has been suggested to be beneficial in some of these conditions (6,7).

Nevertheless, controversy exists about the optimal dietary n-3 PUFA composition to maintain normal human brain function, in different stages of the life span (6-11). One issue relevant to this controversy regards the liver's capacity to convert circulating α -linolenic acid (α -LNA, 18:3n-3) or eicosapentaenoic acid (EPA, 20:5n-3) to DHA (12). Changes in this capacity with development, aging or disease, and in relation to diet, would likely impact brain PUFA content, metabolism and function (13-16). This controversy has been addressed by examining the relation of brain DHA consumption to the ability of the liver and brain to synthesize DHA from circulating α -LNA, in rats fed each of three diets differing in their n-3 PUFA composition.

Methods and Models

Figure 1 illustrates the brain DHA cascade at the synapse (12). It shows DHA esterified at the stereospecifically numbered (*sn*)-2 position of synaptic membrane phospholipids, from where it can be released by a phospholipase A₂ (PLA₂), particularly Ca²⁺-independent iPLA₂ Type VI (17-20). Most of the released DHA will be rapidly reincorporated into an available lysophospholipid after being converted to DHA-CoA, through the action of an acyl-CoA synthetase, with the consumption of two molecules of ATP (21). A smaller fraction will be converted to docosanoids (including neuroprotectins and neuroregulins) by cyclooxygenase (COX), lipoxygenase, or cytochrome P450 enzymes, or lost by β -oxidation within mitochondria or by other pathways (22-25).

DHA recycling within brain phospholipids, as well DHA loss due to brain metabolism, can be experimentally accessed by infusing radiolabeled DHA intravenously for 5 min in awake rats, and using analytical procedures to follow passage of the label (DHA* in Figure 1) through the acyl-CoA and phospholipid pools of high-energy microwaved brain (26,27). In this way, several kinetic parameters can be determined. One is the coefficient of incorporation k^* (ml/sec/g wet wt brain) of plasma unesterified DHA into stable brain lipids,

$$k^* = \frac{c_{brain}^*(T)}{\int_0^T c_{plasma}^* dt} \quad (\text{Eq. 1})$$

where t is time after beginning tracer infusion, $c_{brain}^*(T)$ (nCi/g) is brain radioactivity at time T of sampling (5 min), and c_{plasma}^* (nCi/ml) is plasma radioactivity due to DHA* (metabolites do not appear in plasma within 5-min).

The rate of DHA incorporation from plasma into brain, J_{in} (nmol/sec/g brain), equals the product of k^* and the unlabeled unesterified plasma DHA concentration, c_{plasma} (nmol/ml),

$$J_{in} = k^* \cdot c_{plasma} \quad (\text{Eq. 2})$$

J_{in} also is the rate of brain DHA metabolic consumption, because circulating α -LNA does not contribute significantly ($< 1\%$ converted) to brain DHA, and DHA cannot be synthesized *de novo* from 2-carbon fragments in vertebrate tissue (28-31). k^* and J_{in} are independent of changes in cerebral blood flow, and thus they reflect only brain DHA metabolism (24,32,33). Eqs. 1 and 2 can be used to determine k^* and J_{in} for α -LNA or other PUFAs, in brain or other organs.

To estimate organ rates of DHA synthesis from α -LNA in an unanesthetized rat, radiolabeled α -LNA is infused intravenously for 5 min, and its distribution and that of its elongated products are measured in different lipid compartments of the high-energy microwaved organ. A synthesis (conversion) coefficient $k_{i(\alpha-LNA \rightarrow DHA)}^*$ (ml/sec/g) is defined as,

$$k_{i(\alpha-LNA \rightarrow DHA)}^* = \frac{c_{tissue,i(DHA)}^*}{\int_0^T c_{plasma(\alpha-LNA)}^* dt} \quad (\text{Eq. 3})$$

where $c_{tissue,i(DHA)}^*$ (nCi/g) is the labeled DHA concentration in stable organ lipid i . The synthesis-conversion rate (nmol/s/g) then equals,

$$J_{in,i(\alpha-LNA \rightarrow DHA)} = k_{i(\alpha-LNA \rightarrow DHA)}^* c_{plasma(\alpha-LNA)} \quad (\text{Eq. 4})$$

With regard to the liver, we assume that the DHA formed from α -LNA eventually will be secreted when esterified in phospholipids and triglycerides within very low-density lipoproteins (34). The rate of DHA secretion (nmol/s/g) then is,

$$J_{secretion(liver)} = \sum_i J_{in,i(\alpha-LNA \rightarrow DHA)} / \lambda_{\alpha-LNA \rightarrow CoA} \quad (\text{Eq. 5})$$

$\lambda_{\alpha-LNA-CoA}$ is liver α -LNA-CoA specific activity divided by plasma α -LNA specific activity at the steady state. $\lambda_{\alpha-LNA-CoA}$ corrects for contributions of other than unesterified plasma α -LNA to the liver precursor α -LNA-CoA pool.

Kinetic parameters were determined using Eqs. 1-5 in unanesthetized male rats that had been fed, for 15 weeks post-weaning (starting at 21 days of age), one of three diets: (1) a high DHA containing diet (DHA 2.3% of total fatty acids, 5.1% α -LNA, 4% fat); (2) a DHA-free diet containing 4.6% α -LNA (of total fatty acids), 10% fat; or (3) a DHA-free diet containing 0.2% α -LNA, 10% fat. The compositions of each of the diets can be found in the original publications (12,35-37).

The latter two diets are termed n-3 PUFA “adequate” and “deficient,” respectively (35,36, 38), following the convention of Bourre (1,2). This convention was based on measuring a number of endpoints in brain and retina in relation to α -LNA dietary content, including brain accumulation of docosapentaenoic acid (DPA, 22:5n-6 (omega-6)), performance on learning tasks, enzyme activities and electrophysiological parameters.

Results

DHA consumption and synthesis rates in unanesthetized rats

An organ's ability to convert (synthesize) circulating α -LNA to DHA is given in terms of a coefficient $k_{i(\alpha-LNA \rightarrow DHA)}$ (Eq. 3), which represents serial steps involving elongation and

desaturation by elongases, desaturases and other enzymes within the organ (39). Table 1 gives experimental values for this coefficient in the brain and liver of unanesthetized rats that had been fed each of the three diets, with regard to i = phospholipid and triacylglycerol (37). $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}$ in brain was not evidently related to diet, whereas in liver the coefficient was increased in relation to reduced dietary and plasma levels of n-3 PUFAs. The liver coefficients in rats fed the high DHA and n-3 PUFA adequate diets were 10 fold the respective brain coefficients, and 100 fold in rats fed the n-3 PUFA deficient diet.

The differences between the brain and liver conversion-synthesis coefficients in relation to diet (Table 1) correlated with organ expression of enzymes involved in the conversion (40,41). Thus, activity levels of the $\Delta 5$ and $\Delta 6$ desaturases, and of elongases 2 and 5, were much higher in liver than brain in animals on the n-3 PUFA “adequate” diet, and their mRNA levels were upregulated in liver but not brain in the animals fed the n-3 PUFA deficient compared with adequate diet (39).

Table 2 summarizes additional data from the three dietary experiments. It provides whole brain and unesterified plasma concentrations of DHA and α -LNA, rates of DHA consumption by the 1.5 g rat brain (J_{in} (Eq. 2) $\times$ 1.5 g), rates of DHA synthesis from α -LNA within brain (Eq. 4 applied to brain), and rates of secretion of DHA derived from circulating α -LNA by the 11.5 g rat liver ($J_{secretion(liver)}$ (Eq. 5) multiplied by 11.5 g).

Unesterified plasma DHA declined in relation to reduced dietary n-3 PUFA content, as did brain DHA. Brain DHA equaled 17.6 $\mu\text{m/g}$ wet wt in rats fed the high DHA diet, 11.4 $\mu\text{m/g}$ in rats fed the n-3 PUFA adequate diet, and 7.14 $\mu\text{m/g}$ in rats fed the deficient n-3 PUFA diet. In the latter case, brain DHA consumption, J_{in} , was markedly reduced due to the low plasma DHA concentration. For each diet, the rate of DHA synthesis from α -LNA by brain was less than the brain's DHA consumption rate, whereas the rate of liver DHA secretion exceeded the brain's DHA consumption rate 5-10 fold.

PUFA incorporation and consumption rates of the human brain

J_{in} also has been determined for DHA and arachidonic acid (AA, 20:4n-6) in healthy human volunteers on uncontrolled diets, using positron emission tomography and the intravenous infusion of the positron-emitting radiotracers, $[1\text{-}^{11}\text{C}]\text{DHA}$ and $[1\text{-}^{11}\text{C}]\text{AA}$, respectively, and applying Eqs. 1 and 2. Whole-brain J_{in} equaled 3.8 mg/day (11.6 $\mu\text{m/day}$) for DHA compared with 17.8 mg/day for AA (42,43).

Discussion

In unanesthetized rats fed for 15 weeks post-weaning each of three diets containing different n-3 PUFA contents, the rate of brain DHA synthesis from circulating α -LNA was markedly less than the rate of brain DHA consumption, whereas the rate of liver synthesis-secretion of DHA, calculated following 5 min of intravenous $[1\text{-}^{14}\text{C}]\alpha\text{-LNA}$ infusion, was 5-10 fold greater. With the diet containing 4.6% α -LNA but no DHA, the brain DHA concentration was 11.4 $\mu\text{m/g}$. While this concentration was less than the 17.6 $\mu\text{m/g}$ in rats on the high DHA diet, earlier studies (1,2,36,38) concluded that it was sufficient to maintain normal brain function and metabolism, and thus that the 4.6% α -LNA diet should be considered “adequate.” A parsimonious conclusion from these dietary studies is that normal brain function and metabolism can be maintained in the adult rat by hepatic conversion and secretion of DHA derived from circulating α -LNA, provided sufficient α -LNA is in the diet. This conclusion is further supported by our recent report, based on infusing rats fed a high-DHA containing diet (Diet 1) intravenously with $[\text{U-}^{13}\text{C}]\alpha\text{-LNA}$ for more than 1 hour so as to produce a steady-state liver DHA synthesis-secretion rate. At the steady state, this synthesis-secretion rate exceeded the brain DHA consumption rate 30-fold (44). As this rate was more than was

calculated using the 5-min [$1\text{-}^{14}\text{C}$] α -LNA infusion method (Table 2), the latter method underestimates the liver synthesis-secretion rate because measurements are made before steady-state liver metabolism is established.

The different values of conversion-synthesis coefficients $k_{i(\alpha\text{-LNA}\rightarrow\text{DHA})}$ (Eq. 3) in liver and brain, and the 10-fold increased liver coefficient in rats fed the n-3 PUFA deficient compared with adequate diet (Table 1), reflect differences in transcription of the $\Delta 5$ and $\Delta 6$ desaturases and elongases 2 and 5 that mediate conversion of α -LNA to DHA (39-41). The increased expression of the liver enzymes in rats on the deficient diet was not found to be related to changes in the nuclear transcription factors, peroxisomal proliferator-activated receptor α and sterol-regulatory element binding protein-1 (39).

In contrast to our data suggesting that the rat liver can synthesize sufficient DHA for the brain, a number of clinical and animal studies involving ingestion of isotopic [$\text{U-}^{13}\text{C}$] α -LNA and measuring its subsequent whole body oxidation and pharmacokinetics have led to the conclusion that DHA synthesis from α -LNA by the human liver, whatever the dietary α -LNA content, is insufficient to maintain brain DHA integrity (45-47). Clearly, this issue remains controversial and deserves additional study.

We do not know as yet to what extent our data as well as animal data of others (1,2,36,38), which suggest that the adult rat liver is capable of synthesizing sufficient DHA from dietary or circulating α -LNA to maintain a normal brain DHA content, apply to the human condition. One calculation suggests that they do. For example, using an average ingestion of 1400 mg/day of α -LNA (48,49), and evidence that 1-10% of ingested α -LNA is converted to DHA (45-47), gives calculated liver synthesis rates of 14 to 140 mg/day, 3.7 to 36-fold, respectively, the human brain DHA consumption rate of 3.8 mg/day (see Results) (43). In the future, the liver synthesis might be determined directly by infusing [$\text{U-}^{13}\text{C}$] α -LNA intravenously for an extended period of time and measuring the rate of appearance of esterified [^{13}C]DHA in plasma (44).

Additional clinical data also argue that liver synthesis of DHA from α -LNA in humans not eating high amounts DHA or EPA, or even having no dietary DHA or EPA, is sufficient to maintain normal brain function and a normal life expectancy. Thus, mortality due to different causes and mortality in general did not differ significantly between vegetarians and omnivores in one study, despite lower plasma DHA levels in vegetarians (12 mg/L in vegans, 29 mg/L in vegetarians, and 50 mg/L in omnivores) (50,51).

An issue that has yet to be resolved concerns the range of brain DHA concentrations that produce optimal outcomes in life span, cognition and behavior. In rats, some of these outcomes have been worked out by Bourre when he established the n-3 PUFA adequate diet (1,2). Another issue is whether dietary DHA requirements for brain are increased in certain disease states such as liver disease, which is associated with low plasma PUFA levels (14), and in brain conditions that involve excitotoxicity and neuroinflammation, where excess dietary DHA might be neuroprotective (25). Phase III clinical trials could test whether DHA is protective in these conditions, in which case DHA might be considered a “therapeutic” agent rather than an essential nutrient.

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Figure 1.

Brain docosahexaenoic acid (DHA) recycling at the synapse. DHA at the *sn*-2 position of a phospholipid is liberated by activation (star) of synaptic PLA₂ at the synapse, secondary to neuroreceptor activation. A fraction of the unesterified DHA is converted to docosanoids, whereas the remainder is transported by a fatty acid binding protein (FABP) to the endoplasmic reticulum, where it is converted to DHA-CoA by an acyl-CoA synthetase, with the consumption of 2 ATPs. It then is esterified into an available lysophospholipid by an acyltransferase, or lost by β-oxidation in mitochondria or peroxisomes, or through other pathways (not shown). The brain unesterified DHA pool (not shown) rapidly exchanges with unesterified plasma DHA and labeled DHA*, which have dissociated from albumin. The DHA incorporation rate J_{in} equals the amount metabolized within brain and is calculated by equations in the right corner, where λ represents the ratio of DHA-CoA to plasma DHA specific activity. Modified from (12).

Table 1

Brain and liver synthesis (conversion) coefficients of DHA derived from α -linolenic acid in rats fed different n-3 PUFA containing diets. Data from (16,29,36,37).

Diet during 15 weeks post-waning	BRAIN	LIVER
	$k_{i(\alpha-LNA \rightarrow DHA)}^* \text{ ml/s/g} \times 10^{-4} (i = \text{PL, TG})$	
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, phospholipids; TG, triacylglycerol; DHA, docosahexaenoic acid

Table 2

DHA synthesis rates from α -LNA by liver but not by brain exceed brain consumption rates with each of three diets. Data from (16,29,36,37,52).

Diet during 15 weeks post-weaning	C _{brain} (DHA) [C _{brain} (α -LNA)]	C _{plasma} (DHA) [C _{plasma} (α -LNA)]	Estimated rate DHA consumption by 1.5 g brain, J _{in}	Estimated rate DHA formation from α -LNA by 1.5 g brain,	Estimated rate DHA secretion by 11.5 g liver
	$\mu\text{mol/g}$	nmol/ml	$\mu\text{mol/day}$	$\mu\text{mol/day}$	$\mu\text{mol/day}$
High DHA, fishmeal containing <i>NIH-3T1-18-4</i> diet (2.3% FA)	17.6 $\pm$ 0.3 [#] [0.010 $\pm$ 0.002]	26 $\pm$ 12 [14 $\pm$ 13]	0.23	0.002	1.57
High α -LNA diet (4.6% FA); no DHA	11.4 $\pm$ 0.8 [0.16 $\pm$ 0.003]	6.5 $\pm$ 2.6 [27 $\pm$ 6]	0.29	0.0016	2.19
Low α -LNA diet (0.2% FA); no DHA	7.14 $\pm$ 0.24 [ND]	0.23 $\pm$ 0.10 [1.0 $\pm$ 0.45]	0.06	0.0000006	0.82

[#] Mean $\pm$ SD; FA = fatty acid; ND, not detected.