Regulation by diet and liver of brain metabolism of nutritionally essential polyunsaturated fatty acids*

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Abstract: *It is possible to inject radiolabeled polyunsaturated fatty acids (PUFAs) intravenously to quantify rates of brain and liver PUFA metabolism in the intact organism, in relation to diet, aging or disease. Because circulating α-linolenic acid (α-LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6) in plasma do not contribute to brain docosahexaenoic acid (DHA, 22:6n-3) or arachidonic acid (AA, 20:4n-6), respectively, and DHA and AA cannot be synthesized de novo in vertebrate tissue, rates of incorporation of circulating DHA or AA into brain provide exact measurements of their rates of consumption by brain. Using positron emission tomography imaging, we reported that the adult human brain consumes AA and DHA at rates of 17.8 and 4.6 mg/day, respectively, and that the rate of AA consumption doesn't change with age. In unanesthetized adult rats fed an n-3 PUFA "adequate" diet containing 4.6% (of total fatty acids) α-LNA as its only n-3 PUFA, the liver secretes DHA derived from circulating α-LNA ten-times faster than the brain consumes DHA; thus the liver is capable of supplying all the brain's DHA. With a low dietary α-LNA level, rat liver coefficients of α-LNA conversion to DHA are increased because of increased liver elongase and desaturase activities, and DHA loss from brain is slowed due to downregulated DHA-metabolizing enzymes, including Ca^{2+}-independent phospholipase A_2 ($iPLA_2$). The n-3 PUFA "deficient" diet also increases brain expression of AA-metabolizing enzymes, cytosolic $cPLA_2$, secretory $sPLA_2$ and cyclooxygenase-2, and the brain docosapentaenoic acid (22:5n-6) concentration. These changes, plus reduced expression of brain derived neurotrophic factor (BDNF) caused by the "deficient" diet, likely increase brain vulnerability to excitotoxicity and inflammation.*

Key words: *docosahexaenoic acid, liver, brain, rat, n-3 PUFAs, imaging, metabolism, phospholipase A_2, BDNF, diet, arachidonic acid*

Introduction

Brain structure and function depend on interactions between arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) at multiple sites [1-3]. These long-chain polyunsaturated fatty acids (PUFAs) and their respective shorter-chain PUFA precursors, linoleic acid (LA, 18:2n-6) and α-linolenic acid (α-LNA, 18:3n-3), are nutritionally essential and cannot be synthesized *de novo* in vertebrate tissue.

Animal studies with different proportions of PUFAs in the diet have identified broad dietary requirements for maintaining optimal brain function [4], and have demonstrated that metabolic and behavioral defects arise from severe n-3 PUFA dietary deprivation. Additionally, clinical studies indicate that low dietary consumption of n-3 PUFAs or low plasma DHA concentrations are correlated with a number of brain diseases and with cognitive and behavioral defects in development and aging [5-7], and that dietary n-3 PUFA supplementation may be beneficial in some of these conditions [3, 8].

Effects on the brain of minor n-3 PUFA dietary deprivation associated with small declines in plasma DHA concentrations of the order found in the clinic have rarely been studied in animal models. Additionally, controversy exists about which dietary PUFA compositions are optimal for human brain function [3, 8-12]. The liver's *in vivo* capacity to convert α-LNA to DHA, or LA to AA, has not be quantified in animals or in humans, although changes in this capacity with development, aging or disease likely impact brain PUFA metabolism [13-17].

To address these and related issues, we have developed methods and models to examine PUFA metabolism in the intact organism. The methods include brain imaging with quantitative autoradiography or positron emission tomography (PET), intravenous injection of radiolabeled PUFAs to examine incorporation, turnover and synthesis rates of PUFAs in brain or liver, enzyme assays to evaluate activities of lipid metabolizing enzymes, and molecular techniques to examine transcriptional regulation and protein levels of these enzymes. We have used these approaches to measure AA and DHA brain consumption rates in unanesthetized rats and in human subjects, and to quantify dietary effects in rats on brain and liver PUFA metabolism. We shall discuss the results of these experiments in this paper, when considering three general questions: (1) What are the rates of brain consumption of AA and DHA in rats and humans in relation to diet? (2) How does brain DHA depend on dietary n-3 PUFA composition and the liver's ability to convert α-LNA to DHA? (3) How do brain lipid enzymes and trophic factors respond to dietary n-3 PUFA deprivation?

Methods and models

AA and DHA are found in high concentrations in the stereospecifically numbered (*sn*)-2 position of brain membrane phospholipids, from where they can be released by selective phospholipase A_2 (PLA_2) enzymes [18-23]. After release, most of the unesterified AA or DHA will

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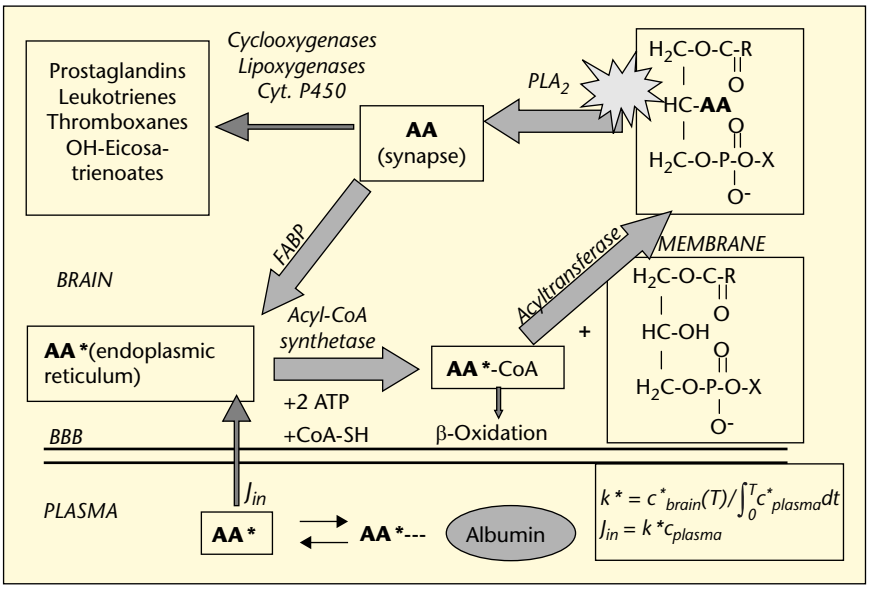

Figure 1. *Model of brain arachidonic acid cascade at the synapse. Arachidonic acid (AA), esterified at the sn-2 position of a phospholipid, is liberated by activation (star) of PLA_2 at the synapse. A fraction of the unesterified AA is converted to eicosanoids by cyclooxygenase, lipoxygenase or P450 enzymes, whereas the remainder is transported by a fatty acid binding protein (FABP) to the endoplasmic reticulum. From there, AA is activated to arachidonoyl-CoA by an acyl-CoA synthetase with the consumption of two ATPs, then esterified into an available lysophospholipid by an acyltransferase. Unesterified AA also can be lost by β-oxidation in mitochondria or peroxisomes, or by other pathways (not shown). The endoplasmic reticulum compartment is in very rapid equilibrium with unesterified plasma AA that has been released from circulating albumin, whereas the synaptic compartment does not exchange with plasma AA. This allows injecting radiolabeled AA* intravenously and determining the incorporation rates J_{in} of unesterified unlabeled plasma AA into individual membrane phospholipids, as well as AA turnover rates and half-lives in those phospholipids. From [37].*

be rapidly reincorporated into an available lysophospholipid *via* the acyl-CoA pool, through serial actions of an acyl-CoA synthetase and acyltransferase with the consumption of two molecules of ATP *(figure 1)* [24]. A small fraction, however, will be lost through any of a number of catabolic pathways, including β-oxidation and conversion to eicosanoids or docosanoids by cyclooxygenases (COXs), lipoxygenases, or cytochrome P450 [25-29]. Neither AA nor DHA is converted significantly (< 1%) in brain from its respective precursor, LA or α-LNA *(figure 2)* [30, 31], and neither can be synthesized *de novo* in vertebrate tissue [32]. However, the quantity of AA or DHA that is lost from brain by metabolism will be rapidly and stoichiometrically replaced by the unesterified PUFA in plasma [33]. Replacement occurs independently of changes in cerebral blood flow at a rate J_{in} (Eq. 2 below) [27, 30, 31, 34-39].

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; LA, linoleic acid; PET, positron emission tomography; PUFA, polyunsaturated fatty acid; PLA_2, phospholipase A_2; α-LNA, α-linolenic acid; BDNF, brain derived nerve growth factor; CREB, cAMP response element-binding protein; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; MAP, mitogen activated protein; *sn*, stereospecifically numbered; COX, cyclooxygenase

In unanesthetized rodents and in human subjects, we have quantified J_{in} by infusing intravenously injected albumin-bound radiolabeled AA or DHA, then imaging regional brain radioactivity. We also have quantified J_{in} into individual phospholipids, triacylglycerols and cholesteryl esters of rodent liver and brain, after subjecting the organ to high energy microwaving to stop its metabolism, then measuring PUFA specific activities in organ lipid compartments.

For neuroimaging, an incorporation coefficient k^* (ml/sec/g brain) is calculated as regional brain radioactivity divided by the integrated plasma radioactivity of the unesterified AA or DHA (input function),

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

where t is time after beginning tracer infusion, $c^*_{brain}(T)$ nCi/g is brain radioactivity at time T of sampling (often 5 min), and $c^*_{plasma}(T)$ nCi/ml is plasma radioactivity. J_{in} nmol/sec/g brain equals k^* multiplied by the unlabeled unesterified plasma AA or DHA concentration, c_{plasma} nmol/ml,

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

The half-life $t_{1/2}$ is calculated from the esterified brain concentration c_{brain} and the incorporation rate,

$$t_{1/2} = 0.693 c_{brain}/J_{in} \qquad \text{(Eq. 3)}$$

The incorporation rate J_{in}, determined following the intravenous infusion of a radiolabeled PUFA using Eq. 3, equals the rate of PUFA loss from brain, J_{loss} (see above),

$$J_{loss} = 0.693 c_{brain}/t_{1/2} \qquad \text{(Eq. 4)}$$

This is illustrated, for example, in *figure 3* (left) for DHA, where the half-life of DHA loss from rat brain was calculated from the rate of decline of brain [4,5-^{3}H]DHA following its intracerebral injection [36].

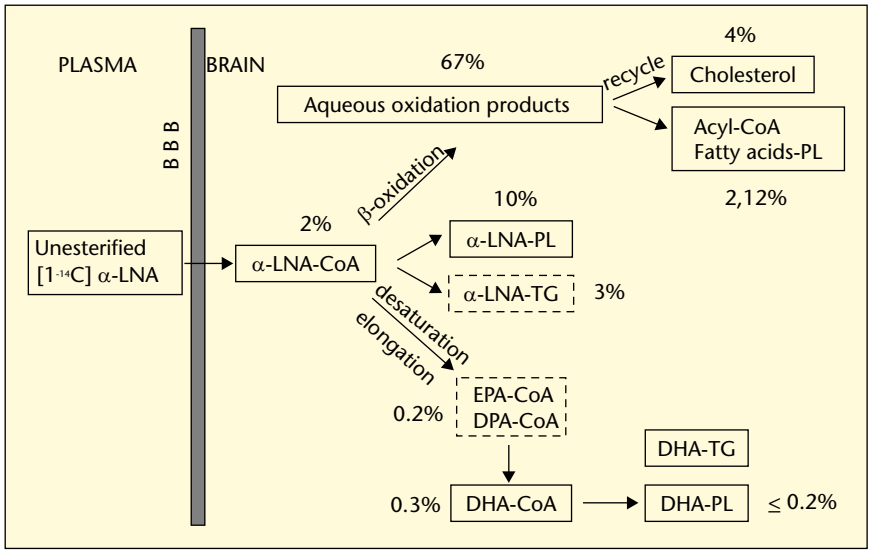

Figure 2. *Fractional distribution of [1-^{14}C]α-LNA in different lipid compartments of rat brain, following 5 min of its intravenous infusion in unanesthetized rats on a high 2.3% DHA containing diet. Less than 1% of the tracer has been elongated to EPA or DHA in the acyl-CoA, phospholipid(PL) or triacylglycerol (TG) pools. From [30].*

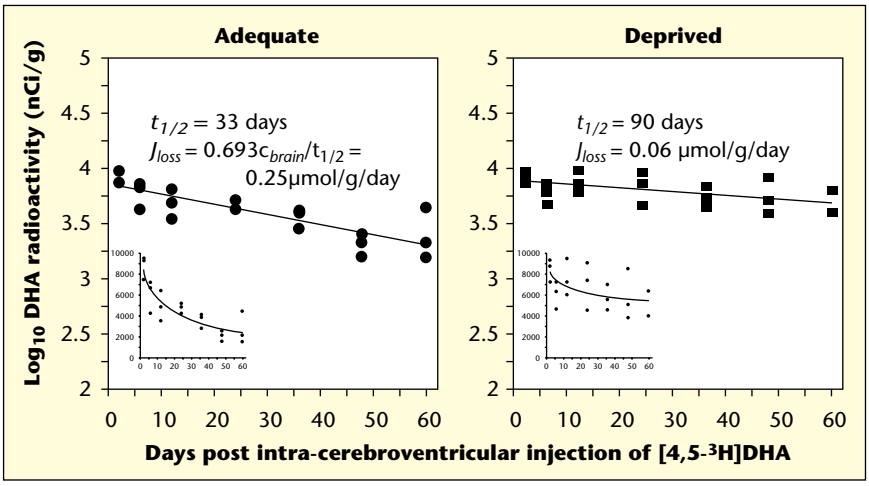

Figure 3. *Fifteen weeks of dietary n-3 PUFA deprivation in post-weaning rats prolongs half-life and slows DHA loss in rat brain phospholipid. [4,5-^{3}H]DHA was injected into the brain. Radioactivity due to it was followed in individual phospholipids for 60 days, from which half-lives $t_{1/2}$ were calculated (Eq. 3.) J_{loss} was calculated from half-life as illustrated in figure. Adapted from DeMar et al. [36].*

Half-lives for net DHA or AA loss from brain (Eqs. 3 or 4), which are of the order of weeks to months in rats [36, 37], are much longer than their respective half-lives due to recycling (deacylation-reacylation) *(figure 1)* [26, 40]. These can be minutes to hours [37, 41]. Recycling is associated with neurotransmission and rapid, neuroreceptor-initiated PLA_2-mediated release of AA or DHA from membrane phospholipid [18, 42], and it is accompanied by high rates of ATP consumption [43].

We have extended our *in vivo* fatty acid model to quantify coefficients and rates of conversion and esterification of circulating α-LNA to DHA into "stable" brain and liver lipids *i* [16, 17, 30, 31, 44]. The appropriate equations relate [1-^{14}C]DHA radioactivity within "stable" lipids *i* following intravenous [1-^{14}C]α-LNA infusion, to the organ's integrated exposure to the tracer in plasma. For the liver, the conversion coefficient is given in units of mL/sec/g liver,

$$k^*_{i(\alpha-LNA\rightarrow DHA)} = \frac{c^*_{liver(DHA),i}(T)}{\int_0^T c^*_{plasma(\alpha-LNA)}dt} \quad \text{(Eq. 5)}$$

whereas the conversion rate in units of nmol/sec/g liver equals,

$$J_{in,i(\alpha-LNA\rightarrow DHA)} = k^*_{i(\alpha-LNA-DHA)} c_{plasma(\alpha-LNA)} \quad \text{(Eq. 6)}$$

where $c^*_{liver(DHA),i}(T)$ nCi/g is DHA radioactivity in "stable" lipid *i*, $c^*_{plasma(\alpha-LNA)}$ nCi/mL is plasma radioactivity due to unesterified [1-^{14}C]α-LNA, and $c_{plasma(\alpha-LNA)}$ nmol/mL is the plasma concentration of unesterified unlabeled α-LNA. The rate of secretion by liver of the DHA that it has synthesized from total plasma α-LNA can be estimated by summing equations 6 for *i* = phospholipid, triacylglycerol and cholesteryl ester, then dividing by a "dilution" factor $\lambda_{\alpha-LNA-CoA}$ [17, 27, 44]. This factor equals the steady-state ratio of specific activity of liver α-LNA-CoA to specific activity of plasma unesterified α-LNA, during infusion of [1-^{14}C]α-LNA,

$$\text{Rate of secretion} = \sum_i J_{in,i(\alpha-LNA\rightarrow DHA)} / \lambda_{\alpha-LNA-CoA} \quad \text{(Eq. 7)}$$

Results and questions

Question 1: What are the rates of brain consumption of AA and DHA in rats and humans in relation to diet?

Studies in unanesthetized rats

To examine how brain PUFA metabolism is related to dietary PUFA composition, we quantified brain consumption rates of DHA in relation to the liver's ability to convert circulating α-LNA to DHA in rats that had been fed, for 15 weeks post-weaning (starting at 21 days of age), one of three diets (*table 1*, row 1): (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. We term the latter two diets n-3 PUFA "adequate" and "deficient", respectively, following the convention

Table 1. *Plasma and brain parameters in unanesthetized rats fed different diets for 15 weeks. Row 1: dietary composition; row 2: unesterified plasma concentrations of α-LNA and DHA (in brackets); row 3: unesterified plasma concentrations of AA and DPAn-6 (in brackets); row 5: AA and DPAn-6 (in brackets) concentrations in brain phospholipids; row 6: incorporation coefficient calculated by Eq. 1; row 7, incorporation rate calculated by Eq. 2; row 8: whole brain DHA incorporation (consumption) rate for 1.5 g rat brain.*

			Diet during 15 weeks post-weaning		
1	**Parameter**	**Units**	**High DHA diet (5.1% α-LNA, 2.3% DHA, 4% fat)**	**High α-LNA diet (4.6% α-LNA, no DHA, 10% fat)**	**n-3 PUFA inadequate diet (0.2% α-LNA, no DHA, 10% fat)**
2	**c_{plasma}(α-LNA) [c_{plasma}(DHA)]**	nmol/ml	41 ± 13$^{\#a}$ [26 ± 12]^a	27 ± 6$^{\#}$ [6.5 ± 2.6]^b	1.0 ± 0.45* [0.23 ± 0.10]*^{b}
3	**c_{plasma}(AA) [c_{plasma}(DPAn-6]**	nmol/ml	22.5 ± 5.6 [2.3 ± 1.2]^c	25 ± 4.8 [ND]^b	34 ± 5.9 [8.7 ± 1.1]*^{b}
4	**DHA concentration in brain phospholipid, c_{brain}**	µmol/g	17.6 ± 2.8^a 13.8 ± 4.9^c	12.0 ± 2.4^d	7.6 ± 1.5*^{d}
5	**Concentrations of AA and [DPAn-6] in brain phospholipid**	µmol/g	11.1 ± 2.9^c [0.1 ± 0.04]^c	9.4 ± 1.1^d [0.25 ± 0.06]^d	9.8 ± 1.5^d [4.4 ± 1.8]*^{d}
6	**DHA incorporation coefficients, *k****	ml/s/g x 10^{-4}	2.2 ± 0.2^f	1.99 ± 0.3^e	2.83 ± 0.6*^{e}
7	**Rate DHA incorporation, $J_{in}$$^{\#}$**	nmol/s/g x 10^{-4}	17.4 ± 2.0^f	22.0 ± 5.0^e	0.23 ± 0.05*^{e}
8	**Daily rate DHA consumption by whole (1.5 g) brain**	µmol/day	0.23^f	0.29^e	0.003^e

$^{\#}$ Mean ± SD; AA, arachidonic acid, DHA, docosahexaenoic acid, α-LNA, α-linolenic acid, DPA, docosapentaenoic acid, ND, not detected; *Differs significantly from mean in high α-LNA (n-3 PUFA adequate) diet rats; $^{\#}$ net rate for brain phospholipids. ^{a} [30]; ^{b} [17]; ^{c} [31]; ^{d} [36]; ^{e} [68]; ^{f} [69].

of Bourre [4]. The rats fed the "deficient" compared with "adequate" diet had increased scores on behavioral measures of depression and aggression [45].

The unesterified plasma α-LNA concentration in rats fed the n-3 PUFA "adequate" diet was 36% less than in rats fed the DHA-containing diet, but 27 times higher than in rats fed the "deficient" diet (*table 1*, row 2). Unesterified plasma AA did not differ markedly among rats on the three diets, whereas the plasma concentration of the AA elongation product, docosapentaenoic acid (DPAn-6, 22:5n-6), while low in rats fed the high DHA or n-3 PUFA "adequate" diet, equaled 8.7 nmol/mL in rats fed the "deficient" diet (row 3, *table 1*).

The DHA concentration in brain phospholipid (*table 1*, row 4) was lower in rats fed the "adequate" than high DHA diet, but was reduced by an additional 4.4 μmol/g in rats fed the "deficient" diet. While AA concentrations in brain phospholipid were about the same in rats on each of the three diets (*table 1*, row 5), brain DPAn-6 was elevated by 4.2 μmol/g in rats fed the "deficient" diet, compensating for the reduced DHA concentration.

DHA incorporation coefficients k* (Eq. 1) did not vary markedly among the three dietary groups (*table 1*, row 6), whereas the net rate of DHA incorporation into brain, J_{in}, thus its rate of loss from brain, was reduced in rats fed the deficient diet (*table 1*, rows 7 and 8), due to the low plasma DHA concentration with this diet (Eq. 2). Reduced values of J_{in} for DHA in rats fed the "deficient" diet corresponded to a 3-fold prolongation of the DHA half-life in brain phospholipid (*figure 3*, right) [36]. Values of J_{in} for AA can be found elsewhere in unanesthetized mice and rats [34, 35, 46, 47].

PUFA consumption by the human brain

We also have determined J_{in} for AA and DHA in the human brain using PET and the positron emitting tracers, [1-^{11}C]AA and [1-^{11}C]DHA, respectively [48-51] (Umhau *et al.*, unpublished results). Whole-brain J_{in} in healthy adults equaled 17.8 mg/day per 1500 g brain for AA *(figure 4)* [49] and 4.6 mg/day per 1500 g brain for DHA (Umhau *et al.*, unpublished results). Furthermore, J_{in} for AA did not decline with healthy aging [49].

Dietary intakes of n-3 PUFAs for maintaining optimal human brain PUFA metabolism are not agreed on, but they now might be estimated by relating dietary PUFA composition to PET-determined brain DHA incorporation (consumption) rates. Different committees have recommended eicosapentaenoic acid (EPA, 20:5n-3) + DHA intakes of 0.11-0.16 g/day [52], 0.2 g/day [10], 0.65 g/day [11], and 1.6 g/day [12]. Another committee recommended that adult men and women should consume 1.6 g/day and 1.1 g/day, respectively, of α-LNA, plus an additional 10% (0.16-0.11 g) representing EPA + DHA [52]. Our PET-determined J_{in} for DHA, 4.6 mg/day (see above), equals 2.5-5% of the estimated average daily dietary intake of EPA + DHA in the United States, 100-200 mg/day [9].

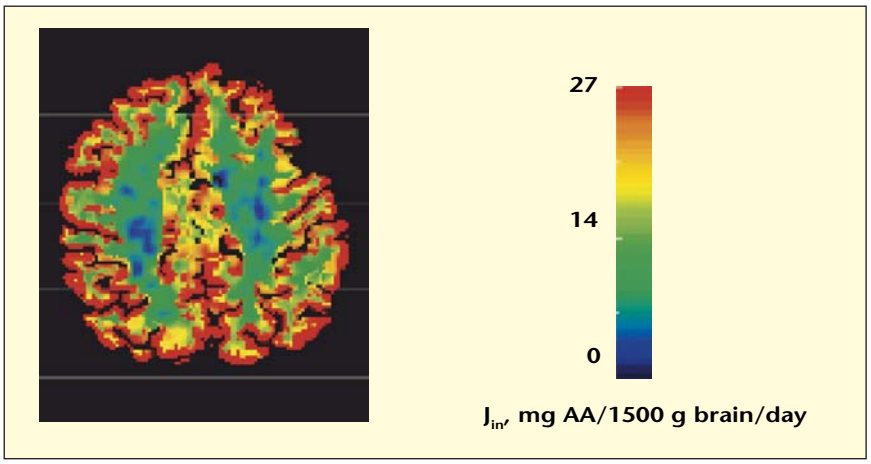

Figure 4. *Horizontal section showing regional incorporation rates of plasma unesterified arachidonic acid into human brain, after correction for partial voluming. Rates are given in terms of color-coding. The global rate, obtained by integrating regional rates for whole brain, equaled 17.8 mg/1500 g brain/day. From [49].*

Question 2: How does brain DHA depend on dietary n-3 PUFA composition and the liver's ability to convert α-LNA to DHA?

A large fraction of the world's population does not eat meat or fish for various reasons, but the effects, if any, of low dietary levels of DHA and

Table 2. *Calculated liver parameters in unanesthetized rats fed each of three diets, correspond to plasma and brain data of table 1a. Row 2: conversion coefficients calculated by Eq. 5; rows 3 and 4: secretion rates per g and per total liver, calculated by Eq. 6.*

			Diet During 15 Weeks Post-weaning		
1	**Parameter**	**Units**	**High DHA diet (5.1% α-LNA, 2.3% DHA, 4% fat)**[a]	**High α-LNA diet (4.6% α-LNA, no DHA, 10% fat)**[b]	**n-3 PUFA inadequate diet (0.2% α-LNA, no DHA, 10% fat)**[b]
2	**Conversion coefficients liver,** $k^{*}_{i(\alpha\text{-}LNA\rightarrow DHA)}$ **(*i* = PL, TG)**	ml/s/g × 10^{-4}	0.03, 0.1	0.053, 0.219	0.44, 1.45
3	**Net DHA conversion rate per g liver** $\sum_i J_{in,i(\alpha\text{-}LNA\rightarrow DHA)}$	nmol/s/g × 10^{-4}	6.6	7.45	1.99
4	**Net daily DHA secretion rate, per 11.5 g rat liver#**	μmol/day	1.57	2.19	0.82

PL, phospholipid; TG, triacylglycerol; #Calculated by Eq. 7 with $\lambda_{\alpha\text{-}LNA\text{-}CoA}$ equal to 0.47, 0.34 and 0.24 for high DHA, 4.6% α-LNA, 0.2% α-LNA diet, respectively.[a] [44]; [b] [17].

EPA on their brain function have not been identified [53]. In such subjects, the brain's DHA content must depend on the liver's ability to synthesize and secrete DHA from circulating α-LNA.

To address the issue of liver synthesis, we estimated the liver's ability to synthesize DHA from α-LNA in unanesthetized rats fed each of the three diets discussed above. Row 2 of *table 2* gives the calculated conversion coefficients $k^*_{i(\alpha-LNA\rightarrow DHA)}$ (Eq. 5) of unesterified α-LNA to DHA into "stable" liver lipids *i* = phospholipid (PL) and triacylglycerol (TG), whereas row 3 gives the sum of rates $J_{in,i(\alpha-LNA\rightarrow DHA)}$ of DHA synthesis followed by incorporation (Eq. 6) into these lipids. Assuming that the liver secretes its newly formed esterified DHA within circulating lipoproteins [54], row 4 presents liver DHA secretion rates, calculated by Eq. 7, in units of μmol/day per 11.5 g rat liver.

Conversion coefficients of unesterified plasma α-LNA into liver phospholipid and triacylglycerol DHA were 2-fold greater in rats fed the n-3 PUFA "adequate" than high DHA diet, and were further increased 7-fold in rats fed the "deficient" diet. The increases corresponded to increased liver activities of the Δ5 and Δ6 desaturases and elongases 2 and 5 that mediate conversion of α-LNA to DHA and of LA to AA [55] (Igarashi *et al.*, unpublished results). Net liver DHA synthesis and secretion rates were much less in rats on the n-3 PUFA "deficient" than "adequate" diet (*table 2*, rows 3 and 4), reflecting the low plasma α-LNA concentration with the "deficient" diet (Eq. 6). The liver's net DHA synthesis rate in rats fed the DHA-free n-3 PUFA "adequate" diet was about 10-fold the brain's DHA consumption rate (*table 1*, row 8). Thus, liver secretion was sufficient to supply the brain's DHA.

In summary, in rats fed an n-3 PUFA "adequate" diet containing 4.6% α-LNA, brain DHA is maintained entirely by the DHA formed and secreted from circulating α-LNA by the liver. When dietary α-LNA is reduced, the liver increases its coefficients for DHA synthesis by upregulating activities of relevant desaturases and elongases.

Question 3: How do brain lipid enzymes and trophic factors respond to dietary n-3 PUFA deprivation?

Enzymes of the brain AA and DHA cascades

DHA-loss half life in rat brain (Eq. 4) was prolonged 3-fold in rats fed the n-3 PUFA "deficient" compared with the "adequate" diet (*figure 3*, right) [36]. This prolongation resulted from the brain's ability to downregulate expression of some of its DHA-metabolizing enzymes. Thus, in rats fed the "deficient" compared with "adequate" diet, brain mRNA, protein and activity levels of DHA-selective Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) [22] and of COX-1 were downregulated [56], as illustrated in *figure 5*. The two affected enzymes are known to be functionally coupled in different tissues [57].

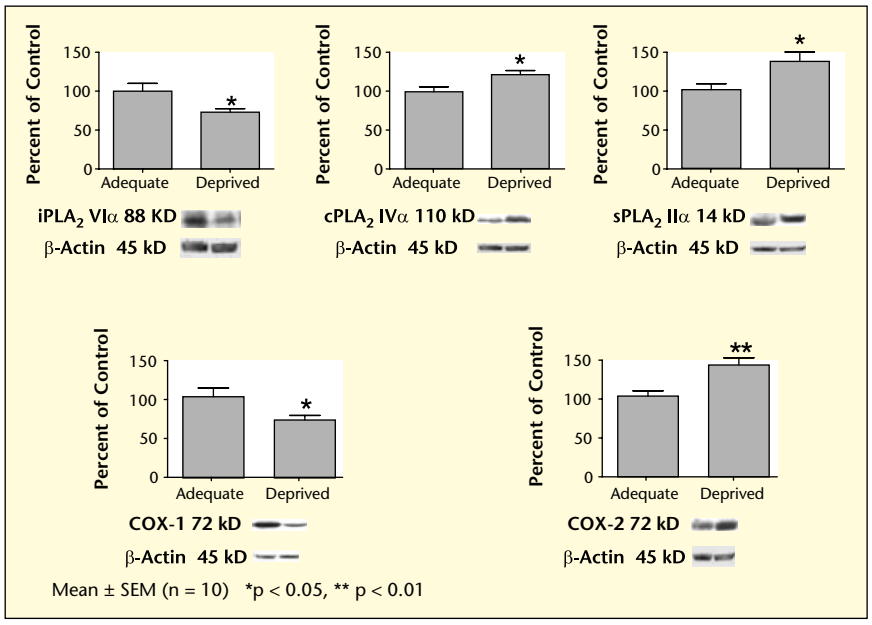

Figure 5. *Fifteen weeks of n-3 PUFA dietary deprivation, compared with an n-3 PUFA "adequate" diet, decreases rat frontal cortex $iPLA_2$ and COX-1 protein but increases $sPLA_2$, $cPLA_2$ and COX-2 protein. From [56].*

Also illustrated in *figure 5*, the 15-week n-3 PUFA deficiency upregulated brain mRNA, protein and activity levels of AA-selective $cPLA_2$, secretory $sPLA_2$ and COX-2 [56], enzymes that often are functionally coupled [23, 57, 58]. These changes, in the context of an increased brain DPAn-6 concentration (*table 1*, row 3), imply that the "deficient" diet upregulated brain n-6 PUFA metabolism.

Excess AA metabolism can contribute to neuronal damage in experimental ischemia, glutamate excitotoxicity, neuroinflammation, and cerebral trauma [42, 59-63]. This implies that n-3 PUFA dietary deficiency would increase brain vulnerability to these insults by increasing brain n-6 PUFA metabolism, whereas dietary n-3 PUFA supplementation would be neuroprotective. In this regard, a low dietary n-3 PUFA content has been suggested to increase brain vulnerability in a number of human diseases, including Alzheimer disease and bipolar disorder, in which neuroinflammation and excitotoxicity play a role, and n-3 PUFA supplementation may be helpful in some of these diseases [5, 6, 64, 65].

BDNF and CREB

Another way in which dietary n-3 PUFAs may be neuroprotective is by upregulating brain trophic factors. For example, brain derived neurotrophic factor (BDNF) promotes neuronal survival, plasticity, differentiation and growth [66]. Transcription of the BDNF gene is regulated by the cAMP response element-binding protein (CREB), following CREB's phosphorylation by protein kinases including p38 mitogen activated protein (MAP) kinase [67]. In rats fed the n-3 PUFA "deficient" compared with "adequate" diet, *figure 6* shows that brain mRNA and protein levels of BDNF, CREB DNA binding activity, the phosphorylated CREB protein level and p38 MAP kinase activity were reduced significantly [67].

In summary, rats subjected to our 15-week dietary n-3 PUFA deprivation have a reduced brain DHA concentration and a prolonged DHA half-life, accompanied by reduced activities of presumably DHA-selective $iPLA_2$ and COX-1; an increased brain DPAn-6 concentration accompanied by increased activities of AA-selective $cPLA_2$, $sPLA_2$ and COX-2; and reduced expression of BDNF that corresponds to reduced CREB DNA binding activity and p38 MAP-kinase activity.

Conclusions

In response to each of the three questions presented in the Introduction, we have shown that: (1) Regional and global brain AA and DHA consumption rates can be and have been quantified in unanesthetized rats, and in humans using PET. (2) In the absence of dietary DHA, a normal brain DHA content can be maintained by liver conversion of α-LNA to circulating DHA, provided sufficient α-LNA is in

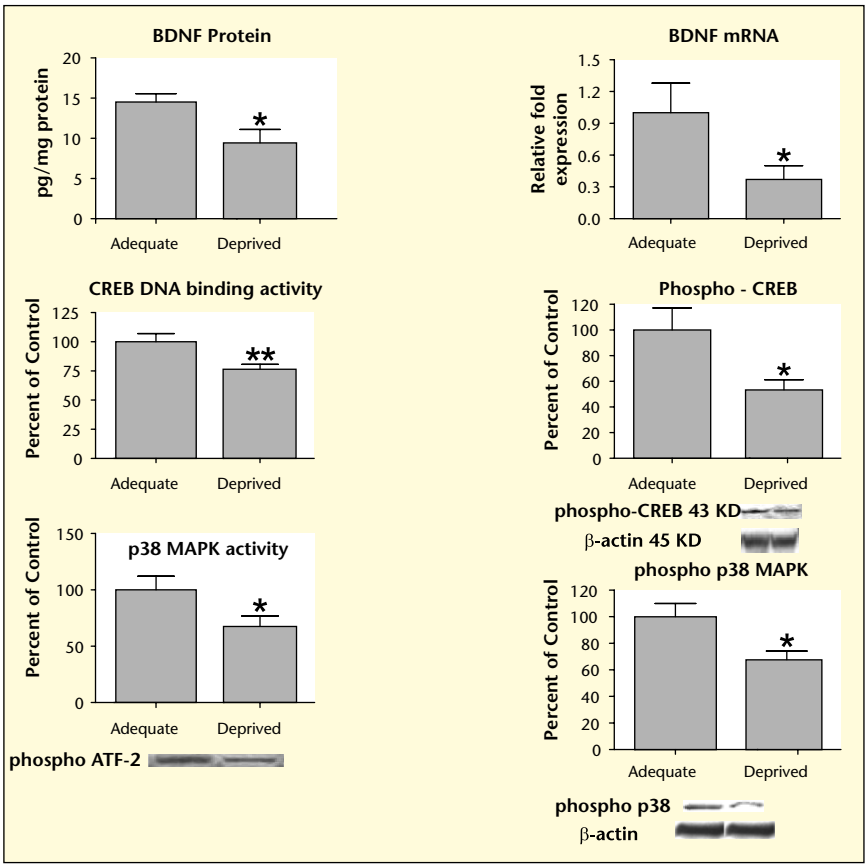

Figure 6. *Fifteen weeks of n-3 PUFA dietary deprivation, compared with an n-3 PUFA adequate diet, downregulates rat frontal cortex p38 MAP kinase activity and phospho p38 MAP kinase protein, CREB DNA binding activity and phospho-CREB protein, and BDNF protein and mRNA. From [67].*

the diet. Liver but not brain conversion coefficients are increased by further α-LNA deprivation, in relation to increased expression of liver elongases and desaturases. (3) Brain DHA reduction caused by 15 weeks of dietary n-3 PUFA deprivation in rats is associated with slowed DHA loss from brain and reduced expression of presumably DHA-metabolizing enzymes, tending to conserve brain DHA, by increased expression of AA-metabolizing enzymes and a high DPAn-6 concentration, and by reduced BDNF, phospho-CREB and p38 MAP kinase activity levels. Some of these changes are consistent with neuroprotective effects of n-3 PUFAs.

Now that appropriate quantitative techniques are available for studying the relations among brain and liver PUFA metabolism and diet in animals and humans, future studies using these techniques might address a number of additional relevant questions: (1) To what extent does the liver convert EPA to DHA under different dietary conditions? (2) What are the effects of graded n-3 PUFA dietary deprivation on the markers and kinetics of brain metabolism and function that we have presented in this paper? (3) What are the effects on these markers of dietary n-6 PUFA deprivation? (4) How do liver conversion rates of α-LNA and EPA to secreted DHA vary with age and liver disease? (5) In humans, how do brain AA and DHA consumption rates change with aging or disease, and how might human diets be tailored to maintain normal consumption rates with these variable conditions?

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