Progress in Lipid Research 59 (2015) 54–66

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Is docosahexaenoic acid synthesis from α-linolenic acid sufficient to supply the adult brain?

Anthony F. Domenichiello [1], Alex P. Kitson [1], Richard P. Bazinet *

University of Toronto, Department of Nutritional Sciences, Canada

ARTICLE INFO

Article history:
Received 11 August 2014
Accepted 9 April 2015
Available online 25 April 2015

Keywords::
Docosahexaenoic acid
Alpha-linolenic Acid
DHA
ALA
Brain
Liver
Synthesis
Uptake
Metabolism
Requirement

ABSTRACT

Docosahexaenoic acid (DHA) is important for brain function, and can be obtained directly from the diet or synthesized in the body from α-linolenic acid (ALA). Debate exists as to whether DHA synthesized from ALA can provide sufficient DHA for the adult brain, as measures of DHA synthesis from ingested ALA are typically <1% of the oral ALA dose. However, the primary fate of orally administered ALA is β-oxidation and long-term storage in adipose tissue, suggesting that DHA synthesis measures involving oral ALA tracer ingestion may underestimate total DHA synthesis. There is also evidence that DHA synthesized from ALA can meet brain DHA requirements, as animals fed ALA-only diets have brain DHA concentrations similar to DHA-fed animals, and the brain DHA requirement is estimated to be only 2.4–3.8 mg/day in humans. This review summarizes evidence that DHA synthesis from ALA can provide sufficient DHA for the adult brain by examining work in humans and animals involving estimates of DHA synthesis and brain DHA requirements. Also, an update on methods to measure DHA synthesis in humans is presented highlighting a novel approach involving steady-state infusion of stable isotope-labeled ALA that bypasses several limitations of oral tracer ingestion. It is shown that this method produces estimates of DHA synthesis that are at least 3-fold higher than brain uptake rates in rats.

Contents

* Corresponding author at: Department of Nutritional Sciences, University of Toronto, Fitzgerald Building, 150 College St., Room 306, Toronto, Ontario M5S 3E2, Canada. Tel.: +1 416 946 8276.
E-mail address: richard.bazinet@utoronto.ca (R.P. Bazinet).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plipres.2015.04.002
0163-7827/

1. Introduction

Docosahexaenoic acid (DHA, 22:6n-3) is highly concentrated in the brain, and is important for brain function in part by regulation of cell survival and neuroinflammation [1–5]. DHA cannot be synthesized *de novo* in mammals, and therefore, must be obtained in the diet primarily through fish, nutraceuticals and functional foods [6] or synthesized within the body from α-linolenic acid (ALA, 18:3n-3). While fish oil also contains the n-3 PUFA eicosapentaenoic acid (EPA), DHA is the main n-3 PUFA in the brain as it is concentrated at levels of about 10,000 nmol/g brain (10–15% of brain fatty acids or about 5 g in an adult brain [7,8]), at least 50-fold more than EPA and 200-fold more than ALA [8,9].

In mammals, DHA synthesis rates from ALA are suggested to be low relative to dietary intake and tissue demand, however, debate exists as to whether the rate of DHA synthesis is sufficient to meet functional requirements for DHA. Estimates of DHA synthesis in humans are based on appearance of labeled DHA following oral ingestion of stable-isotope ALA, or changes in blood DHA following acute or chronic increases in ALA ingestion. Stable isotope methods have typically resulted in estimates of percent conversion of ALA to DHA being less than 1% of the ingested stable-isotope ALA, although estimates vary widely, ranging from 0–9.2% (Table 1). Also, there is typically no increase in plasma total lipid or phospholipid DHA when ALA intake is increased in humans (reviewed in [10,11]), supporting the conclusion that DHA synthesis from ingested ALA is not an efficient process in humans.

However, there is evidence that DHA synthesis from ALA can be sufficient to maintain brain function. For example, vegetarians and vegans, in which DHA derived from ALA is the sole source of DHA, have plasma DHA levels that are 0–40% lower than omnivores [12–14] and have neurological disease rates comparable to omnivores [15–18] suggesting that ALA-derived DHA is sufficient to maintain brain function in these individuals. In addition, dietary ALA, with no DHA, is sufficient to completely restore brain DHA in rats [19] and non-human primates [20] following *in utero* DHA depletion, although retinal DHA was not completely restored in non-human primates. Taken together, evidence suggests that ALA-derived DHA is sufficient to maintain brain DHA levels and preserve function.

In addition to a biological precedent for dietary ALA supplying adequate DHA for the brain, there is also environmental rationale to pursue this possibility. Concern has been raised regarding the environmental sustainability of current recommendations for DHA intake [21], as fish are the primary dietary source of DHA [22] and the world's fish stocks are declining [23]. Although controversial [24] it has been estimated that 100% of the world's fish taxa will have collapsed by 2048 [23], indicating that strategies to reduce non-essential demands on fisheries be considered. Therefore, determining if DHA can be supplied by synthesis from ALA will be important to reduce the pressure on declining fish stocks. To accomplish this, it is essential that the extent to which ALA can be converted into DHA in humans is evaluated and compared to the requirement for DHA.

This review critically examines the methodologies used to estimate DHA synthesis from ALA in humans and presents evidence suggesting that DHA synthesis capacity in humans may be greater than previously estimated. Studies measuring DHA synthesis in adult humans will also be reviewed in the context of the brain. Additionally, a novel technique to measure DHA synthesis, that can be used in humans, the steady-state infusion method, is presented and evaluated as a means to determine, for the first time, a quantitative DHA synthesis-secretion rate in adult humans. In 2009, Barcelo-Coblijn and Murphy elegantly argued that ALA is a significant contributor to tissue DHA [25]. Herein, we provide an update of the literature with a focus on brain DHA homeostasis.

2. Current intakes of n-3 PUFA and relation to brain function

In the North America, mean dietary intakes of DHA in adult (20–39 years of age) men and women are about 70 and 60 mg/d respectively, while intakes of ALA in adult men and women are about 1700 and 1300 mg/d, respectively [26]. Preformed DHA is found primarily in marine sources, while ALA is found in seeds and seed oils including flax, canola, and soy [26]. The Institute of Medicine (IOM) recommends an adequate intake for ALA of 0.6–1.2% of total calories [27]. There is only one documented case of specific n-3 PUFA deficiency observed in a patient undergoing total parenteral nutrition, with 0.6% of fatty acids as ALA in a dietary emulsion (equivalent to 0.12–0.21% of calories based on IOM acceptable macronutrient distribution ranges), that developed neuropathy and blurred vision that was reversed upon increasing ALA in the emulsion 10-fold [28]. There is no specific recommendation for DHA; however, the IOM does state that 0–10% of the requirement for ALA can be made up from EPA and DHA [27], corresponding to approximately 0–0.12% of calories, or 0–160 mg/d based on a 2000 kcal diet. Recommendations for daily intake of EPA and DHA for primary prevention of coronary heart disease range from 200 to 3000 mg/d (reviewed in [29]), but we are not aware of any specific recommendations regarding DHA intakes pertaining to the adult brain.

DHA is highly concentrated in the brain and retina, and reductions in brain and retina DHA in rodents and non-human primates are associated with cognitive impairments such as severe learning deficits and anxiety, as well as visual impairments such as lower electroretinogram amplitude and longer electroretinogram recovery time (reviewed in [30]). Supplemental DHA is associated with improved visual acuity in pre-term infants [31], and infant formula containing DHA and arachidonic acid (ARA, the main n-6 PUFA in the brain) improves cognitive development up to one year post-partum [32]. However, the effect of these PUFA treatments later in childhood is not clear [33,34]. Lower post-mortem brain DHA is present in major depressive disorder relative to controls [35–37], however, supplemental EPA, but not DHA, appears to be effective in the management of depressive symptoms [38,39].

Table 1
Summary of published studies that have used stable-isotope labeled ALA to measure DHA synthesis.

Reference	Subjects (No. and sex)	Dose (mg)	Blood fraction	Time (days)	Conversion to DHA	Method
Emken et al. 1994 [134]	7M	2.8 g	TL	2	3.79%	AUC
Pawlosky et al. 2001 [136]	4M, 4F	1 g	TL	7	0.05%	Modeling
Burdge et al. 2002 [132]	6M	700 mg	TL	21	ND	AUC
Burdge et al. 2002 [133]	6F	700 mg	TL	21	9.2%	AUC
Burdge et al. 2003 [131]	14M	700 mg	TL	2	0.04%	AUC
Pawlosky et al. 2003 [135]	5M, 5F	1 g	TL	7	0.05–0.08%	Modeling
McCloy et al. 2004 [138]	6F	47 mg	TL	7	0.34% dose/l plasma	AUC
Hussein et al. 2005 [140]	12M	400 mg	TL	14	<0.01%	Modeling
Goyens et al. 2005 [139]	14M, 15F	190 mg	PL	9	0.08%	Modeling
Gillingham et al. 2013 [137]	14M, 25F	45 mg	TL	1	0.17–0.22% of dose recovered	Single blood sample

Although somewhat controversial [40], brain DHA may also be lower in Alzheimer's disease [41–44] as compared to normal aging in which brain DHA is relatively stable [45] and prospective studies demonstrate a protective effect of fish intake on Alzheimer's disease incidence (reviewed in [29] and [46]). However, it should be mentioned that clinical trials investigating the use of fish oil supplements to prevent/reverse cognitive decline associated with Alzheimer's disease have produced mostly neutral findings in their pre-registered endpoints [47–50]. The effect of DHA on brain function has previously been reviewed in detail [46,51–55].

3. Current model for brain DHA uptake

PUFA such as DHA are present in the circulatory system in either the unesterified form, bound to albumin, or in the esterified form as cholesteryl esters, phospholipids and triacylglycerides. To enter the brain, DHA must cross the blood brain barrier (BBB), a process that can be mediated either by receptor-facilitated transport or passive diffusion. The endothelium of brain capillaries contains lipoprotein receptors [56], however, lipoprotein receptor knock-out mice do not have lower brain DHA levels [57,58]. It has also been suggested that the major plasma pool supplying the brain is the unesterified DHA pool [59]. Additionally, in rodents, unesterified DHA crosses the BBB rapidly in a non-competitive manner suggesting that the mechanism by which DHA crosses the BBB is via passive diffusion [60,61]. Based on the model that plasma unesterified DHA is the major DHA pool that enters the brain, brain DHA uptake rates in the rat can be measured by infusing radiolabeled unesterified-DHA and measuring how much gets incorporated into the brain, after correcting for plasma radioactivity (i.e. brain exposure to radioactivity) and the pool size [62]. More recently, this concept was applied to humans using positron emission tomography to image the incorporation of [1-^{11}C]-DHA into the brain and quantify a rate of DHA uptake into the brain [63]. The rate of DHA uptake into the brain is assumed to be replacing DHA that is consumed in the brain, and therefore, can be used as an estimate for the brain DHA requirement. It has been reported that the brain DHA uptake rate in humans is between 2.4 and 3.8 mg/day [63,64]. Based on current estimates of ALA consumption in adult males of 1700 mg/day, the percent conversion of ALA to DHA would need to be 0.14–0.22% to match the brain DHA requirement [65]. Therefore, it is possible that even a small amount of DHA synthesis may be sufficient to meet adult brain DHA uptake demands. We have found that in rats DHA synthesis rates are at least 3-fold higher than brain DHA uptake rates indicating that rats may synthesize enough DHA to support the brain [66]. However, the conclusion that ALA is sufficient to support the brain will depend on what proportion of synthesized DHA is available to the brain (i.e. the brain-body partition coefficient for DHA). It is also important to recognize that if another plasma DHA pool contributes to brain DHA, current estimates of brain DHA uptake will be underestimates. It is possible that the plasma lysophosphatidylcholine (LPC) pool is a major contributor to brain DHA, especially in the rodent pup where i.v. injections of radiolabeled-LPC-DHA resulted in 12-fold higher brain radioactivity compared to pups injected with radiolabeled-unesterified-DHA [67,68]. Additionally, the orphan receptor Mfsd2a has recently been shown to transport LPC-DHA *in vitro*, and ablation of Mfsd2a resulted in decreased uptake of LPC-DHA and lowered brain DHA composition compared to wild-type controls [69]. Serum LPC-DHA levels, as measured by liquid-chromatography tandem mass spectrometry, range from 1.5 to 30 μM [70–73], while measures of serum unesterified-DHA range from 1 to 4 μM to [74–76] as measured by TLC-GC-FID. However, measurement of LPC- and unesterified DHA in single studies in rodents shows that unesterified DHA ranges from 10-fold higher than LPC-DHA to approximately equal [73,77]. The apparent discrepancy between the contribution of non-esterified and LPC DHA to brain phospholipid DHA may be explained by different half-lives and different experimental procedures between laboratories. A more comprehensive comparison of non-esterified DHA and LPC-DHA concentrations using the same technique within studies is required. Regardless, current estimates of brain DHA uptake may be underestimates if LPC-DHA proves to be a major contributor to adult brain DHA uptake.

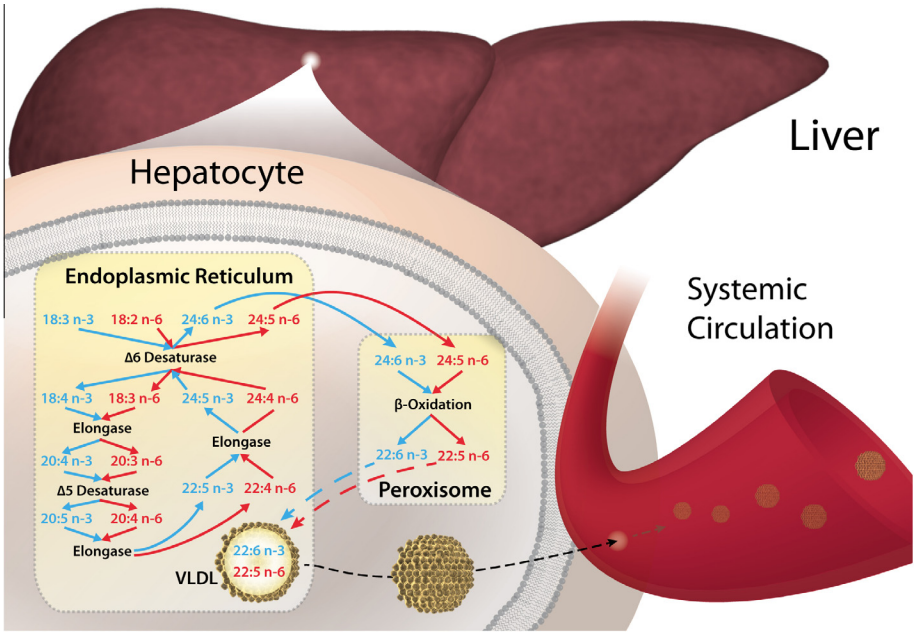

Fig. 1. DHA is synthesized from ALA in the liver by a series of desaturations, elongations and a β-oxidation. Enzymes involved in the synthesis of DHA from ALA are also used by n-6 PUFA and n-9 fatty acids (not shown) leading to competition between n-3 PUFA, n-6 PUFA, and n-9 fatty acids for these enzymes. This competition is most apparent for the Δ6 desaturase, where 4 PUFA (2 n-3 PUFA and 2 n-6 PUFA) compete for a single enzyme. The desaturations and elongations occur in the endoplasmic reticulum and the β-oxidation occurs in the peroxisome, to where 24-carbon PUFA are transferred. The final products (DHA and 22:5n-6) are then transferred back to the endoplasmic reticulum where they along with other PUFA can be esterified to lipoproteins (eg. VLDL) and secreted into the blood.

4. Pathway of DHA synthesis

Fig. 1 depicts the synthesis pathway of DHA from ALA. The desaturase and elongase enzymes that are used to synthesize longer chain PUFA (for example DHA) are most highly expressed in the liver as compared to heart or brain [78–80], corresponding to more than 30-fold higher rates of DHA synthesis in this organ [81]. ALA is desaturated by the rate-limiting Δ6-desaturase enzyme in the endoplasmic reticulum (ER) to form 18:4n-3 [82,83], followed by elongation to 20:4n-3 and desaturation by Δ5-desaturase to form 20:5n-3 (EPA). EPA can be elongated further to 22:5n-3 (docosapentaenoic acid – DPAn-3), and 24:5n-3. 24:5n-3 is desaturated by Δ6-desaturase forming 24:6n-3, which is transferred from the ER to the peroxisome where it is β-oxidized to form 22:6n-3 (DHA) [11,82,84,85]. DHA is then transferred back to the ER where it can undergo esterification, lipoprotein packaging and secretion to the blood.

The pathway is active towards both n-3 and n-6 PUFA as well as n-9 fatty acids, resulting in potential competition for enzyme activity between the families of fatty acids. This is particularly important for the rate-limiting enzyme, Δ6-desaturase, which is active towards both ALA and linoleic acid (LNA), as well as 24-carbon n-3 and n-6 PUFA [86,87]. Dietary PUFA down regulate the expression and activity of the enzymes involved in DHA synthesis in the liver [78,79,81,88]; thus, decreasing the hepatic DHA synthesis rate [89]. The brain is capable of synthesizing DHA [81,90], however, brain DHA synthesis is approximately 100-fold lower than brain DHA uptake and consumption rates, indicating that brain DHA synthesis does not contribute significantly to brain DHA homeostasis [91]. Interestingly, dietary n-3 PUFA deprivation does not affect the expression of the desaturases or elongases or the DHA synthesis rate in the brain, in contrast to increased synthesis found in the liver [81]. DHA synthesis-secretion in the liver is at least 3–10-fold greater than brain DHA consumption rates [66,92], which, combined with the finding of up-regulated hepatic DHA synthesis during n-3 deprivation, suggests that hepatic DHA synthesis is capable of maintaining brain DHA homeostasis.

Recently, alternative mechanisms for DHA synthesis have been proposed [84,93–95]. An experiment performed in baboons determined that the Δ6-desaturase enzyme also has Δ8-desaturase activity [93]. Based on this finding the authors proposed an alternative pathway for DHA synthesis from ALA that functions in parallel with the classical pathway and involves an initial elongation of ALA to 20:3n-3 followed by Δ8-desaturation to make 20:4n-3, which is then desaturated and elongated to become DHA [93]. Another recent study questioned the Δ6-desaturation as the sole rate-limiting step in the synthesis pathway. The authors found that the elongation of DPA n-3 to 24:5n-3 may be another crucial control point in DHA synthesis [94]. This reaction is catalyzed by the enzyme elovl2, and lack of expression of this enzyme in heart is believed to be the reason why heart tissue has very low DHA synthesis rates [96]. These novel insights into DHA synthesis merit further investigation to determine how much they contribute to DHA synthesis *in vivo*.

5. Estimates of DHA synthesis from ALA in humans

5.1. Evidence from ALA feeding

The simplest means of estimating DHA synthesis in humans is measuring changes in DHA status in response to acute or chronic increases in dietary ALA consumption, and these studies have been previously reviewed in detail [10,11,97]. In general, these studies increase subjects' ALA consumption and measure DHA in the blood. While most studies report that plasma and erythrocyte EPA increase with ALA feeding, most do not detect an increase in plasma or erythrocyte DHA [98–117]. Reviews of these studies have pointed out two important points pertaining to the lack of plasma DHA increases after ALA feeding. Firstly, in humans with low DHA diets (vegans and vegetarians), ALA feeding increases plasma DHA [97]. Additionally, plasma DHA tends to increase to a greater extent when ALA consumption is increased in combination with decreased LNA consumption [10,11].

It should be recognized that these studies only measure DHA in blood lipids (plasma, erythrocytes, or leukocytes) as opposed to tissues. While plasma DHA may be a reliable marker for dietary DHA intake, the applicability of this pool to the brain is not agreed upon. This is because most of these studies measure percent composition of DHA in the esterified blood lipid pools, which are not thought to be available to the brain [62]. A recent rodent study performed in our laboratory highlights this point [66]. We fed rats a diet that was either low in n-3 PUFA (0.25% fatty acids as ALA) or contained either ALA or DHA. After 15 weeks on these diets, levels of DHA in the body and plasma were significantly higher in rats fed DHA compared to rats fed the ALA and control diet (2.4 and 11-fold higher, respectively, for the body and 2 and 5-fold higher, respectively, for plasma). However, brain DHA levels were not different between ALA- and DHA-fed rats, similar to previous studies in rats [19] and non-human primates [20], suggesting that changes in blood DHA concentration do not necessarily reflect the magnitude of changes in brain DHA, with some exceptions [118,119]. Interestingly, graded ALA deprivation from 4.6% (considered "adequate" to maintain brain function and DHA concentrations) to 0.2% (considered "inadequate" based on decreased DHA concentration and metabolism) of fatty acids in a diet lacking DHA results in decreased brain DHA only when the ALA content of the diet is decreased to 0.8% or lower [120]. This indicates that extreme cases of ALA deprivation are required to affect brain DHA concentrations. Accordingly, the only recorded case of n-3 PUFA deficiency in humans resulted from total parenteral feeding of an emulsion containing only 0.6% of fatty acids as ALA [28]. This supports the hypothesis that extremely low ALA intakes are required to significantly affect brain DHA levels and function, assuming however, that the neurological impairments observed with ALA deficiency are caused by decreases in brain DHA.

It is possible that though plasma esterified DHA is unchanged with chronic increases in ALA feeding, dietary ALA may be sufficient to maintain brain DHA concentrations, possibly via the plasma unesterified fatty acid pool. The plasma unesterified fatty acid pool is 10–100-fold smaller than the esterified pools [89,121,122] and is maintained largely via the adipose (fasting state) and hydrolysis from plasma lipoproteins (post-prandial) [123]. Also, the DHA concentration of the plasma unesterified fatty acid pool decreases only when extreme n-3 PUFA deprivation occurs [120]. Moreover, few studies have examined the effect of increasing dietary DHA intake on unesterified DHA concentrations in humans, with some studies reporting an increase and others reporting no increase [76,121,124–127]. Adipose, the tissue that maintains plasma unesterified fatty acid concentrations, has been estimated to contain 1–4 and 20–50 g of DHA in the infant [128,129] and adult [130], respectively. Using the previously measured brain DHA uptake rate of 3.8 mg/day in adult humans, it can be calculated that adult human adipose contains enough DHA to supply the brain for 14–36 years. It is important to note that the estimate for how long adipose DHA can supply the brain is an overestimate because DHA released from the adipose is used by other tissues as well as the brain. Therefore, to determine the actual amount of time that adipose DHA can supply the brain, the proportion of DHA that is released from the adipose and taken up into the brain (brain-body partition coefficient) must be determined.

5.2. Evidence from stable isotope administration

DHA synthesis from ALA in humans has been examined by administering an oral dose of stable isotope-labeled ALA and measuring the appearance of labeled DHA in blood lipids over time. Through repeated blood sampling, concentration–time plots of the appearance of labeled n-3 PUFA are obtained, and the area under the curve (AUC) for DHA is compared to either the AUC for all labeled PUFA [131–136] or expressed relative to the administered dose to calculate the fractional conversion of ALA to DHA [137,138]. The fractional conversion of DHA from ALA is, therefore, a measure of the percentage of labeled n-3 PUFA that appears in the plasma as DHA or the percentage of a single oral dose of ALA administered at one time that appears in the plasma as DHA.

Estimates of fractional conversion of an oral dose of ALA to DHA using this technique have ranged from below the detection limit in one study to 9.8%, however, the majority of studies using this technique report fractional DHA conversion of <1% (Table 1). Alternatively, the relative conversion of each intermediate within the pathway can be estimated by using compartmental modeling. This approach is based on the assumption that the relative concentration of pathway intermediates in plasma is representative of the relative concentrations in liver, the primary site of DHA synthesis. The amount of orally administered ALA utilized for DHA synthesis using this technique has been estimated to be between 0.01 and 0.08% [135,136,139,140]. Taken together, these measures have led to a general consensus that DHA synthesis in humans is insufficient to meet DHA demands; however, care must be taken in interpreting these estimates of DHA synthesis in humans, especially in reference to the brain.

In general, there are considerations regarding the oral administration of an ALA tracer to estimate DHA synthesis, as this type of experiment represents DHA synthesis from postprandial ALA only, rather than total DHA synthesis from ALA. For example, the extent to which orally administered ALA is available for DHA synthesis is not known. Fatty acids absorbed in the intestine are packaged into lipoproteins, and the majority are transported through lymphatic circulation and secreted into the blood through the thoracic duct (Fig. 2). Fatty acids are taken up by tissues following hydrolysis of lipids by endothelial lipase and lipoprotein lipase or by endocytosis of the lipoprotein. Approximately 72% and 64% of orally administered ^{13}C-ALA is β-oxidized 168 h after dosing in humans [138] and after 24 h in rats [141], respectively. This value for β-oxidation of ALA in humans is similar to that of oleic and elaidic acids, but slightly higher than LA, at least between 9 and 24 h post dose [138]. Studies in rats demonstrate that the adipose AUC makes up 75% of the whole-body AUC for orally gavaged ^{2}H-ALA after 600 h with progressive enrichment of adipose tissue with ALA [142]. Balance studies performed in rodents have also found that the majority of dietary ALA that is not β-oxidized accumulates in the adipose tissue [143]. Moreover, in adult females after one week it has been estimated that upon an oral dose of labeled ALA, up to 57% of the tracer is in the adipose [138]. The fate of ALA that is deposited into adipose tissue is not clear, however, the adipose fatty acid half-life is approximately 1 year [144,145] indicating that long-term storage would make a large proportion of oral ALA tracer unavailable for DHA synthesis measures. Taken together, this indicates that the major fate of orally administered ALA tracer, that is not β-oxidized, is adipose sequestration with a long half-life. In fact, enrichment of plasma with gavaged ALA peaks at only 5% of the whole-body tracer content and progressively declines over time [142]. Moreover, in rats, less than 5% of ^{2}H-EPA, DPAn-3 and DHA derived from gavaged ^{2}H-ALA is found in plasma with the majority found in nervous system, liver, and adipose with a progressive enrichment in nervous tissue [142]. Thus, the amount of tracer that is found in plasma represents a very small proportion of the total tracer that is provided orally, and is likely an underestimate of the total whole-body DHA synthesized and accreted [142]. This suggests that DHA synthesis measures from ingested ALA tracer likely represent only DHA synthesized from postprandial ALA, but do not necessarily reflect the total pool of ALA that is available for DHA synthesis. As fractional conversion of DHA from ingested ALA represents only the proportion of the dose that is found in the blood compartment, which is a very small portion of the DHA synthesized from ALA, these estimates of fractional conversion are likely underestimates of actual DHA synthesis in humans [142,146]. Estimates of DHA synthesis from ALA using this method range from <0.01 to 1% of oral dose of ALA [137,138,140].

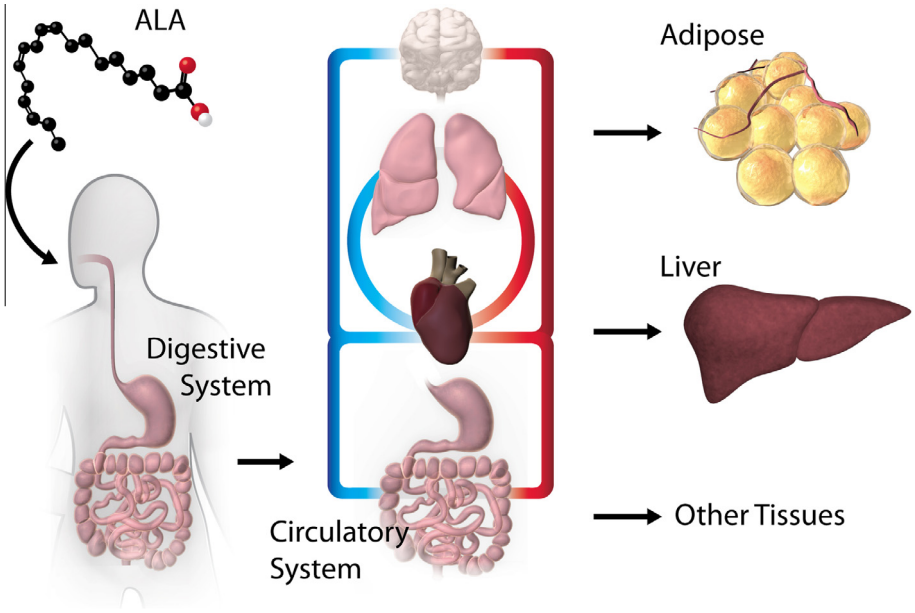

Fig. 2. When ALA is administered orally it is absorbed into the lymphatic system and then deposited into systemic circulation. This is problematic for human tracer studies that administer ALA orally and measure the appearance of labeled n-3 PUFA products in the plasma, as a large portion of the tracer will get taken up into the tissues and adipose and not reach the liver for the duration of the study.

Fractional DHA synthesis has also been estimated by comparing the plasma AUC for labeled n-3 PUFA to estimate the percentage of plasma ALA that is converted into DHA. In these studies fractional conversion is measured by determining what percentage of the total labeled n-3 PUFA that appeared in the plasma was labeled DHA. By adjusting for the appearance of labeled fatty acids, this method is less likely to underestimate fractional DHA synthesis rates by accounting for loss of label associated with adipose sequestration. The fractional conversion of ^{13}C- or ^{2}H-ALA to DHA in young men using this technique has been measured as 3.8% after 48 h [134] and below the detection limit after 504 h in one study [132], and 9.2% after 504 h in young women [133]. However, the extent to which the fractional conversion quantifies actual DHA synthesis is not clear, as it is only a relative measure [147]. In addition, the AUC comparisons do not take into account differences in the plasma half-lives of the different n-3 PUFA. It has been estimated that the half-life for plasma esterified ALA is 1 h, while that of DHA is 20 h [136]. This difference in plasma half-life would result in equal amounts of DHA and ALA eliciting a much greater AUC for DHA than that of ALA. Therefore, these methods are also susceptible to factors that affect plasma half-life of DHA, such as diet [66,135].

Compartmentalized modeling procedures are another method to determine DHA synthesis from orally administered ALA and also provide measures of rate of flow of labeled fatty acids between compartments, half-lives, loss rates, as well as conversion rates from one fatty acid to another. Compartmentalized modeling describes the flow of materials, in this case n-3 PUFA, from one compartment to another. For modeling n-3 PUFA metabolism, stable isotope-labeled ALA is provided orally and the appearance of ALA and its longer-chain derivatives, including DHA, is measured over time. Each fatty acid between ALA and DHA is considered a "compartment" in the model, and when the data is corrected for unlabeled n-3 PUFA concentrations the transfer of label from one compartment to another describes the rate constants for conversions between fatty acids within the DHA synthesis pathway. A major advantage of this type of modeling is that it can potentially yield conversion rates in μg/h rather than relative data such as percent conversion. However, numerous assumptions are required for this type of modeling that likely affect conclusions rendered from the data. For example, the kinetics that are modeled in this analysis are based on oral consumption of an ALA tracer, and as such may not represent the kinetics of all sources of ALA that compose steady-state serum ALA concentration, such as ALA secreted from adipose or liver stores. Also, the majority of the tracer is lost to uptake by adipose or other tissues and/or β-oxidation based on very low appearance of the ALA tracer in plasma after ingestion [136]. This type of modeling is an approximation of hepatic conversion of ALA into longer-chain n-3 PUFA based on appearance of label in plasma [136], however, important differences in plasma and hepatic n-3 PUFA composition (eg. ratio of DHA to ALA is 2-fold higher in liver than in plasma total lipids [148,149]) suggest this approximation is limited. The rate constants that are calculated, therefore, represent the cumulative process involved in conversion of one plasma tracer to another, including uptake by the liver, conversion, and secretion back into the plasma [136]. This will also lead to an underestimation of DHA synthesis as it has been reported that after the consumption of a labeled ALA tracer, approximately 15% of DHA is synthesized fully in the liver before appearing in the plasma based on comparison of compartmental DHA metabolism [139]. Interestingly, in one study the compartmental model predicted that the amount of dietary DHA required to maintain serum DHA concentration was 2.2-fold higher than what was directly measured by food duplicate, and the authors concluded that maintenance of DHA status requires greater DHA output from body store utilization or ALA synthesis than was measured in this study [136]. Estimates of fractional DHA synthesis from ALA using this method range from 0.01 to 0.08% [135,136,139,140].

5.2.1. Considerations for oral stable isotope studies

A factor that contributes to the significant variation in estimates of DHA synthesis in humans, and therefore, adds significant uncertainty to conclusions regarding DHA synthesis, is heterogeneity between studies in background fatty acid intake. Dietary fatty acid composition has significant effects on the DHA synthesis rate [81,89,134,135]. Specifically, DHA is known to down regulate enzymes involved in its own synthesis [79,81,150,151]. In addition, n-6 PUFA may compete with n-3 PUFA for the enzymes involved in DHA synthesis [134,152–154]. For example, higher fractional conversion of ALA into DHA has been shown in response to increased ALA:LNA ratio in the diet using compartmentalized modeling in humans [114].

Although methods utilizing oral administration of stable-isotope-labeled ALA to estimate DHA synthesis in humans may not directly measure a DHA synthesis rate, these measures do have utility in comparing DHA synthesis between individuals or groups in the same study [137,147,155]. In general, conclusions can be drawn about the relative differences in DHA synthesis between groups, such as the finding that women utilize a greater proportion of n-3 DPA for DHA synthesis as compared with men [156]. However, based on factors discussed previously, absolute DHA synthesis rates cannot be quantified with this method.

Ingested fatty acid tracers also appear to poorly model the pharmacokinetics of *in situ* PUFA metabolism, in addition to having only a fraction of the tracer appear in the blood. For example, compartmental analysis revealed that stable isotope-labeled EPA is 40% less effectively utilized for DHA synthesis when ingested as compared with EPA that has been synthesized from ALA [157]. This may also be true for ALA, in that ingested labeled ALA may poorly represent unlabeled ALA derived from body stores, although this has not been examined.

The use of stable isotope tracers to measure DHA synthesis has another general consideration, as one must by definition change the substrate concentration in the form of an administered tracer. This may increase flux through a pathway, result in substrate inhibition, or result in additional effects that might otherwise not occur under normal circumstances. Therefore, one must use the smallest amount of tracer that allow for reliable quantitation of the measure of interest so as not to influence the physiological process being measured. There is also some concern regarding deuterium exchange while using deuterium-labeled stable isotopes, in which deuterium atoms are exchanged with unlabeled hydrogen atoms. Though this exchange rate has not been quantified in DHA synthesis studies, hydrogen exchange between water and fatty acids has been found to be negligible under typical experimental conditions [158], suggesting that deuterium exchange is a quantitatively minor process. Also, deuterium exchange would most likely affect tracer/tracee ratio of both products and substrates in DHA synthesis (assuming all fatty acids have equal deuterium exchange rates). Therefore, studies calculating DHA synthesis as "percent of oral dose" are susceptible to underestimation if using ^{2}H-ALA, while studies calculating percent conversion based on comparisons between AUCs of ^{2}H-ALA and ^{2}H-DHA would likely be unaffected.

6. Evidence that DHA synthesis affects blood DHA levels

In addition, there is evidence that significant changes in DHA status can occur independent of changes in n-3 PUFA intake, likely through increased synthesis of DHA from ALA. For example,

women have higher DHA in plasma phospholipids and erythrocytes compared with men [159], which is associated with much higher rates of DHA synthesis in women [132,133,156]. The higher DHA synthesis in women corresponds to higher hepatic expression of the Δ5- and Δ6-desaturase enzymes in female compared with male rodents [148,160]. Female rats also have higher expression of fatty acid binding protein in hepatocytes [161], suggesting that binding and trafficking of ALA towards DHA synthesis may be higher in females as compared with males, and it is also possible that the half-life of DHA in the plasma is longer in women. Another example of DHA status being affected independent of changes in n-3 PUFA intake is altered fatty acid profiles associated with single nucleotide polymorphisms (SNP) in the human Fatty Acid Desaturase 2 gene (FADS2), the gene that encodes the Δ6-desaturase enzyme. The majority of these polymorphisms affect EPA concentrations, but not DHA concentrations, in phospholipids of plasma [162], serum [163], and erythrocytes [164]; while analysis of a particular haplotype (with 28 SNP) has shown increased levels of DHA in plasma total lipids in the Northern Swedish Population Health Study [165]. Also, a Δ6-desaturase SNP associated with increased Δ6-desaturase product:precursor ratios is associated with increased DHA percent composition in maternal erythrocytes during pregnancy [166] and colostrum [167] and a SNP with lower Δ6-desaturase activity is associated with lower levels of DHA in erythrocytes in pregnancy and breast milk [168]. A recent study using orally administered ALA tracer found that some minor allele variants were associated with lower labeled EPA enrichment in the plasma as well as lower concentrations of ARA and EPA [137]. These studies provide some evidence that DHA levels can be altered with no change in n-3 PUFA intake, with evidence that these changes are due, at least in part, from differences in DHA synthesis.

7. Estimates of DHA synthesis rates in rodents

Examination of DHA synthesis in rodent models allows for more invasive analytical methods which can assist in validation of less invasive methods that can be applied to human subjects. Estimates of DHA synthesis from ALA based on isotope administration can be compared with whole-body DHA synthesis-accretion rates in ALA-fed animals to validate the isotope method. For example, rates of DHA synthesis in rats achieved using the balance method (described below) and steady-state ^{2}H-ALA infusion (which can be applied to humans and is also described below) provide estimates of 4.4 μmol/d and 1.5 μmol/d, respectively. In this way, the balance method validates the steady-state infusion method and suggests that the infusion method can provide an accurate measure of DHA synthesis in humans.

There is concern that the rat is a more rapid converter of ALA to DHA as compared with humans [169], resulting in concern regarding the generalizability of DHA synthesis measures in rats to humans. This notion stems largely from the comparison of microsomal desaturase enzyme activities measured in rats and humans [169,170]. However, no study has directly compared human and rat desaturase activities or DHA synthesis rates. Moreover, the methods used to estimate DHA synthesis rates from ALA in the rat differ from those in the human, and the method used to measure DHA synthesis rates in the human have not been validated in the rat. To examine this, our laboratory orally administered $^{2}H_5$-ALA to rats and sampled blood over a 6-h experiment to measure $^{2}H_5$-DHA and apply calculations used previously in studies providing a single oral bolus of labeled ALA in humans [66]. Depending on the calculation used, the percentage of ALA dose converted to DHA ranged from 0.12% to 0.64%, which are not higher than previous estimates of DHA synthesis in humans using the same calculations (Table 2) [134,137,138], suggesting that DHA synthesis estimated by oral dose methodology is similar between rats and humans and that the rat may be a suitable model for validation of human DHA synthesis methods.

7.1. Measurements of DHA synthesis from balance studies

The balance method, developed by Cunnane et al. for use in examination of essential fatty acid accretion and metabolism, requires feeding rats a diet with ALA as the only n-3 PUFA then measuring the accretion of DHA in the rat whole body [128,143,171]. Previously published balance studies have reported that the DHA synthesis rate in rats to be between 4.4 and 15 μmol/day [66,143,172]. The balance method does provide an estimate of the net DHA synthesis and accretion; however, it cannot account for DHA that has been synthesized and then metabolically consumed. Even so, this potential limitation to the balance study would, at most, result in an underestimate in the actual DHA synthesis rate.

Interestingly, balance studies also provide more evidence that the major fate of orally ingested ALA and DHA is apparent β-oxidation. By feeding rats only either ALA or DHA, it has been shown that β-oxidation of these fatty acids is between approximately 60% after 8 weeks of feeding [172] and 90% after 15 weeks [66], with no differences between ALA and DHA loss rates.

7.2. Measurements of DHA synthesis from steady-state ALA infusion studies

The steady-state infusion model, developed by Rapoport et al. [173] and recently applied with modifications by our laboratory [66], involves infusing isotope-labeled unesterified ALA, such that plasma concentration of the tracer achieves steady-state, and measuring the appearance of labeled DHA in the plasma [173]. The major strength of this method is that it provides a whole-body DHA synthesis rate (μmol or mg synthesized per unit time) as opposed to many methods using ingestion of an oral bolus which provide relative estimates of DHA synthesis from ingested ALA only (the exception being some applications of compartmental modeling [136], but these are still based on ingested ALA tracers). Another advantage of the steady-state infusion method is that it measures DHA derived from serum ALA, rather than only postprandial ALA, and as such likely represents the entirety of ALA that is available to the liver for DHA synthesis. By more closely representing the substrate pool available for DHA synthesis (serum ALA from

Table 2
Summary of fractional conversion estimates in rats applying calculations used in human studies (adapted from Domenichiello et al. [66]).

Method	Description	Human Conversion to DHA	Rat Conversion to DHA
McCloy et al. [138]	% oral dose of ALA appearing as AUC DHA*	0.99%	0.31%
Emken et al. [134]	% of total n-3 AUC as DHA AUC	3.8%	0.64%
Gillingham et al. [137]	% recovery of oral ALA tracer in blood sample at conclusion of study	0.10–0.25%	0.12%

* corrected for plasma volume.

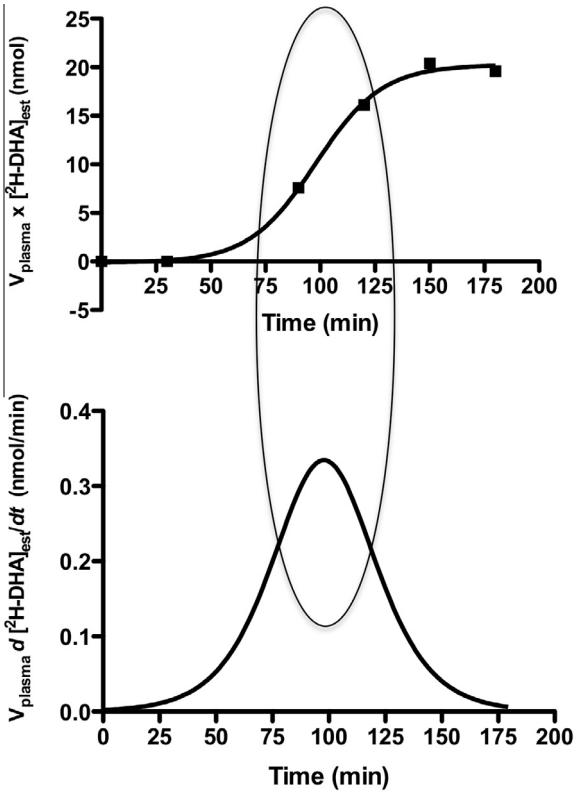

Fig. 3. Quantitation of DHA synthesis rate using the steady-state infusion method. Upon infusion of labeled ALA at steady-state, appearance of labeled DHA in the plasma is sigmoidal (top panel). The first derivative of this curve can be determined and is equal to the rate of appearance of labeled DHA in the plasma at every time point during the infusion (bottom panel). The maximal first derivative (circled in the top and bottom panel) is taken to be the maximal rate of DHA appearance in the plasma (S_{max}). S_{max} is assumed to be the point where tissue uptake of labeled DHA is negligible meaning that the appearance of labeled DHA at this point is due solely to DHA synthesis from ALA. By correcting S_{max} for the tracee to tracer ratio the DHA synthesis rate can be determined.

all sources, not just post-prandial, gut-derived ALA), the steady-state infusion method may be a more representative estimate of DHA synthesis relative to oral dosing methodology.

The steady-state infusion model involves infusing unesterified albumin-bound labeled ALA intravenously at a constant rate to achieve a steady state concentration in the blood, and through repeated blood sampling the appearance of labeled DHA in esterified lipids is measured. The appearance of labeled DHA using this method can be fit to a sigmoidal curve (Fig. 3). Generally, the first derivative at any point of a curve represents the rate-of-change of the measured variable at that point, and as such the derivative of the sigmoidal DHA appearance curve represents the rate-of-change of labeled DHA (i.e. the rate of change of the concentration of labeled DHA in plasma) at that point. By determining the maximal first derivative of the sigmoidal labeled-DHA curve, the maximum rate-of-change of labeled DHA is obtained. This maximal rate-of-change is taken as the labeled DHA synthesis rate from labeled ALA, and by correcting this rate by the unlabeled ALA concentration the whole-body DHA synthesis rate is obtained (see description of calculations, Fig. 3).

There are several important limitations of this method, including that the serum concentration of labeled DHA represents the equilibrium between synthesis-secretion and tissue uptake. While the contribution of tissue uptake to labeled DHA concentration is lowest relative to synthesis-secretion at the maximal first derivative, it is necessarily defined as zero in the calculations. The half-life of serum DHA is approximately 20 h [136] suggesting that tissue uptake DHA would not be a major contributor to changes in labeled DHA over the time-course of most experiments (infusions are typically 3 h, with the maximal derivative obtained within 2 h). Despite this, the assumption of zero tissue uptake at the maximal first derivative means that the steady-state infusion yields only a lower bound estimate on whole-body DHA synthesis measures. There is also potential for dilution of the ALA tracer in the liver acyl-CoA pool (the pool that is primarily utilized for DHA synthesis), such that measured tracer-tracee ratios in the plasma may not represent the ratio in the liver, the primary site of DHA synthesis–secretion. This dilution has been estimated in the rat where after a 5 min infusion of radiolabeled ALA the tracer:-tracee ratio was 60–80% lower in liver acyl-CoA compared with plasma unesterified ALA [81,89]. While the dilution factor is unlikely to be measurable in the human, it should be noted that this limitation will also result in an underestimation of the actual DHA synthesis rate (for more details refer to [66]). Additionally, it should also be pointed out that due to the small pool size of unesterified ALA in the plasma, minimal amounts of labeled unesterified ALA should be infused during these studies to avoid altering the pathway that is being studied (as previously discussed). Finally, synthesis is measured only over a short period of time (i.e. 2–3 h) in the rat and may miss any diurnal variation that occurs in the synthesis rate.

The achievement of a steady-state of ALA tracer concentration is essential for calculating a DHA synthesis rate as the rate of DHA synthesis is not influenced by changes in plasma ALA tracer. This is in contrast to the single oral dosing method used in humans, in which labeled ALA concentrations in the plasma do not reach steady state, therefore, limiting the possibility of estimating DHA synthesis rates as discussed previously [155]. The steady-state infusion method bypasses ingestion, digestion, and absorption of the labeled ALA, and therefore, measures the whole-body DHA synthesis rate from circulating unesterified ALA, whereas estimates of DHA synthesis from orally provided ALA model only post-prandial ALA. The method provides a measure of whole-body DHA synthesis, and would account for synthesized DHA that occurs when other secretory tissues, such as adipose or gut, take up the labeled ALA and secrete labeled DHA back into circulation. The steady-state infusion method has been used to estimate DHA synthesis rates in rats, and found DHA synthesis rate to be approximately 1.5 μmol/day [66], which is lower, but in broad agreement with the rates determined by the balance method in rats fed the same diet (4.4 μmol/day) [66] and growing rats fed a similar diet (11–14 μmol/day [143,172]). Using the steady-state method, the DHA synthesis rate is at least 3-fold higher than the daily brain DHA uptake rate in rats [66], suggesting that DHA synthesis may be sufficient to provide the brain with DHA. Importantly, the steady-state infusion method can be performed in humans, and is currently being applied as part of a larger clinical study (ClinicalTrials.gov Identifier: NCT01251887).

8. Concluding remarks

There is considerable debate as to whether the human capacity to synthesize DHA from ALA is sufficient to meet brain DHA requirements. This debate has been further complicated by lack of agreement regarding the brain DHA requirement, and methodological inconsistencies in attempts to quantify the rate of DHA synthesis from ALA. The IOM did not assign a dietary reference intake for DHA, and other recommendations for DHA and EPA intake pertain to cardiovascular disease prevention [27,29,174,175] and not specifically to support the brain, in part reflecting uncertainty in the role of dietary DHA in maintaining

brain DHA. Fortunately, an estimate of human brain DHA uptake is now available (2.4–3.8 mg/day [63,64]), and novel approaches to measure whole-body DHA synthesis from serum ALA using steady-state isotope infusion will allow for quantitative comparison of DHA synthesis rates to brain DHA uptake rates, as done previously in rats [66]. This approach will supplement previous measurements of DHA synthesis from ingested stable isotope ALA, which provide estimates of DHA synthesis from postprandial ALA, and produce a more complete understanding of DHA homeostasis in humans.

Despite limitations in comparing rates of DHA synthesis and brain DHA uptake rates in humans to date, there is considerable evidence from animals showing that brain DHA levels are similar when fed ALA as the only n-3 PUFA as opposed to DHA or ALA+DHA, as reviewed extensively by [65], although there are some exceptions [119] possibly related to dose-, duration-, and species-specific effects. The brain has mechanisms whereby it can conserve DHA that may explain similar brain DHA between DHA- and ALA-fed rats [176]. For example, the expression of DHA-catabolizing enzymes, such as group VIB calcium-independent phospholipase A_2, can be reduced, resulting in decreased catabolism of DHA and a longer brain DHA half-life [120,176,177]. The effect of altering brain DHA turnover on brain function is not clear. Also, n-3 PUFA deficiency increases aspects of ARA turnover and decreases DHA turnover [120,176–180], suggesting that the brain may metabolize ARA to spare DHA. The effect of increased utilization of ARA relative to DHA by the brain, on brain function in health or disease is not currently known. However, vegans and vegetarians have similar prevalence of neurological diseases as compared with omnivores suggesting that any altered brain DHA metabolism in these individuals does not manifest neurologically [15–18,181–184].

Studies that have used ingested stable-isotope ALA to measure DHA synthesis in humans have for the most part reported that DHA synthesis from ALA is thought to be an inefficient process (generally <1% conversion). The calculations used in these studies are inconsistent [147], and we have shown that they yield different values for percent conversion depending on the calculation used [66]. In addition, these methods may only provide relative as opposed to absolute quantifications of DHA synthesis rates [97,147] and only represent the DHA synthesized from postprandial ALA. However, if the brain DHA uptake rate is an accurate measure of the brain DHA requirement than a low fractional conversion may still be sufficient to supply DHA to the brain.

It must be stressed that the focus of this review is the capacity of DHA synthesis from ALA to supply the brain in healthy adults. Situations that may affect DHA synthesis rates and/or brain DHA uptake rates (such as diet, development, genetics, brain injury, disease or aging) must be examined to determine if ALA-derived DHA can meet brain DHA requirements in these cases. For example, during infancy the brain accretes a large amount of DHA as it grows and post-mortem studies have found that breast-fed infants have significantly higher brain DHA concentrations than infants fed formula that contains ALA but not DHA [185]. Therefore, this may be an instance where DHA synthesis from ALA is not sufficient to supply the brain, and preformed DHA is required. However, methods are now available that can be applied to both rodents and humans to measure brain DHA uptake and DHA synthesis rates, allowing for estimation of sufficiency of DHA synthesis and recommendations for DHA intake.

In 2009, Rapoport and colleagues developed an *in vivo* steady-state, stable-isotope infusion method to measure the DHA synthesis rate from serum ALA in rats [173]. By infusing the tracer intravenously this method avoids some of the considerations of oral tracer administration. The steady-state infusion method allows for the direct quantification of the DHA synthesis rate, rather than a relative conversion measure. Importantly, the synthesis rates measured using this method [66] are in line with rates that were measured using balance studies [143,173]. Application of this method to humans would represent the first quantification of the DHA synthesis rate from blood ALA in humans, which could be compared to the brain DHA uptake rate. It is of importance to know how much DHA can be synthesized by humans, in order to properly set guidelines for ALA and DHA consumption

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

AFD holds an Ontario Graduate Scholarship, distributed by the Province of Ontario and the University of Toronto. APK holds an Ontario Women's Health Scholars Award, funded by the Ontario Ministry of Health and Long-Term Care. RPB acknowledges funding from the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada and RPB holds a Canada Research Chair in Brain Lipid Metabolism.

References

[1] Orr SK, Palumbo S, Bosetti F, Mount HT, Kang JX, Greenwood CE, et al. Unesterified docosahexaenoic acid is protective in neuroinflammation. J Neurochem 2013;127:378–93.
[2] Bazan NG, Molina MF, Gordon WC. Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. Annu Rev Nutr 2011;31:321–51.
[3] Desai A, Kevala K, Kim HY. Depletion of brain docosahexaenoic acid impairs recovery from traumatic brain injury. PLoS ONE 2014;9:e86472.
[4] Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, et al. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest 2005;115:2774–83.
[5] Akbar M, Calderon F, Wen Z, Kim HY. Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. Proc Natl Acad Sci USA 2005;102:10858–63.
[6] Patterson AC, Stark KD. Direct determinations of the fatty acid composition of daily dietary intakes incorporating nutraceuticals and functional food strategies to increase n-3 highly unsaturated fatty acids. J Am Coll Nutr 2008;27:538–46.
[7] Martinez M. Abnormal profiles of polyunsaturated fatty acids in the brain, liver, kidney and retina of patients with peroxisomal disorders. Brain Res 1992;583:171–82.
[8] Igarashi M, Ma K, Gao F, Kim HW, Greenstein D, Rapoport SI, et al. Brain lipid concentrations in bipolar disorder. J Psychiatr Res 2010;44:177–82.
[9] Chen CT, Domenichiello AF, Trepanier MO, Liu Z, Masoodi M, Bazinet RP. The low levels of eicosapentaenoic acid in rat brain phospholipids are maintained via multiple redundant mechanisms. J Lipid Res 2013;54:2410–22.
[10] Brenna JT, Salem Jr N, Sinclair AJ, Cunnane SC. Alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostaglandins Leukot Essent Fatty Acids 2009;80:85–91.
[11] Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev 2005;45:581–97.
[12] Welch AA, Shakya-Shrestha S, Lentjes MA, Wareham NJ, Khaw KT. Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the product-precursor ratio [corrected] of alpha-linolenic acid to long-chain n-3 polyunsaturated fatty acids: results from the EPIC-Norfolk cohort. Am J Clin Nutr 2010;92:1040–51.
[13] Rosell MS, Lloyd-Wright Z, Appleby PN, Sanders TA, Allen NE, Key TJ. Long-chain n-3 polyunsaturated fatty acids in plasma in British meat-eating, vegetarian, and vegan men. Am J Clin Nutr 2005;82:327–34.
[14] Mann N, Pirotta Y, O'Connell S, Li D, Kelly F, Sinclair A. Fatty acid composition of habitual omnivore and vegetarian diets. Lipids 2006;41:637–46.
[15] Giem P, Beeson WL, Fraser GE. The incidence of dementia and intake of animal products: preliminary findings from the Adventist Health Study. Neuroepidemiology 1993;12:28–36.
[16] McCarty MF. Does a vegan diet reduce risk for Parkinson's disease? Med Hypotheses 2001;57:318–23.
[17] Orlich MJ, Singh PN, Sabate J, Jaceldo-Siegl K, Fan J, Knutsen S, et al. Vegetarian dietary patterns and mortality in adventist health study 2. JAMA Inter Med 2013;173:1230–8.

[18] Beezhold BL, Johnston CS, Daigle DR. Vegetarian diets are associated with healthy mood states: a cross-sectional study in seventh day adventist adults. Nutr J 2010;9:26.
[19] Andre A, Juaneda P, Sebedio JL, Chardigny JM. Effects of aging and dietary n-3 fatty acids on rat brain phospholipids: focus on plasmalogens. Lipids 2005;40:799–806.
[20] Anderson GJ, Neuringer M, Lin DS, Connor WE. Can prenatal N-3 fatty acid deficiency be completely reversed after birth? Effects on retinal and brain biochemistry and visual function in rhesus monkeys. Pediatr Res 2005;58:865–72.
[21] Jenkins DJ, Sievenpiper JL, Pauly D, Sumaila UR, Kendall CW, Mowat FM. Are dietary recommendations for the use of fish oils sustainable? CMAJ 2009;180:633–7.
[22] Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. Am J Clin Nutr 2011;93:950–62.
[23] Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, et al. Impacts of biodiversity loss on ocean ecosystem services. Science 2006;314:787–90.
[24] Jaenike J. Comment on "Impacts of biodiversity loss on ocean ecosystem services". Science 2007;316:1285 [author reply].
[25] Barcelo-Coblijn G, Murphy EJ. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. Prog Lipid Res 2009;48:355–74.
[26] Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 2006;83:1526S–35S.
[27] Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). New York, USA: Institute of Medicine; 2005.
[28] Holman RT, Johnson SB, Hatch TF. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 1982;35:617–23.
[29] Harris WS, Mozaffarian D, Lefevre M, Toner CD, Colombo J, Cunnane SC, et al. Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. J Nutr 2009;139:804S–19S.
[30] Brenna JT. Animal studies of the functional consequences of suboptimal polyunsaturated fatty acid status during pregnancy, lactation and early post-natal life. Matern Child Nutr 2011;7(Suppl. 2):59–79.
[31] Makrides M, Gibson RA, McPhee AJ, Collins CT, Davis PG, Doyle LW, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. JAMA 2009;301:175–82.
[32] Colombo J, Carlson SE, Cheatham CL, Fitzgerald-Gustafson KM, Kepler A, Doty T. Long-chain polyunsaturated fatty acid supplementation in infancy reduces heart rate and positively affects distribution of attention. Pediatr Res 2011;70:406–10.
[33] Colombo J, Carlson SE, Cheatham CL, Shaddy DJ, Kerling EH, Thodosoff JM, et al. Long-term effects of LCPUFA supplementation on childhood cognitive outcomes. Am J Clin Nutr 2013;98:403–12.
[34] Makrides M, Gould JF, Gawlik NR, Yelland LN, Smithers LG, Anderson PJ, et al. Four-year follow-up of children born to women in a randomized trial of prenatal DHA supplementation. JAMA 2014;311:1802–4.
[35] McNamara RK, Hahn CG, Jandacek R, Rider T, Tso P, Stanford KE, et al. Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. Biol Psychiatry 2007;62:17–24.
[36] McNamara RK, Jandacek R, Rider T, Tso P, Stanford KE, Hahn CG, et al. Deficits in docosahexaenoic acid and associated elevations in the metabolism of arachidonic acid and saturated fatty acids in the postmortem orbitofrontal cortex of patients with bipolar disorder. Psychiatry Res 2008;160:285–99.
[37] McNamara RK, Jandacek R, Tso P, Dwivedi Y, Ren X, Pandey GN. Lower docosahexaenoic acid concentrations in the postmortem prefrontal cortex of adult depressed suicide victims compared with controls without cardiovascular disease. J Psychiatr Res 2013;47:1187–91.
[38] Martins JG, Bentsen H, Puri BK. Eicosapentaenoic acid appears to be the key omega-3 fatty acid component associated with efficacy in major depressive disorder: a critique of Bloch and Hannestad and updated meta-analysis. Mol Psychiatry 2012;17:1144–9 [discussion 63–7].
[39] Sublette ME, Ellis SP, Geant AL, Mann JJ. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. J Clin Psychiatry 2011;72:1577–84.
[40] Cunnane SC, Plourde M, Pifferi F, Begin M, Feart C, Barberger-Gateau P. Fish, docosahexaenoic acid and Alzheimer's disease. Prog Lipid Res 2009;48:239–56.
[41] Soderberg M, Edlund C, Kristensson K, Dallner G. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids 1991;26:421–5.
[42] Igarashi M, Ma K, Gao F, Kim HW, Rapoport SI, Rao JS. Disturbed choline plasmalogen and phospholipid fatty acid concentrations in Alzheimer's disease prefrontal cortex. J Alzheimer's Dis JAD 2011;24:507–17.
[43] Fraser T, Tayler H, Love S. Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease. Neurochem Res 2010;35:503–13.
[44] Cunnane SC, Schneider JA, Tangney C, Tremblay-Mercier J, Fortier M, Bennett DA, et al. Plasma and brain fatty acid profiles in mild cognitive impairment and Alzheimer's disease. J Alzheimer's Dis JAD 2012;29:691–7.
[45] Carver JD, Benford VJ, Han B, Cantor AB. The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. Brain Res Bull 2001;56:79–85.
[46] Crawford MA, Bazinet RP, Sinclair AJ. Fat intake and CNS functioning: ageing and disease. Ann Nutr Metab 2009;55:202–28.
[47] Chiu CC, Su KP, Cheng TC, Liu HC, Chang CJ, Dewey ME, et al. The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:1538–44.
[48] Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C, et al. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. JAMA 2010;304:1903–11.
[49] Shinto L, Quinn J, Montine T, Dodge HH, Woodward W, Baldauf-Wagner S, et al. A randomized placebo-controlled pilot trial of omega-3 fatty acids and alpha lipoic acid in Alzheimer's disease. J Alzheimer's Dis JAD 2014;38:111–20.
[50] Jiao J, Li Q, Chu J, Zeng W, Yang M, Zhu S. Effect of n-3 PUFA supplementation on cognitive function throughout the life span from infancy to old age: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr 2014;100:1422–36.
[51] Joffre C, Nadjar A, Lebbadi M, Calon F, Laye S. N-3 LCPUFA improves cognition: The young, the old and the sick. Prostaglandins Leukot Essent Fatty Acids 2014;91:1–20.
[52] Lukiw WJ, Bazan NG. Docosahexaenoic acid and the aging brain. J Nutr 2008;138:2510–4.
[53] Bazan NG. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Curr Opin Clin Nutr Metab Care 2007;10:136–41.
[54] Innis SM. Dietary (n-3) fatty acids and brain development. J Nutr 2007;137:855–9.
[55] Rapoport SI. Translational studies on regulation of brain docosahexaenoic acid (DHA) metabolism in vivo. Prostaglandins Leukot Essent Fatty Acids 2013;88:79–85.
[56] Meresse S, Delbart C, Fruchart JC, Cecchelli R. Low-density lipoprotein receptor on endothelium of brain capillaries. J Neurochem 1989;53:340–5.
[57] Chen CT, Ma DW, Kim JH, Mount HT, Bazinet RP. The low density lipoprotein receptor is not necessary for maintaining mouse brain polyunsaturated fatty acid concentrations. J Lipid Res 2008;49:147–52.
[58] Taha AY, Chen CT, Liu Z, Kim JH, Mount HT, Bazinet RP. Brainstem concentrations of cholesterol are not influenced by genetic ablation of the low-density lipoprotein receptor. Neurochem Res 2009;34:311–5.
[59] Purdon D, Arai T, Rapoport S. No evidence for direct incorporation of esterified palmitic acid from plasma into brain lipids of awake adult rat. J Lipid Res 1997;38:526–30.
[60] Ouellet M, Emond V, Chen CT, Julien C, Bourasset F, Oddo S, et al. Diffusion of docosahexaenoic and eicosapentaenoic acids through the blood-brain barrier: an in situ cerebral perfusion study. Neurochem Int 2009;55:476–82.
[61] Hamilton JA, Brunaldi K. A model for fatty acid transport into the brain. J Mol Neurosci 2007;33:12–7.
[62] Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: review and critical analysis. Brain Res Brain Res Rev 1992;17:187–214.
[63] Umhau JC, Zhou W, Carson RE, Rapoport SI, Polozova A, Demar J, et al. Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. J Lipid Res 2009;50:1259–68.
[64] Umhau JC, Zhou W, Thada S, Demar J, Hussein N, Bhattacharjee AK, et al. Brain docosahexaenoic acid [DHA] incorporation and blood flow are increased in chronic alcoholics: a positron emission tomography study corrected for cerebral atrophy. PLoS ONE 2013;8:e75333.
[65] Barcelo-Coblijn G, Murphy EJ. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. Prog Lipid Res 2009;48:355–74.
[66] Domenichiello AF, Chen CT, Trepanier MO, Stavro PM, Bazinet RP. Whole body synthesis rates of DHA from alpha-linolenic acid are greater than brain DHA accretion and uptake rates in adult rats. J Lipid Res 2014;55:62–74.
[67] Thies F, Pillon C, Moliere P, Lagarde M, Lecerf J. Preferential incorporation of sn-2 lysoPC DHA over unesterified DHA in the young rat brain. Am J Physiol 1994;267:R1273–9.
[68] Lagarde M, Bernoud N, Brossard N, Lemaitre-Delaunay D, Thies F, Croset M, et al. Lysophosphatidylcholine as a preferred carrier form of docosahexaenoic acid to the brain. J Mol Neurosci MN 2001;16:201–4 [discussion 15–21].
[69] Nguyen LN, Ma D, Shui G, Wong P, Cazenave-Gassiot A, Zhang X, et al. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. Nature 2014;509.
[70] Abdolahi A, Georas SN, Brenna JT, Cai X, Thevenet-Morrison K, Phipps RP, et al. The effects of aspirin and fish oil consumption on lysophosphatidylcholines and lysophosphatidic acids and their correlates with platelet aggregation in adults with diabetes mellitus. Prostaglandins Leukot Essent Fatty Acids 2014;90:61–8.
[71] Block RC, Kakinami L, Jonovich M, Antonetti I, Lawrence P, Meednu N, et al. The combination of EPA+DHA and low-dose aspirin ingestion reduces platelet function acutely whereas each alone may not in healthy humans. Prostaglandins Leukot Essent Fatty Acids 2012;87:143–51.
[72] Barber MN, Risis S, Yang C, Meikle PJ, Staples M, Febbraio MA, et al. Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. PLoS ONE 2012;7:e41456.

[73] Balogun KA, Albert CJ, Ford DA, Brown RJ, Cheema SK. Dietary omega-3 polyunsaturated fatty acids alter the fatty acid composition of hepatic and plasma bioactive lipids in C57BL/6 mice: a lipidomic approach. PLoS ONE 2013;8:e82399.
[74] Taha AY, Cheon Y, Faurot KF, Macintosh B, Majchrzak-Hong SF, Mann JD, et al. Dietary omega-6 fatty acid lowering increases bioavailability of omega-3 polyunsaturated fatty acids in human plasma lipid pools. Prostaglandins Leukot Essent Fatty Acids 2014;90:151–7.
[75] Bakewell L, Burdge GC, Calder PC. Polyunsaturated fatty acid concentrations in young men and women consuming their habitual diets. Br J Nutr 2006;96:93–9.
[76] Du Plooy WJ, Venter CP, Muntingh GM, Venter HL, Glatthaar II, Smith KA. The cumulative dose response effect of eicosapentaenoic and docosahexaenoic acid on blood pressure, plasma lipid profile and diet pattern in mild to moderate essential hypertensive black patients. Prostaglandins Leukot Essent Fatty Acids 1992;46:315–21.
[77] Brossard N, Croset M, Normand S, Pousin J, Lecerf J, Laville M, et al. Human plasma albumin transports [13C]docosahexaenoic acid in two lipid forms to blood cells. J Lipid Res 1997;38:1571–82.
[78] Cho HP, Nakamura M, Clarke SD. Cloning, expression, and fatty acid regulation of the human delta-5 desaturase. J Biol Chem 1999;274:37335–9.
[79] Cho HP, Nakamura MT, Clarke SD. Cloning, expression, and nutritional regulation of the mammalian Delta-6 desaturase. J Biol Chem 1999;274:471–7.
[80] Leonard AE, Kelder B, Bobik EG, Chuang LT, Lewis CJ, Kopchick JJ, et al. Identification and expression of mammalian long-chain PUFA elongation enzymes. Lipids 2002;37:733–40.
[81] Igarashi M, Ma K, Chang L, Bell JM, Rapoport SI. Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain. J Lipid Res 2007;48:2463–70.
[82] Voss A, Reinhart M, Sankarappa S, Sprecher H. The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. J Biol Chem 1991;266:19995–20000.
[83] Bernert Jr JT, Sprecher H. Studies to determine the role rates of chain elongation and desaturation play in regulating the unsaturated fatty acid composition of rat liver lipids. Biochim Biophys Acta 1975;398:354–63.
[84] Brenna JT, Kothapalli KS, Park WJ. Alternative transcripts of fatty acid desaturase (FADS) genes. Prostaglandins Leukot Essent Fatty Acids 2010;82:281–5.
[85] Sprecher H. Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochim Biophys Acta 2000;1486:219–31.
[86] Sprecher H, Chen Q, Yin FQ. Regulation of the biosynthesis of 22:5n–6 and 22:6n–3: a complex intracellular process. Lipids 1999;34(Suppl.):S153–6.
[87] Stroud CK, Nara TY, Roqueta-Rivera M, Radlowski EC, Lawrence P, Zhang Y, et al. Disruption of FADS2 gene in mice impairs male reproduction and causes dermal and intestinal ulceration. J Lipid Res 2009;50:1870–80.
[88] Wang Y, Botolin D, Christian B, Busik J, Xu J, Jump DB. Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases. J Lipid Res 2005;46:706–15.
[89] Igarashi M, DeMar Jr JC, Ma K, Chang L, Bell JM, Rapoport SI. Upregulated liver conversion of alpha-linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet. J Lipid Res 2007;48:152–64.
[90] Moore SA, Yoder E, Murphy S, Dutton GR, Spector AA. Astrocytes, not neurons, produce docosahexaenoic acid (22:6 omega-3) and arachidonic acid (20:4 omega-6). J Neurochem 1991;56:518–24.
[91] Igarashi M, DeMar Jr JC, Ma K, Chang L, Bell JM, Rapoport SI. Docosahexaenoic acid synthesis from alpha-linolenic acid by rat brain is unaffected by dietary n-3 PUFA deprivation. J Lipid Res 2007;48:1150–8.
[92] Igarashi M, DeMar Jr JC, Ma K, Chang L, Bell JM, Rapoport SI. Upregulated liver conversion of alpha-linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet. J Lipid Res 2007;48:152–64.
[93] Park WJ, Kothapalli KS, Lawrence P, Tyburczy C, Brenna JT. An alternate pathway to long-chain polyunsaturates: the FADS2 gene product Delta8-desaturates 20:2n–6 and 20:3n–3. J Lipid Res 2009;50:1195–202.
[94] Gregory MK, Gibson RA, Cook-Johnson RJ, Cleland LG, James MJ. Elongase reactions as control points in long-chain polyunsaturated fatty acid synthesis. PLoS ONE 2011;6:e29662.
[95] Gregory MK, Cleland LG, James MJ. Molecular basis for differential elongation of omega-3 docosapentaenoic acid by the rat Elovl5 and Elovl2. J Lipid Res 2013;54:2851–7.
[96] Igarashi M, Ma K, Chang L, Bell JM, Rapoport SI. Rat heart cannot synthesize docosahexaenoic acid from circulating alpha-linolenic acid because it lacks elongase-2. J Lipid Res 2008;49:1735–45.
[97] Plourde M, Cunnane SC. Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. Applied physiology, nutrition, and metabolism =. Physiol Appl Nutr Metab 2007;32:619–34.
[98] Kwon JS, Snook JT, Wardlaw GM, Hwang DH. Effects of diets high in saturated fatty acids, canola oil, or safflower oil on platelet function, thromboxane B2 formation, and fatty acid composition of platelet phospholipids. Am J Clin Nutr 1991;54:351–8.
[99] Mutanen M, Freese R, Valsta LM, Ahola I, Ahlstrom A. Rapeseed oil and sunflower oil diets enhance platelet in vitro aggregation and thromboxane production in healthy men when compared with milk fat or habitual diets. Thromb Haemost 1992;67:352–6.
[100] Chan JK, McDonald BE, Gerrard JM, Bruce VM, Weaver BJ, Holub BJ. Effect of dietary alpha-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. Lipids 1993;28:811–7.
[101] Kelley DS, Nelson GJ, Love JE, Branch LB, Taylor PC, Schmidt PC, et al. Dietary alpha-linolenic acid alters tissue fatty acid composition, but not blood lipids, lipoproteins or coagulation status in humans. Lipids 1993; 28:533–7.
[102] Freese R, Mutanen M, Valsta LM, Salminen I. Comparison of the effects of two diets rich in monounsaturated fatty acids differing in their linoleic/alpha-linolenic acid ratio on platelet aggregation. Thromb Haemost 1994; 71:73–7.
[103] Allman MA, Pena MM, Pang D. Supplementation with flaxseed oil versus sunflowerseed oil in healthy young men consuming a low fat diet: effects on platelet composition and function. Eur J Clin Nutr 1995;49:169–78.
[104] Cunnane SC, Hamadeh MJ, Liede AC, Thompson LU, Wolever TM, Jenkins DJ. Nutritional attributes of traditional flaxseed in healthy young adults. Am J Clin Nutr 1995;61:62–8.
[105] Mantzioris E, James MJ, Gibson RA, Cleland LG. Differences exist in the relationships between dietary linoleic and alpha-linolenic acids and their respective long-chain metabolites. Am J Clin Nutr 1995;61:320–4.
[106] Nordstrom DC, Honkanen VE, Nasu Y, Antila E, Friman C, Konttinen YT. Alpha-linolenic acid in the treatment of rheumatoid arthritis. A double-blind, placebo-controlled and randomized study: flaxseed vs. safflower seed. Rheumatol Int 1995;14:231–4.
[107] Ezaki O, Takahashi M, Shigematsu T, Shimamura K, Kimura J, Ezaki H, et al. Long-term effects of dietary alpha-linolenic acid from perilla oil on serum fatty acids composition and on the risk factors of coronary heart disease in Japanese elderly subjects. J Nutr Sci Vitaminol 1999;45:759–72.
[108] Li D, Sinclair A, Wilson A, Nakkote S, Kelly F, Abedin L, et al. Effect of dietary alpha-linolenic acid on thrombotic risk factors in vegetarian men. Am J Clin Nutr 1999;69:872–82.
[109] Finnegan YE, Minihane AM, Leigh-Firbank EC, Kew S, Meijer GW, Muggli R, et al. Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. Am J Clin Nutr 2003;77:783–95.
[110] Francois CA, Connor SL, Bolewicz LC, Connor WE. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. Am J Clin Nutr 2003;77:226–33.
[111] James MJ, Ursin VM, Cleland LG. Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. Am J Clin Nutr 2003;77:1140–5.
[112] Wallace FA, Miles EA, Calder PC. Comparison of the effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. Br J Nutr 2003;89:679–89.
[113] de Groot RH, Hornstra G, van Houwelingen AC, Roumen F. Effect of alpha-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. Am J Clin Nutr 2004;79:251–60.
[114] Goyens PL, Spilker ME, Zock PL, Katan MB, Mensink RP. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. Am J Clin Nutr 2006;84:44–53.
[115] Harper CR, Edwards MJ, DeFilippis AP, Jacobson TA. Flaxseed oil increases the plasma concentrations of cardioprotective (n-3) fatty acids in humans. J Nutr 2006;136:83–7.
[116] Bloedon LT, Balikai S, Chittams J, Cunnane SC, Berlin JA, Rader DJ, et al. Flaxseed and cardiovascular risk factors: results from a double blind, randomized, controlled clinical trial. J Am Coll Nutr 2008;27:65–74.
[117] Barcelo-Coblijn G, Murphy EJ, Othman R, Moghadasian MH, Kashour T, Friel JK. Flaxseed oil and fish-oil capsule consumption alters human red blood cell n-3 fatty acid composition: a multiple-dosing trial comparing 2 sources of n-3 fatty acid. Am J Clin Nutr 2008;88:801–9.
[118] Abedin L, Lien EL, Vingrys AJ, Sinclair AJ. The effects of dietary alpha-linolenic acid compared with docosahexaenoic acid on brain, retina, liver, and heart in the guinea pig. Lipids 1999;34:475–82.
[119] Fu Z, Sinclair AJ. Increased alpha-linolenic acid intake increases tissue alpha-linolenic acid content and apparent oxidation with little effect on tissue docosahexaenoic acid in the guinea pig. Lipids 2000;35:395–400.
[120] Kim HW, Rao JS, Rapoport SI, Igarashi M. Regulation of rat brain polyunsaturated fatty acid (PUFA) metabolism during graded dietary n-3 PUFA deprivation. Prostaglandins Leukot Essent Fatty Acids 2011;85:361–8.
[121] Conquer JA, Holub BJ. Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background. J Lipid Res 1998;39:286–92.
[122] Demar Jr JC, Ma K, Chang L, Bell JM, Rapoport SI. Alpha-Linolenic acid does not contribute appreciably to docosahexaenoic acid within brain phospholipids of adult rats fed a diet enriched in docosahexaenoic acid. J Neurochem 2005;94:1063–76.
[123] Frayn KN, Summers LK, Fielding BA. Regulation of the plasma non-esterified fatty acid concentration in the postprandial state. Proc Nutr Soc 1997; 56:713–21.
[124] Plourde M, Vohl MC, Vandal M, Couture P, Lemieux S, Cunnane SC. Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men. Br J Nutr 2009;102:1121–4.

[125] Vandal M, Freemantle E, Tremblay-Mercier J, Plourde M, Fortier M, Bruneau J, et al. Plasma omega-3 fatty acid response to a fish oil supplement in the healthy elderly. Lipids 2008;43:1085–9.
[126] Berstad P, Seljeflot I, Veierod MB, Hjerkinn EM, Arnesen H, Pedersen JI. Supplementation with fish oil affects the association between very long-chain n-3 polyunsaturated fatty acids in serum non-esterified fatty acids and soluble vascular cell adhesion molecule-1. Clin Sci (Lond) 2003;105:13–20.
[127] Walker CG, Browning LM, Mander AP, Madden J, West AL, Calder PC, et al. Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans. Br J Nutr 2014;111:679–89.
[128] Cunnane SC, Francescutti V, Brenna JT, Crawford MA. Breast-fed infants achieve a higher rate of brain and whole body docosahexaenoate accumulation than formula-fed infants not consuming dietary docosahexaenoate. Lipids 2000;35:105–11.
[129] Clandinin MT, Chappell JE, Heim T, Swyer PR, Chance GW. Fatty acid utilization in perinatal de novo synthesis of tissues. Early Human Dev 1981;5:355–66.
[130] Luxwolda MF, Kuipers RS, Koops JH, Muller S, de Graaf D, Dijck-Brouwer DA, et al. Interrelationships between maternal DHA in erythrocytes, milk and adipose tissue. Is 1 wt% DHA the optimal human milk content? Data from four Tanzanian tribes differing in lifetime stable intakes of fish. Br J Nutr 2014;111:854–66.
[131] Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. Br J Nutr 2003;90:311–21.
[132] Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men*. Br J Nutr 2002;88:355–63.
[133] Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr 2002;88:411–20.
[134] Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1994;1213:277–88.
[135] Pawlosky RJ, Hibbeln JR, Lin Y, Goodson S, Riggs P, Sebring N, et al. Effects of beef- and fish-based diets on the kinetics of n-3 fatty acid metabolism in human subjects. Am J Clin Nutr 2003;77:565–72.
[136] Pawlosky RJ, Hibbeln JR, Novotny JA, Salem Jr N. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. J Lipid Res 2001;42:1257–65.
[137] Gillingham LG, Harding SV, Rideout TC, Yurkova N, Cunnane SC, Eck PK, et al. Dietary oils and FADS1-FADS2 genetic variants modulate [13C]alpha-linolenic acid metabolism and plasma fatty acid composition. Am J Clin Nutr 2013;97:195–207.
[138] McCloy U, Ryan MA, Pencharz PB, Ross RJ, Cunnane SC. A comparison of the metabolism of eighteen-carbon 13C-unsaturated fatty acids in healthy women. J Lipid Res 2004;45:474–85.
[139] Goyens PL, Spilker ME, Zock PL, Katan MB, Mensink RP. Compartmental modeling to quantify alpha-linolenic acid conversion after longer term intake of multiple tracer boluses. J Lipid Res 2005;46:1474–83.
[140] Hussein N, Ah-Sing E, Wilkinson P, Leach C, Griffin BA, Millward DJ. Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. J Lipid Res 2005;46:269–80.
[141] Leyton J, Drury PJ, Crawford MA. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. Br J Nutr 1987;57:383–93.
[142] Lin YH, Salem Jr N. Whole body distribution of deuterated linoleic and alpha-linolenic acids and their metabolites in the rat. J Lipid Res 2007;48:2709–24.
[143] Cunnane SC, Anderson MJ. The majority of dietary linoleate in growing rats is beta-oxidized or stored in visceral fat. J Nutr 1997;127:146–52.
[144] Hirsch J, Farquhar JW, Ahrens Jr EH, Peterson ML, Stoffel W. Studies of adipose tissue in man. A microtechnic for sampling and analysis. Am J Clin Nutr 1960;8:499–511.
[145] Dayton S, Hashimoto S, Dixon W, Pearce ML. Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. J Lipid Res 1966;7:103–11.
[146] DeMar Jr JC, DiMartino C, Baca AW, Lefkowitz W, Salem Jr N. Effect of dietary docosahexaenoic acid on biosynthesis of docosahexaenoic acid from alpha-linolenic acid in young rats. J Lipid Res 2008;49:1963–80.
[147] Demmelmair H, Iser B, Rauh-Pfeiffer A, Koletzko B. Comparison of bolus versus fractionated oral applications of [13C]-linoleic acid in humans. Eur J Clin Invest 1999;29:603–9.
[148] Kitson AP, Smith TL, Marks KA, Stark KD. Tissue-specific sex differences in docosahexaenoic acid and Delta6-desaturase in rats fed a standard chow diet. Applied physiology, nutrition, and metabolism =. Physiol Appl Nutr Metab 2012;37:1200–11.
[149] Sarkadi-Nagy E, Wijendran V, Diau GY, Chao AC, Hsieh AT, Turpeinen A, et al. The influence of prematurity and long chain polyunsaturate supplementation in 4-week adjusted age baboon neonate brain and related tissues. Pediatr Res 2003;54:244–52.
[150] Zhu H, Fan C, Xu F, Tian C, Zhang F, Qi K. Dietary fish oil n-3 polyunsaturated fatty acids and alpha-linolenic acid differently affect brain accretion of docosahexaenoic acid and expression of desaturases and sterol regulatory element-binding protein 1 in mice. J Nutr Biochem 2010;21:954–60.
[151] Nara TY, He WS, Tang C, Clarke SD, Nakamura MT. The E-box like sterol regulatory element mediates the suppression of human Delta-6 desaturase gene by highly unsaturated fatty acids. Biochem Biophys Res Commun 2002;296:111–7.
[152] Brenner RR, Peluffo RO. Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic, and linolenic acids. J Biol Chem 1966;241:5213–9.
[153] Mohrhauer H, Christiansen K, Gan MV, Deubig M, Holman RT. Chain elongation of linoleic acid and its inhibition by other fatty acids in vitro. J Biol Chem 1967;242:4507–14.
[154] Holman RT. Biological activities of and requirements for polyunsaturated acids. Prog Chem Fats Other Lipids 1971;9:607–82.
[155] Vermunt SH, Mensink RP, Simonis MM, Hornstra G. Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha-linolenic acid. Lipids 2000;35:137–42.
[156] Pawlosky R, Hibbeln J, Lin Y, Salem Jr N. n-3 fatty acid metabolism in women. Br J Nutr. 2003;90:993–4 [discussion 4–5].
[157] Lin YH, Llanos A, Mena P, Uauy R, Salem Jr N, Pawlosky RJ. Compartmental analyses of 2H5-alpha-linolenic acid and C-U-eicosapentaenoic acid toward synthesis of plasma labeled 22:6n–3 in newborn term infants. Am J Clin Nutr 2010;92:284–93.
[158] Yamanaka A, Goto AS, Korenaga T. D/H exchange in C-H bonds of fatty acids: implication for geographical discrimination of food materials. Food Chem 2013;138:1720–2.
[159] Lohner S, Fekete K, Marosvolgyi T, Decsi T. Gender differences in the long-chain polyunsaturated fatty acid status: systematic review of 51 publications. Ann Nutr Metab 2013;62:98–112.
[160] Extier A, Langelier B, Perruchot MH, Guesnet P, Van Veldhoven PP, Lavialle M, et al. Gender affects liver desaturase expression in a rat model of n-3 fatty acid repletion. J Nutr Biochem 2010;21:180–7.
[161] Ockner RK, Burnett DA, Lysenko N, Manning JA. Sex differences in long chain fatty acid utilization and fatty acid binding protein concentration in rat liver. J Clin Invest 1979;64:172–81.
[162] Bokor S, Legry V, Meirhaeghe A, Ruiz JR, Mauro B, Widhalm K, et al. Single-nucleotide polymorphism of CD36 locus and obesity in European adolescents. Obesity (Silver Spring) 2010;18:1398–403.
[163] Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet 2006;15:1745–56.
[164] Rzehak P, Heinrich J, Klopp N, Schaeffer L, Hoff S, Wolfram G, et al. Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. Br J Nutr 2009;101:20–6.
[165] Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, Johansson AC, et al. Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. Am J Hum Genet 2012;90:809–20.
[166] Koletzko B, Lattka E, Zeilinger S, Illig T, Steer C. Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children. Am J Clin Nutr 2011;93:211–9.
[167] Morales E, Bustamante M, Gonzalez JR, Guxens M, Torrent M, Mendez M, et al. Genetic variants of the FADS gene cluster and ELOVL gene family, colostrums LC-PUFA levels, breastfeeding, and child cognition. PLoS ONE 2011;6:e17181.
[168] Xie L, Innis SM. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. J Nutr 2008;138:2222–8.
[169] Rodriguez A, Sarda P, Nessmann C, Boulot P, Leger CL, Descomps B. Delta6- and delta5-desaturase activities in the human fetal liver: kinetic aspects. J Lipid Res 1998;39:1825–32.
[170] Biagi PL, Hrelia S, Stefanini GF, Zunarelli P, Bordoni A. Delta-6-desaturase activity of human liver microsomes from patients with different types of liver injury. Prostaglandins Leukot Essent Fatty Acids 1990;39:39–42.
[171] Yang J, Chen ZY, Cunnane SC. Application of the balance method to determining accumulation, metabolism, and apparent oxidation of linoleic and alpha-linolenic acids in the pregnant rat. Metab Clin Exp 1994;43:940–4.
[172] Poumes-Ballihaut C, Langelier B, Houlier F, Alessandri JM, Durand G, Latge C, et al. Comparative bioavailability of dietary alpha-linolenic and docosahexaenoic acids in the growing rat. Lipids 2001;36:793–800.
[173] Rapoport SI, Igarashi M, Gao F. Quantitative contributions of diet and liver synthesis to docosahexaenoic acid homeostasis. Prostaglandins Leukot Essent Fatty Acids 2010;82:273–6.
[174] Kris-Etherton PM, Harris WS, Appel LJ. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. Arterioscler Thromb Vasc Biol 2003;23:151–2.
[175] Simopoulos AP, Leaf A, Salem Jr N. Workshop statement on the essentiality of and recommended dietary intakes for Omega-6 and Omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 2000;63:119–21.
[176] DeMar Jr JC, Ma K, Bell JM, Rapoport SI. Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids. J Neurochem 2004;91:1125–37.
[177] Rao JS, Ertley RN, DeMar Jr JC, Rapoport SI, Bazinet RP, Lee HJ. Dietary n-3 PUFA deprivation alters expression of enzymes of the arachidonic and

docosahexaenoic acid cascades in rat frontal cortex. Mol Psychiatry 2007;12:151–7.
[178] Rao JS, Lee HJ, Rapoport SI, Bazinet RP. Mode of action of mood stabilizers: is the arachidonic acid cascade a common target? Mol Psychiatry 2008;13:585–96.
[179] Rao JS, Ertley RN, Lee HJ, DeMar Jr JC, Arnold JT, Rapoport SI, et al. N-3 polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism. Mol Psychiatry. 2007;12:36–46.
[180] Green JT, Liu Z, Bazinet RP. Brain phospholipid arachidonic acid half-lives are not altered following 15 weeks of N-3 polyunsaturated fatty acid adequate or deprived diet. J Lipid Res 2010;51:535–43.
[181] Anderson C, Checkoway H, Franklin GM, Beresford S, Smith-Weller T, Swanson PD. Dietary factors in Parkinson's disease: the role of food groups and specific foods. Mov Disord 1999;14:21–7.
[182] Logroscino G, Marder K, Cote L, Tang MX, Shea S, Mayeux R. Dietary lipids and antioxidants in Parkinson's disease: a population-based, case-control study. Ann Neurol 1996;39:89–94.
[183] Beezhold BL, Johnston CS. Restriction of meat, fish, and poultry in omnivores improves mood: a pilot randomized controlled trial. Nutr J 2012;11:9.
[184] Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). Am J Clin Nutr 2009;89:1613S–9S.
[185] Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr 1994;60:189–94.