α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans

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A R T I C L E I N F O

Article history:
Received 15 January 2009
Accepted 15 January 2009

Keywords:
ISSFAL Statement
Omega-3 fatty acid supplementation
Human PUFA nutrition

A B S T R A C T

Blood levels of polyunsaturated fatty acids (PUFA) are considered biomarkers of status. Alpha-linolenic acid, ALA, the plant omega-3, is the dietary precursor for the long-chain omega-3 PUFA eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Studies in normal healthy adults consuming western diets, which are rich in linoleic acid (LA), show that supplemental ALA raises EPA and DPA status in the blood and in breast milk. However, ALA or EPA dietary supplements have little effect on blood or breast milk DHA levels, whereas consumption of preformed DHA is effective in raising blood DHA levels. Addition of ALA to the diets of formula-fed infants does raise DHA, but no level of ALA tested raises DHA to levels achievable with preformed DHA at intakes similar to typical human milk DHA supply. The DHA status of infants and adults consuming preformed DHA in their diets is, on average, greater than that of people who do not consume DHA. With no other changes in diet, improvement of blood DHA status can be achieved with dietary supplements of preformed DHA, but not with supplementation of ALA, EPA, or other precursors.

1. Introduction

The ability of mammals, and humans in particular, to metabolize alpha-linolenic acid (ALA, 18:3n-3) to its longer chain and more unsaturated forms including eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPAn-3, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) is an important nutritional question since there is evidence that enhanced EPA and DHA status is important for optimal health. The principal n-3 fatty acids, ALA, EPA and DHA, are believed to each have a constellation of physiological functions and therefore it is important to understand the extent of their metabolism from ALA in various mammals in order to achieve an understanding of the n-3 PUFA that must be consumed to support desirable tissue levels.

This statement addresses the degree to which the supplementation of the diet with ALA, EPA or DHA supports the tissue and blood levels of the major omega-3 PUFA in animals and in humans. “Supplementation” in this context refers specifically to the addition of a fatty acid to a diet that is otherwise not changed, which can be achieved by fortification of normal foods or by consumption of caplets. In vivo metabolic studies of various mammals, including tracer studies in humans using stable isotopically labeled fatty acids are considered. Since there is antagonism between n-3 and n-6 essential fatty acids (EFA) for tissue composition, their interplay is also briefly considered.

2. Alpha-linolenic acid supplementation—tissue compositional studies in animals

Several reviews concerning the issue of ALA metabolism to EPA, DPAn-3 and DHA have appeared recently [13–17]. Rodent studies show that diets containing ALA as the only n-3 PUFA leads to tissue composition reflecting the full diversity of n-3 polyunsaturates. However, observations from several laboratories have indicated that the tissue concentrations of the long-chain n-3 polyunsaturates, particularly DHA, are lower in an ALA-based diet than one in which the preformed LCPUFA are present. For example, one study showed that rat brain and retinal DHA were greater in pups fed a diet with preformed EPA and DHA compared to pups fed diets containing only ALA. Even when the ALA intake was increased by a factor of 10 greater than the EPA/DHA levels, the retinal DHA content remained below the value obtained for
retina in the preformed DHA diet and led to a diminution of the arachidonic acid (AA) content [20]. Similarly, in guinea pigs, both the brain and retina DHA levels were greater when a diet containing 1% ALA and 1.8% DHA was fed relative to one with only 7.1% ALA [22]. In the heart, the DHA level was over 7-fold greater in the DHA diet relative to the ALA only diet. The liver was an even more extreme case, with DHA 17-fold greater when preformed DHA was in the diet. Gliol cell phospholipids of neonatal rats contained more DHA when the dams were fed a diet containing DHA than when they were fed only ALA [24]. When DHA is added to a 1 wt% ALA diet fed to the artificially reared rat pup, there is a significant increase in the DHA content of the brain and the liver DHA content more than doubles [26].

When rodents or guinea pigs are fed a diet with high levels of ALA, increased tissue content of EPA and DPA-n-3 is commonly observed. In guinea pigs fed a high ALA diet, the DPA-n-3 content of the brain, retina, heart and liver were at a higher level than in the DHA diet, as may be expected from much higher ALA content in the diet relative to the DHA [22]. Similarly, when guinea pigs were fed a high level of ALA, many tissues had very substantial increases in ALA, EPA and DPA-n-3 but comparatively little increase in DHA [29]. In a study of several tissues of the suckling rat, increasing the ALA content of the maternal diet led to increased ALA, EPA and DPA-n-3 in the whole body, skin and epididymal fat pads; however, there was no effect on the DHA content of these tissues nor on the brain or muscles [32]. The predominant fate of ALA is catabolism [16,34] and carbon recycling to acetate [36]. In rodents, only about 16% of an ALA dose is found in rat tissues, mainly adipose, and 6% was elongated/desaturated [38].

As carnivores with regular DHA intakes, cats express extremely low levels of in vivo desaturases such that it has only been observed in PUFA deficiency [40]; even a diet with the very high ALA content of 17 wt% led to plasma PC with EPA, DPA-n-3 and DHA below detectable limits [42]. Adding 2.3% EPA and 0.4% DHA to this diet with low ALA (0.9%) led to cat plasma PC with 8% EPA, 0.8% DPA-n-3 and 4.1% DHA. In dogs, omnivorous animals, increasing the EPA and DHA content of the diet led to an increase in plasma DHA even though the reference diet with high ALA content contained more than 10-fold more n-3 fatty acid [44]. Similarly, ALA diets do not support the DHA content of canine milk as does providing much lower levels of preformed EPA/DHA [46].

Piglets fed a diet with 1.7% of fatty acids as ALA supplemented with DHA (0.7%) had lung phospholipid DHA markedly increased compared to the control group consuming 1.7% ALA as the only dietary omega-3 [47]. Similarly, when DHA was added to piglet formula containing ALA, there was a significant increase in DHA in liver TG, PE, PC, PS and PI [48]. Increasing levels of menhaden oil in piglet formula led to increases in EPA, DPA-n-3 and DHA in plasma in comparison to a base formula containing ALA [49]. Brain, liver and adipose DHA increased in a dose-response manner when DHA-containing oil up to 1.66% of fatty acids is fed to piglets over 28 days [50].

Non-human primate studies are perhaps the most enlightening of all animal studies because PUFA metabolism is similar among omnivorous primates [51], and the primate brain is a much larger proportion of body weight than in subprimates. Several studies have been conducted in perinatal baboons investigating the relative efficacy of ALA and preformed DHA to supply DHA to developing tissue. In 6-week-old neonates, 0.23% of a dose of ALA was found in the brain as DHA, whereas 1.7% of a DHA dose was found in the brain as DHA, giving a ratio of efficacy of 7.1 [52]. This ratio was 20:1 for ALA and preformed DHA provided to pregnant animals [53]. When DHA was added to a formula containing ALA fed to baboon neonates, there was a significant DHA increase in the brain, retina, liver, erythrocytes and plasma relative to those fed the same formula without DHA, and the increase in retinal DHA was directly related to retinal function [54]. Very recent studies demonstrate a dose-response between dietary DHA and cerebral cortex DHA, accompanied by global changes in gene expression at moderate and high DHA levels [55,56]. These studies indicate that dietary ALA contributes to DHA deposition in fetal and neonatal brain, but the relative efficacy of dietary preformed DHA is far higher.

3. Alpha-linolenic acid supplementation—tissue compositional studies in adults

Reviews by Plourde and Cunnane [16] and Burdge and Calder [14] of human studies of the effects of ALA supplementation on plasma fatty acid composition have been published recently and the results are summarized in Table 1.

Many of these studies of human plasma fatty acid composition involved a high dosage of ALA feeding (up to 40g/d) and for a prolonged period of time (up to 42 weeks). There was in most cases a significant increase in the plasma EPA content upon ALA supplementation. Generally, where it was reported, the DPA-n-3 was also increased after ALA supplementation. However, with few exceptions, these studies did not find a significant increase in plasma DHA. Burdge and Calder [14] concluded that ALA supplementation of human subjects generally led to an increase in EPA and in several studies, in DPA-n-3, but little or no effects on DHA content in plasma fractions and in circulating blood cells. A cross-study analysis of ALA supplementation in humans found increased plasma ALA and EPA but no increase in DHA [57]. Of importance, the size of the plasma DHA pool is far greater that of EPA; therefore it may take longer time until a small contribution of ALA conversion to the plasma DHA pool is detected. A flax oil supplementation study found no increase in either breast milk DHA or in plasma DHA [21]. Overall, these data are very consistent with the idea that ALA is effective in increasing the EPA and DPA-n-3 content in the human blood stream but has little effect on DHA content.

Notably, the studies that reported a significant increase in plasma DHA levels altered the oils in the diet, changing both ALA and LA. Long-term effects of ALA supplementation were investigated by substituting perilla oil, high in ALA, for soy oil in foods for 20 Japanese elderly subjects. At 3 months, changes in serum fatty acids were consistent with results shown in Table 1 since all but DHA increased; at 10 months DHA increased 21%, and then returned to baseline 3 months after being switched back to soy [25]. These data suggest that modest long-term changes in dietary ALA intake may be of value for increasing DHA status. However, perilla oil contains about one-third of the LA as soy oil, hence this result is confounded by a reduction in LA as well as ALA supplementation. These data are consistent with results showing that the DHA status can be improved by switching from oils rich in omega-6 PUFA to a blend incorporating an oil containing a substantial quantity of ALA and less LA. A study in India showed considerable increase of DHA in plasma but not in platelets, as well as in EPA, by partially substituting canola oil for sunflower (75% LA) or groundnut (peanut) oil to obtain cooking oils with 25–40% linoleic acid (LA) and 4% ALA [58]. Typical safflower and peanut oils have very low ALA (<0.1%), while canola is an excellent source of ALA (~11%) and is usually not higher in LA than peanut oil. Diets with very low ALA with high LA may be driven low omega-3 LCPUFA status in these subjects. Further research to confirm these findings is needed.

Supplementation trials have been conducted with purified EPA ethyl ester, and all consistently show increase in both EPA and DPA-n-3 but no changes in DHA in the blood. Table 2 outlines seven studies of EPA supplementation in adults, all of which show
increases in EPA but no increases in DHA. Purified EPA ethyl ester (3.6 g) was given for 4 weeks to healthy volunteers, both platelet and erythrocyte phospholipid EPA was significantly increased but there was no change in DHA [2,3]. Highly purified EPA (3.8 g/d) was fed to healthy men for 7 weeks and serum phospholipid EPA and DPA{n}-3 were significantly increased but DHA decreased [6]. In middle aged men, after 4 g/d of EPA was fed for 6 weeks, there was a marked increase in plasma phospholipid EPA and DPA{n}-3 was significantly increased but DHA was unaltered [8]. In another study of healthy subjects, 4 g/d of EPA was fed for 4 weeks and platelet EPA was increased many-fold, DPA{n}-3 was significantly increased but DHA was unaltered [10]. In type 2 diabetic patients, consuming 4 g/d of EPA for 6 weeks led to a 5-fold increase in plasma phospholipid EPA and a significant increase in DPA{n}-3, but no change in DHA [11]. In one psychiatric trial of schizophrenics, 1, 2 or 4 g EPA/d for 12 weeks produced significant increases in erythrocyte membrane EPA but not DHA [59].

One other omega-3 PUFA, stearidonic acid (SDA, 18:4n-3), has also been studied as a precursor for long-chain omega-3 in humans. SDA is an intermediate metabolite between ALA and EPA in the omega-3 biosynthetic pathway. Supplementation of SDA led to an elevated increase in EPA compared to ALA supplementation, but no increase in DHA [9,60], consistent with studies of ALA and EPA.

In contrast to the results with EPA supplementation, many studies have demonstrated that humans given preformed DHA increases in EPA but no increase in DHA. Purified EPA ethyl ester (3.6 g) was given for 4 weeks to healthy volunteers, both platelet and erythrocyte phospholipid EPA was significantly increased but there was no change in DHA [2,3]. Highly purified EPA (3.8 g/d) was fed to healthy men for 7 weeks and serum phospholipid EPA and DPA{n}-3 were significantly increased but DHA decreased [6]. In middle aged men, after 4 g/d of EPA was fed for 6 weeks, there was a marked increase in plasma phospholipid EPA and DPA{n}-3 but no increase in DHA [8]. In another study of healthy subjects, 4 g/d of EPA was fed for 4 weeks and platelet EPA was increased many-fold, DPA{n}-3 was significantly increased but DHA was unaltered [10]. In type 2 diabetic patients, consuming 4 g/d of EPA for 6 weeks led to a 5-fold increase in plasma phospholipid EPA and a significant increase in DPA{n}-3, but no change in DHA [11]. In one psychiatric trial of schizophrenics, 1, 2 or 4 g EPA/d for 12 weeks produced significant increases in erythrocyte membrane EPA but not DHA [59].

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supplements rapidly incorporate the fatty acid into the blood stream [6,8,10,11,57,62]. There is a dose-dependent rise in plasma phospholipid DHA when various doses of DHA were given for 14 days [62]. In a cross-study, meta-regression analysis of the dose-response relationship of plasma phospholipid DHA, a plateau is reached at a daily value of about 3 g DHA/d in studies of 1–6 months in duration. Steady state levels of DHA reached within about 1 month in plasma and 4–6 months in erythrocytes after DHA supplementation had begun [57]. Human autopsy studies show that infants consuming formulas without preformed DHA have lower brain DHA than infants receiving DHA via breast milk, and this relationship was not detected for arachidonic acid [63–65].

4. Competition of n-3 and n-6 fatty acids

This statement is concerned primarily with omega-3 supplementation, rather than dietary adjustments of other components. Nevertheless, it is clear that the metabolism of n-3 fatty acids depends on other nutrients, in particular, n-6 fatty acids, due to the competition of n-3 and n-6 fatty acids for the same enzymes and transport systems. They also compete for incorporation into more complex lipids that comprise mammalian tissues, and high levels of n-6 PUFA replace, and reduce, n-3 PUFA.

The main n-6 fatty acids are LA and arachidonic acid, the former being a major dietary constituent in western countries and those parts of the developing world with substantial intakes of seed oils [66]. The intake of LA has increased dramatically mainly due to the intake of soybean oil in the US [67], as well as sunflower, safflower, and others. The intake of n-3 fatty acids has been relatively constant over the past decades, especially in the USA, though it has risen in countries where canola oil has been introduced in the last two decades.

There is concern that increased intake of LA has led to an increase in AA in tissue lipids and a decrease in n-3 content. Evidence of n-3 and n-6 fatty acid antagonism has come from tissue compositional studies as well as from radioisotope studies performed in vitro. Early studies of rat liver microsomes showed that the delta-6 desaturase activity measured in vitro with various substrates was subject to competitive inhibition by other substrates. In particular, desaturation of ALA to 18:4n-3 was inhibited by LA and, conversely, LA conversion to 18:3n-6 was inhibited by ALA [68]. Altered ratios of n-3 and n-6 fatty acids can markedly alter tissue fatty acid compositions in rodents [69,70], pigs [71,72] and humans [43], including alteration of the nervous system [73,74].

In view of the important interaction of n-3 and n-6 essential fatty acids for tissue fatty acid content, Lands has integrated dietary data on rodent tissue composition and provided empirical equations that predict some features of tissue essential fatty acid composition [75], and enable relative changes in plasma PUFA to be estimated based on comparison of any particular set of diets. A recently updated calculator is available in which the values for LA, ALA, long-chain n-6 (meaning 20 and 22 carbon length polyunsaturates) and long-chain n-3 fatty acid intakes can be entered, and plasma phospholipid n-6 and n-3 long-chain polyunsaturated content can be predicted: [http://efaeducation.nih.gov/sig/diet-balance.html].

This calculator can be used to estimate how increasing ALA against a constant and Western dietary level of n-6 fatty acid dietary intake (approx 6 en%) will lead to increases in plasma phospholipid long-chain n-3 fatty acids. (HUFA are defined as highly unsaturated fatty acids with 20- or 22 carbons and three or more double bonds). For example, if typical American values (in en%) for LA (6.82) and n-6 HUFA (0.08) are used as the background diet, then adding n-3 PUFA in increments of 0.3 en% for ALA are equivalent to adding n-3 HUFA increments of 0.02 en% in terms of the effects on n-3 HUFA content in plasma phospholipids, suggesting an equivalency factor of about 15–1 for ALA and n-3 HUFA. It must be cautioned that the term n-3 HUFA here indicates the sum of all of the n-3 HUFA: EPA, DPAn-3 and DHA; however, only the EPA and DPAn-3 are increasing in this example while in most studies there is no significant increase of DHA. In other words, the equations summarizing many dietary experiments indicate that dietary EPA is about 15-fold more effective than dietary ALA in increasing the EPA/DPAn-3 content of human plasma phospholipids. An estimate has been made concerning how recommended intake of n-3 HUFA can be decreased when LA intake is decreased in order to decrease competition for tissue lipid content [76]. In depth consideration of the impact of reductions in n-6 PUFA are the subject of separate treatments [77].

5. Isotope studies of in vivo ALA and EPA metabolism

Early studies of in vivo ALA metabolism in rodents indicated that conversion of ALA through EPA and to DHA does occur [78]. Subsequently, stable isotope labeled fatty acids were employed to demonstrate elongation and desaturation of ALA to EPA and DHA in human subjects [79]. This was followed by several studies in human infants where metabolism of ALA to EPA and DHA was evident [80–82], as well as a later paper showing a downward trend in conversion efficiency with gestational age at birth in preterm infants [83]. A very recent study using a whole body natural isotope tracer approach reports that an average of 42% of DHA is biosynthesized from ALA in 1-month-old infants consuming formulas with 0.644%w/w DHA. This drops to 11% by 3 months of age, and 7% at 7 months [84]. Adult metabolism of stable isotope labeled fatty acids in vivo has been reviewed several times [13,15,16]. Generally, the results have clearly shown metabolism of ALA to EPA with decreasing amounts of DPAn-3 and DHA [4,85–91] with one paper finding little or no DHA formed in young men [92]. Burdge [15] has proposed that women have a greater activity of elongation/desaturation than do men and the finding has been confirmed in a second laboratory, though at a lower level of ALA conversion [93]. These findings are consistent with higher plasma DHA concentrations in women compared to that of men [94,95]. The extent of conversion of ALA to DHA is influenced by the dietary long-chain polyunsaturated fatty acid content [4,85–88,91,93,96]. ALA metabolism to EPA and DHA has been observed in humans of all ages from premature infants [80–82,96] to adults in their sixth decade [87,88,91,97]. DHA biosynthesis may be impaired in disease states such as retinitis pigmentosa [98] and is altered by smoking [97].

Non-human primates have been used to investigate ALA metabolism in internal organs and compartments not accessible in humans. Early studies of acute omega-3 deficiency in rhesus monkeys confirmed the importance of a source of omega-3 for neural development [99], and recent work suggests that deprivation of omega-3 early in life cannot be fully reversed in retina with ALA feeding [100]. Studies of DHA in pregnancy show that preformed DHA accumulates at 20–33 times the level of DHA biosynthesized from ALA in liver, retina, and brain, and the amount of ALA found as DHA is <0.1% of dose [53]. Similar results are found in 4-week-old neonates at 2 weeks post-dose [52]. DHA synthesis has also been demonstrated in late-term fetal baboons at levels several fold above those seen for neonate baboons [101]. DHA levels rise in a dose-dependent manner in liver, heart, and retina of 12-week-old baboons consuming preformed DHA [55].

A key point is that although metabolism to DHA was observed in most studies of stable isotope-labeled ALA in humans in vivo, the quantity of labeled DHA produced was very small. Highly
6. Summary

1. ALA conversion to EPA, DPAn-3 and DHA in tracer studies has been observed in nearly all humans studied from birth through late middle age and in both males and females. The majority of evidence from isotopic tracer studies show that conversion of ALA to DHA is of the order of 1% in infants, and considerably lower in adults. This is consistent with measurements of whole body ALA oxidation which is the predominant fate of ALA in both rodents and humans. These “conversion rates” must be viewed as markers of flux through this metabolic pathway but must not be assumed to represent a net change in mass.

2. Very few studies in adults show that blood stream or breast milk DHA concentrations increase following several weeks of increased dietary ALA supply, whereas most studies do not. ALA appears to contribute little to circulating DHA when added to a diet that already contains some ALA and high LA levels. Supplementation of the diet with high levels of ALA leads to small but significant increases in EPA and DPAn-3 although supplementation with preformed EPA is approximately 15-fold more efficacious in this regard.

3. Dietary DHA increases blood and tissue DHA beyond that achievable with consumption of usual intakes of any precursor omega-3 PUFA, against a background of western diets providing ample n-6 fatty acids.

4. For a given dietary concentration of ALA, the conversion of ALA to LCPUFA is decreased by high dietary ratios of LA/ALA. Moreover, n-6 fatty acid intake influences tissue concentrations of the n-3 LCPUFA. Present evidence indicates that n-3 LCPUFA status can be improved by increasing their intake or by decreasing LA intake, and a combination of the two is likely to be most effective.

References


[16] R.A. Bowden, M.T. Claudinim, Maternal dietary 22: 6n-3 is more effective than 20:4n-6 in increasing milk DHA concentrations following several weeks of increased dietary ALA supply, whereas most studies do not. ALA appears to contribute little to circulating DHA when added to a diet that already contains some ALA and high LA levels.


