Review

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

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**Abbreviations:** ALA, alpha-linolenic acid, 18:3 n−6; PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid, 22:6 n−3; EPA, eicosapentaenoic acid, 20:5 n−3; DPA, docosapentaenoic acid, 22:5 n−6; CE, cholesteryl ester; COX, cyclooxygenase; CRP, C-reactive protein; CVD, cardiovascular disease; HDL-C, HDL-cholesterol; IL, interleukins; LTB, leukotrienes; LNA, linoleic acid, 18:2 n−6; NF-κB, nuclear factor κB; PGE2, prostaglandin E2; PPAR, peroxisome proliferator-activated receptor; SAA, serum amyloid A; SCI, spinal cord injury; sICAM-1, soluble intercellular cell-adhesion molecule; sVCAM, soluble vascular cell-adhesion molecule; TAG, triacylglycerol; TXB2, thromboxanes; TNF, tumor necrotic factor.

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1. Introduction to \( n \)-3 fatty acids and their metabolism

All-cis-9,12,15-octadecatrienoic acid or \( \alpha \)-linolenic acid (ALA, 18:3\( \text{n-3} \)), is a polyunsaturated fatty acid (PUFA) abundant in some vegetable oils (Table 1). In the early 1930s, the essentially of ALA and linoleic acid (18:2\( \text{n-6} \); LNA) in rat diets was identified [1], but in humans the first demonstration of this was reported only in the early 1980s [2,3]. Hence, ALA is referred to as the essential precursor of the longer chain \( n \)-3 PUFA (commonly known as \( \omega-3 \) fatty acids) because it is the metabolic precursor from which longer chain \( n \)-3 fatty acids are synthesized. The \( n \)-3 fatty acid family is defined by a double bound beginning at the third carbon from the methyl end. Because mammals cannot insert double bonds more proximal to the methyl end than the ninth carbon atom (\( \Delta-9 \) desaturase), \( n \)-3 fatty acids cannot be synthesized de novo, consequently \( n \)-3 fatty acids have to be present in the diet. These features are shared by another family of fatty acids, the \( n \)-6 fatty acids whose precursor is LNA. As in the case of \( n \)-3 fatty acids, the \( n \)-6 fatty acids are defined by a double bound beginning at the sixth carbon from the methyl end (Fig. 1). Because neither of these fatty acids can be synthesized de novo, ALA and LNA are referred to as essential fatty acids for mammals.

Hence, ALA and LNA serve as the precursor molecules from which the rest of fatty acids belonging to the \( n \)-3 or \( n \)-6 fatty acid family can be synthesized through a series of elongation and desaturation reactions. All the reactions are catalyzed by an enzymatic system consisting in fatty acyl-CoA synthetases, \( \Delta-6 \) and \( \Delta-5 \) desaturases and respective elongases [4–9]. Evidence from several studies in vivo and in vitro, indicate that these two fatty acid families not only share these enzymes, but they also compete for the same enzymes [10–12] (see Fig. 2). Although lower order animals have enzymes to convert \( n \)-6 to \( n \)-3 fatty acids [13], mammals do not possess the enzymatic machinery necessary to convert an \( n \)-3 fatty acid into an \( n \)-6 fatty acid or vice versa. However, a mouse expressing the gene encoding FAT-1 from *C. elegans*, was recently made that converts \( n \)-6 fatty acids into \( n \)-3 fatty acids, thereby increasing endogenous docosahexaenoic acid (DHA, 22:6\( \text{n-3} \)) via the conversion of \( n \)-6 fatty acids, resulting in a reduction in arachidonic acid (ARA, 20:4\( \text{n-6} \)) levels [14]. FAT-1 encodes for an \( n \)-3 fatty acid desaturase in *C. elegans* that recognizes 18 and 20 carbon \( n \)-6 fatty acids and inserts a double bond to form an \( n \)-3 fatty acid [13]. Hence, these mice are the only known mammal able to convert an \( n \)-6 fatty acid into an \( n \)-3 fatty acid and are a useful model to elucidate the role of \( n \)-3 fatty acids in health and disease.

There are two basic metabolic fates for ALA, both of which are highlighted in detail below. First, it is subjected to \( \beta \)-oxidation and extensive carbon recycling [15–21]. Second, it is converted into longer fatty acids via elongation and desaturation [19,22–27] (Fig. 2). For studies on ALA metabolism, the main focus is to firmly establish if it is converted in sufficient quantities to maintain adequate tissue levels of DHA. Although less importance has been given to the EPA or DPAn-3 accumulation as a consequence of ALA metabolism, there is a growing appreciation of how EPA, and to a lesser extent DPAn-3, are positively associated with health benefits, e.g. to improvements in cardiovascular diseases (CVD) [28] and impact cellular function via oxidation to the 3-series of prosta-glandins [29]. All together, the scientific evidence obtained in a variety of models has confirmed that ALA is significantly accumulated and converted to longer \( n \)-3 fatty acids [22,23,30–48]. In addition, these studies provide evidence indicating that the fatty acid accumulation is tissue-dependent [22], suggesting that the metabolism is based upon a tissue-selective need for longer \( n \)-3 fatty acids, such as DHA. In this review, these two metabolic fates of ALA will be discussed in detail as well as the downstream impact of \( n \)-3 fatty acids on health and disease. Additional aspects of ALA metabolism have been extensively reviewed by other colleagues and readers may want to consult these reviews for additional points of view [21,49–51]. Finally, the term essentiality and its exclusive applicability to LNA and ALA is reviewed by Cunnane [49].

2. Conversion of ALA into long chain \( n \)-3 PUFA

Because ALA is the precursor for DHA, it is considered as an adequate dietary source to provide and maintain the required levels of DHA in mammals. This statement is based upon several important points, yet is fraught with controversy. First, the minimal ALA requirement for developing rats is 2.4 g/kg diet (0.4% of energy), while for adult rats to maintain the maximal DHA level the requirement for ALA is 1.3 g/kg diet (0.26% of dietary energy) [52,53]. Similar values were obtained using [U-\( ^{13} \)C]ALA to study its elongation and the results showed that the minimal amount of ALA required to maintain adequate DHA levels in the developing non-human primate fetal brain is 0.45% of energy [26]. However, dietary DHA only needs to account for 0.03% of energy to maintain adequate brain DHA levels in non-human primate fetal brain [26]. This difference between the amounts of dietary ALA needed to support brain DHA levels as compared to preformed dietary DHA is an important, but often overstated point. It is important to understand that DHA is far more efficient in entering the brain phospholipid pools relative to ALA, but yet ALA can and is used to make brain DHA. Furthermore, note that the energy % required to maintain adequate brain DHA levels is not different between rats and non-human primates, suggesting that similar metabolism of ALA may occur in both rodents and non-human primates.

The need for more ALA to maintain adequate brain levels of DHA as compared to preformed DHA is due to the low rate of ALA

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**Table 1**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>g ALA/100 g material &lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Flaxseed</td>
<td><em>Linum usitatissimum</em></td>
<td>22.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td></td>
<td>53.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Perilla</td>
<td><em>Perilla frutescens</em></td>
<td>58.0</td>
</tr>
<tr>
<td>Chia seed</td>
<td><em>Salvia hispanica</em></td>
<td>17.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Camelina oil</td>
<td><em>Camelina sativa</em></td>
<td>38.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Canola oil or rapeseed oil</td>
<td><em>Brassica campestris</em></td>
<td>9.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soybean oil</td>
<td><em>Glycine max</em></td>
<td>6.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soybean green raw</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Walnuts</td>
<td><em>Juglans regia</em></td>
<td>9.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cloudberry</td>
<td><em>Rubus chamaemorus</em></td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blueberry</td>
<td><em>Vaccinium corymbosum</em></td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Longinberry or Cowberry</td>
<td><em>Vaccinium vitis-idaea</em></td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Seeds, oil, berries, etc.

<sup>b</sup> [302].

<sup>c</sup> [303].

<sup>d</sup> [304].
conversion to DHA, which has been estimated differently depending upon the study. For instance in non-human primates, ALA conversion to DHA is reported at 0.23% [24] and 0.57% [25] of the ALA dose, as determined using [U-13C]ALA. It is important to note that the primary tissue site at which elongation occurs was not determined in these studies, but it is suggested that the liver is the major site for conversion using stable and non-stable isotope methods [16,21,25]. Additional studies using a steady-state radiotracer kinetic model, indicates that about 0.2% of the ALA entering the brain undergoes conversion to DHA [16]. However, using this same model, another study reports that 1% of plasma ALA entering the brain is converted to DHA in rats fed either an n-3 adequate or deficient diet, indicating that the n-3 status of the brain is not critical for conversion [18]. This is important, as many investigators in the field have suggested that the dietary status of an animal might impact the ability of the brain or other tissues to convert ALA to DHA. Unlike rat brain, rat liver is capable of increasing the conversion of ALA to DHA during n-3 inadequacy [18], whereas the heart is not capable of this conversion [54]. Additional, in-depth discussion of these papers is found in following sections. However, it is important emphasize that again, the conversion found in non-human primates and rats is not tremendously different, that when combined with the other data, suggests rodent modeling is an important tool in assessing ALA metabolism.

2.1. β-Oxidation and carbon recycling

One major reason that ALA conversion is relatively poor is because of the high percentage of ALA directed toward β-oxidation, which is approximately 60–85% of ALA [15,20]. In the brain, about 67% of the ALA is directed toward β-oxidation [16], but for other fatty acids, such as ARA, this is only about 30% [55–57]. For plasma-derived erucic acid (22:1 n–9), which is found at trace levels in the brain, a similar percentage is directed toward β-oxidation as demonstrated for ALA [55]. However, if this fatty acid is infused directly into the brain ventricles, a much smaller percentage is subjected to β-oxidation. This suggests the ability of the brain to uniquely distinguish the metabolic fate of fatty acids coming in from the plasma as opposed to that derived directly from the cerebral spinal fluid. This notion is consistent with the very high degree of radioactivity present in the choroid plexus of rats infused with radiotracer fatty acids [58,59]. Nonetheless, this high percentage of ALA used for β-oxidation is not exclusive for ALA as other dietary derived PUFA are β-oxidized in similar percentages. For n-6 fatty acids, about 50% are used for β-oxidation and about 65% of DHA undergoes the same fate [20]. Thus, dietary ALA, similar to other dietary fatty acids, undergoes significant utilization by tissues for energy, e.g. heart and muscle, or it is recycled by tissues to be used as a carbon source for production of other fatty acids, amino acids, and sterols, e.g. brain, liver.

It is important to note that carbon recycling of ALA is quite efficient and that in developing rats this carbon is found enriched in brain saturated fatty acids and in cholesterol [19]. Similarly, in developing and adult non-human primates, carbon recycling from ALA also occurs [27], indicating that this process occurs across species. The rapidity by which carbon recycling occurs is best illustrated by ourselves and others, where fatty acid carbons are recycled and saturated fatty acids are formed within a 5–10 min time frame in both liver and brain [16–18,21,55,60]. This rapidity by which carbon recycling occurs is often grossly understated as well as misunderstood because it is generally difficult to view lipid metabolism in the terms of seconds and minutes, rather than hours.

2.2. Elongation and desaturation

ALA is converted to DHA through a series of chain elongation and desaturation processes (Fig. 1). First, ALA is desaturated to 18:4n–3 by Δ-6 desaturase, chain-elongated to 20:4n–3, and then converted to EPA by Δ-5-desaturase. EPA can be elongated by elongase-2 [7,8] to form 22:5n–3 and then to 24:5n–3 followed by a Δ-6 desaturation to form 24:6n–3 [61,62]. All of these reactions occur in the endoplasmic reticulum (ER). However, it is now readily accepted that 24:6n–3 is transferred to the peroxisome where it undergoes one round of β-oxidation forming DHA [61–63]. Any of the n-3 fatty acids synthesized in the ER are either incorporated into phospholipids or further chain-elongated/desaturated to form DHA. Unfortunately, while ample evidence demonstrates that this process occurs in vivo, very little is known about these processes and how they are regulated [63,64], but recent evidence suggest expression of elongase-2 (Elo-2) is critical to this process [7,8,54]. While elongase-5, which elongates 16–22 carbon fatty acids, is regulated by PPARα, elongase-2 is regulated by SREBP-1 [7,8,65]. The involvement of these two different genetic control mechanisms may account for the tissue-selective accumulation of DHA in ALA-fed rats [22]. In addition, this may also account for the lack of influence of dietary n-3 status on the conversion of ALA to DHA in brain [16,18], while the liver is very plastic to dietary modulation of this process [7,17].
Several studies in vivo and in vitro have indicated that \(n\)-3 and \(n\)-6 fatty acids utilize the same enzymes for their elongation and desaturation, thus in essence LNA and ALA levels can influence the metabolic outcome of each other. However, ALA appears to be a much stronger suppressor of \(n\)-6 fatty acid elongation and desaturation than LNA is of \(n\)-3 fatty acid elongation and desaturation. In fact, 10 times more LNA is required to have an equal effect on \(n\)-3 metabolism as ALA does on LNA elongation [3]. In a set of classic studies, the competition between these two fatty acids for the enzymes involved in elongation and desaturation in liver microsomes was demonstrated at several steps of the metabolic pathway [10,12]. For this reason and because mammals cannot interconvert \(n\)-3 and \(n\)-6 fatty acids, the dietary ALA to LNA ratio is an important issue for study and discussion [22,66,67]. In addition, fatty acid elongation and desaturation is subjected to feedback regulation because both ARA and DHA suppress endogenous conversion of LNA and ALA into longer chain fatty acids, respectively [68,69]. However, fatty acid modulation of these reactions appears to be tissue-selective [16,21,54,70]. Reductions in dietary \(n\)-3 fatty acid uptake results in an increased ability of the liver to elongate and desaturate \(n\)-3 fatty acids, whereas the brain is not responsive to dietary levels [16] and the heart does not have the capacity to elongate and desaturate ALA into longer chain \(n\)-3 fatty acids [54]. Thus, we know that \(n\)-3 and \(n\)-6 fatty acid metabolism regulation is more complex than a mere competition at the substrate level because this process is regulated at genetic level as well [6–8,23,39,50,65,71–74].

2.3. Evidence for ALA conversion: studies in cell cultures

The liver is considered the center of lipid metabolism and for this reason the first studies on \(n\)-3 and \(n\)-6 fatty acid metabolism were done using microsomes isolated from liver [10]. These studies demonstrate that liver microsomes contained the necessary enzymes for the elongation and desaturation of ALA to DHA. Incubating HepG2 cells (human hepatocellular liver carcinoma cells) with increasing concentrations of ALA (1.8–72 \(\mu\)M), there is a linear increase in ALA, EPA, and DPA \(n\)-3 levels with increasing ALA concentration [30]. In contrast to EPA and DPA \(n\)-3, the levels of DHA reached saturation at an ALA concentration of 18 \(\mu\)M [30]. What is perhaps more important is that despite the reduction in \(\Delta_6\) desaturase expression by PUFA (LNA and ALA included) there is a marked increase in accumulation of elongated and desaturated ALA and LNA in these cells [75]. This demonstrates the complexity of ALA conversion in HepG2 cells and that the perceived relationship between \(\Delta_6\) desaturase expression and desaturation of ALA and LNA may not be quite as simple as once thought. This leads to the important point that measures of gene expression
may not directly relate to enzyme activity and accumulation of products.

Equivalent results to those in Hep2G cells were obtained in cardiomyocytes [32,76], although results in intact rats suggest that the heart lacks the capacity to elongate ALA [54]. This is principally because the heart lacks elongase-2 expression [7,54], which appears to be absolutely essential for elongating 22:5n–3 to 24:5n–3, i.e. the first step of the Sprecher pathway (Fig. 2). Because these results in Hep2G cells and cardiomyocytes are saturable, it suggests a metabolic block in the step going from DPA–3 to DHA, yet also demonstrates that this process does occur at a rate that may be sufficient to produce DHA for export to other tissues. However, now an emerging use of kinetic analysis has shed new light on these processes and the importance of tissues in maintaining the type of long chain n–3 fatty acid in it.

Whether cells from the central nervous system have a similar capacity to elongate ALA as hepatocytes is a much more debated issue. The conversion of ALA into DHA in brain is particularly important because it is one of tissues with the highest DHA content, whereas brain levels of ALA, or of its metabolic intermediates, EPA and DPA–3, are very low [22,77]. In 1971, the first evidence that [1–14C]18:3–n3 crosses the blood–brain barrier was presented, suggesting the conversion of ALA to DHA in the CNS [78]. Five years later, the same group provided the first evidence that intracranially injected [1–14C]18:3–n3 is converted to DHA [79], demonstrating that like liver, the brain has the capacity to elongate and desaturate ALA. Astrocytes appear to be the major site of lipid metabolism in the brain, akin to the hepatocyte in the liver. Using [1–14C]18:3–n3 as a substrate, the capacity of astrocytes to convert it to ALA to DHA was observed [34–36]. However, a conflicting study suggests that astrocytes have limited capacity to produce DHA from ALA [80]. Although glia make up the majority of cells in the CNS, the question about the ability of neurons to elongate and desaturate ALA to form DHA is much more difficult to answer, in part, because of the experimental difficulty in culturing sufficient quantities of neurons.

One approach to circumvent this problem is to use neuroblastoma cells to model neurons. For instance, using the human neuroblastoma cell line SH-SY5Y, a model system for human neurons, ALA elongation was demonstrated by incubating these cells with 30 μM ALA for 24 h, after which EPA (+330%) and DPA–3 (430%) content in ethanolamine glycerophospholipids (EttnGpl) is increased, while DHA content increased only by 10% [37]. Another study in this cell line demonstrated that after 72 h of incubation, EPA, DPA–3, and DHA content is increased (780%, 850%, and 65% respectively) in EttnGpl and a similar change occurs in choline glycerophospholipids (ChoGpl) [81]. Again, both of these studies indicate a much slower conversion of DPA–3 to DHA, similar to what is seen in liver, yet there is little doubt that neuroblastoma cells elongate and desaturate ALA to longer chain n–3 fatty acids, including DHA.

However, do primary neurons have the ability to convert ALA to DHA? According to experiments done in rat primary cortical neurons, these are unable to elongate ALA to DHA, suggesting that the capacity of neurons to undertake these steps might be limited [34–36]. A recent study confirmed this point, but also demonstrated that primary rat hippocampal neurons were able to convert ALA to DHA [38], suggesting that the neuronal cell type might be an important contributing factor to the ability of neurons to elongate and desaturate ALA to DHA. This is further supported by the capacity of cerebellar granule cells to convert ALA to DHA [35]. Collectively, these studies indicate that it is more difficult for neurons to elongate and desaturate ALA all the way to DHA, suggesting that astrocytes are the major source of DHA for neurons [34–36,82].

These cell culture studies, which model a variety of tissues, including hepatic, cardiac and neural cells, indicate that these cells all have the capacity to synthesize longer chain n–3 fatty acids from ALA, including DHA in cells of liver and neural origin. In addition, these results suggest that compared with EPA and DPA–3, the production of DHA from ALA is metabolically limited at a step beyond DPA–3 formation. However, as discussed previously and in more depth below, these differences may, in part, be due to differential tissue expression of elongase-2, an enzyme required to convert 22:5n–3 to 24:5n–3 [78,54].

### 2.4. Evidence for ALA conversion: studies in animals

ALA conversion into its n–3 fatty acid derivatives has been studied in many different mammalian species including: rats [22,23,83–89], hamsters [41,42], guinea pigs [90], rabbits [91], piglets [71,92], baboons [25], monkeys [93] and cattle [39,40,94–98]. Through inference, the requirement for ALA and its conversion to DHA is supported by studies in which removal of ALA from animals’ diets for a prolonged period of time, including multigenerational models, results in profound n–3 inadequacy, including DHA [52,99–102]. Each of these studies demonstrate that ALA is generally rapidly converted into EPA and DPA–3, but that conversion into DHA is significantly less, although results on the latter aspect are more controversial. As discussed above, the impact of tissue specific elongase expression is beginning to offer a potential mechanistic explanation for these observations from animal studies. More importantly, accumulation of n–3 fatty acids depends on the tissue analyzed, leading to our hypothesis that ALA is converted in a tissue-dependent manner to longer chain n–3 fatty acids in a tissue-selective manner [22]. For instance, most of the studies show that, to a greater or lesser degree, rats fed an ALA-enriched diets (using canola, perilla, or flaxseed oil) have significantly increased ALA, EPA, DPA–3 content in plasma, liver, heart and DPA–3 and DHA in brain [22,23,85,86,88,90,103]. In contrast, hearts from cardiomyopathic hamsters fed a high ALA diet [41] and in ALA-fed hypercholesterolemic rabbits, only ALA and EPA content is increased [91], which is consistent with recent kinetic studies demonstrating that heart does not elongate [1–14C]18:3–n3 past EPA and DPA–3 [54]. The mechanisms accounting for this lack of conversion to DHA in heart appears to be due to the lack of elongase-2 expression [7,54], limiting the first step of the Sprecher pathway where 22:5n–3 is elongated to 24:5n–3 [61,62].

A similar mechanism may account for the limited muscle deposition of DHA in ALA-fed cattle. While these cattle accumulate ALA, EPA and DPA–3, there are limited increases in muscle DHA content [39,40,96,97,104,105]. However, it is important to note that in cattle, the bioremediation of ALA in the rumen limits the bioavailability of ALA [106,107], although this is not an issue in monogastric mammals such as rats and mice. In addition, we have demonstrated a significant increase in PPARγ expression in muscle of flax fed cattle, but not of PPARα [39], suggesting that the presence of ALA differentially influenced expression of these important modulators of expression of genes involved in lipid metabolism. This increase in PPARγ may influence ALA conversion to longer chain n–3 fatty acids in a muscle-specific manner in cattle as well as reduce the impact of late-stage type-2 diabetes in these cattle [108,109], a common issue in the US.

The accumulation of DHA in tissues from ALA-fed animals is somewhat more controversial, yet emerging evidence indicates that this is a tissue-selective process. While DHA accumulation occurs in hearts [90,103], hepatic membranes [85] and livers [83,90] of animals fed a high ALA-containing diet, we have not observed significant changes in liver or heart DHA levels in rats fed a 7% diet over an 8 week period [22], which is consistent with the recent observation that heart is not capable of elongating ALA to DHA [54]. Similar inconsistency in results for DHA accumulation in
brains of ALA-fed animals has also been observed between groups. So, while some of the studies observed DHA accumulation in brain [22,90,110], other studies did not detect any increase in DHA content [83,86]. Despite the heterogeneity of these models, all of the results converge to the same conclusion that ALA is converted to longer chain n–3 fatty acids, including DHA, but that the conversion of ALA to DHA is dependent not only on the type of tissue, but on the individual phospholipid class examined as well. Thus, while ALA and EPA and DPAn–3 are accumulated in plasma, liver and heart tissues, brain tends to accumulate DPAn–3 and DHA [22], suggesting a tissue-selective process.

The impact of the amount of dietary ALA on its own accumulation and conversion to longer chain n–3 fatty acids is an important issue. Unfortunately, there are limited studies addressing this point. In one study, hamsters were fed one of four doses of ALA over 5 weeks containing approximately 1, 10, 20 and 40 g ALA/100 g of total fatty acids, while LNA was kept constant [42]. In this study, the results clearly indicate that ALA content increases dramatically in epididymal adipose tissue and plasma cholesteryl ester (CE) fraction, but by a much lesser extent in red blood cells (RBC), heart, and plasma phospholipid fraction. It is worth mentioning that the brain and liver were not analyzed in this study. The increased ALA content in all of these lipid fractions was positively associated with increasing amounts of ALA in the diet, and therefore decreasing LNA/ALA ratio or n–6/n–3 ratio. While plasma phospholipid ALA and EPA content increased with increased ALA intake, DPAn–3 and DHA content did not change. Heart ALA content increased as did EPA, but no changes in DHA were observed, consistent with what we have seen in rats fed over an 8-week-period with a 7% ALA diet [22]. ALA storage in the epididymal adipose tissue is important as it represents a slow releasable pool of ALA that is utilized over time by other tissues [111]. Again, the importance of this study is that there is a linear increase in ALA and EPA in these hamsters, while that of DPAn–3 was not linear. Unfortunately, brain levels of n–3 fatty acids were not assessed in this model, which focused solely on assessing n–3 fatty acid accretion in adipose, heart, and vascular lipid pools.

Hence, the ratio LNA to ALA is increasingly being considered as one of the factors that influence ALA conversion into longer n–3 fatty acids. There have been a number of attempts to establish the optimal ratio in various animals in order to find the optimal ratio of LNA to ALA that leads to maximal tissue DHA accumulation [23,67,92]. By varying the LNA to ALA ratios from 0.5:1 to 10:1, there is an effect on plasma and brain DHA levels, but there is apparently some degree of complexity in this process [92]. The highest levels of DHA are not found in diets containing the highest ALA content, but rather maximal DHA accumulation occurs when the LNA to ALA ratio is within the range of 3:1–4:1. Interestingly, a similar optimal ratio was obtained by other groups [23,67]. Extending these studies to examine gene expression, the same non-linear effect of the LNA to ALA ratio is observed for brain gene expression in rats [23]. Collectively, these studies demonstrate that the ratio of LNA to ALA is critical for elongation and desaturation of ALA into longer chain fatty acids, a process that undoubtedly is controlled in part by gene expression, which is also subject to the same non-linear relationship to dietary ALA intake. These non-linear effects of dietary ALA levels on its conversion to longer chain n–3 fatty acids may account for the different observations for ALA conversion to DHA between various studies.

As demonstrated thus far, ALA-enriched diets are able to increase tissue n–3 fatty acid content, but can these diets reestablish the n–3 fatty acid content after a period of ALA deficiency? This is an important issue particularly during embryonic development because a deficient DHA supply during the pre- and early postnatal periods (depending on the mammal species) has serious physiological consequences. Thus, female monkeys receiving an ALA-poor diet from 2 months before conception and throughout the pregnancy had a 70–90% decrease in plasma, erythrocytes, and tissue n–3 fatty acid content in the offspring [93]. For the next 3 years, these offspring received a diet containing soybean oil (rich in LNA and to a lesser extent ALA). The recovery of tissue n–3 fatty acid levels was followed and all tissue recovered but at different rates, although the retina never fully recovered and had a content that was 84% of control levels. This lack of recovery of retinal DHA levels is associated with a lower functional response [93]. In rats depleted of ALA using a three generational depletion model, brain DHA levels are completely restored using dietary ALA within 8 weeks [112]. This includes complete repletion of plasmalogen DHA levels and the restoration of these levels by dietary ALA is comparable to that by dietary DHA. Again, this suggests that the brain has a unique plasticity with regards to conversion of ALA to DHA and supports the concept of a tissue-selective process for conversion of ALA to DHA.

### 2.5. Evidence for ALA conversion: studies in humans

Studies in humans face additional difficulties compared to studies in animals or in cell cultures, such as dietary heterogeneity, treatment duration, subject compliance, and the limited number of readily accessible tissues for analysis. Sampling is virtually limited to blood-derived cells, e.g. RBC, neutrophils or plasma compartments, e.g. lipoproteins, whole plasma. It is important to note that blood analysis does not reflect the potential elongation seen in tissue compartments such as brain [22], which is a critical issue. However, it may provide a good approximation of the changes that may take place in tissues like liver or heart. An additional difficulty that hampers the interpretation of outcomes and comparison of these outcomes to those from other studies is the high variability in the means by which ALA is provided, e.g. capsules containing oil, oil, or food containing high levels of ALA. In addition, the varied background diet of each subject is also difficult to unify, a problem that does not exist in animal studies. Regardless of background diets or dietary changes made during studies, high subject compliance during a study is always desired although in humans it might be difficult to certify. Despite all these limitations, results generally confirm what has been shown in cell and animals studies. That is, if humans receive a ALA-enriched source, there is an increase in ALA, EPA and DPAn–3 in plasma [113,114], in RBC [33,43–45,47,48], and in mononuclear cells [114,115]. More controversial and less consistent data are found in regards of the conversion and accumulation of DHA from dietary ALA [116].

As in animals, in humans there is a dose-dependent response to ALA-enriched diets. Thus, a treatment consisting of 30 ml/d (27 g/d) of flax oil (approximately 14 g/d ALA) for 4 weeks is sufficient to significantly increase ALA levels in serum triacylglycerols (TAG, 5.7-fold) and in CE (4-fold), while EPA levels are slightly increased, and no changes in DHA content are observed [117]. Similar results were obtained when patients with an atherogenic lipoprotein phenotype were treated with 15 g ALA/d for 12 weeks. The treatment led to an increase in ALA, EPA levels in plasma and RBC, although there were no changes in DHA [118]. A dietary intake of flaxseed oil (3 g ALA/d) for 12 weeks leads to an increase in plasma EPA levels (60%), DPAn–3 (25%), with no changes in DHA [44]. However, dietary ALA (35 mg/d for 3 months) did not alter plasma TAG and total cholesterol, but did result in higher EPA content in lipoprotein fatty acids [119]. However, this effect was only seen in subjects with a high PUFA/saturated fatty acid ratio, suggesting that this ratio may be an important factor in elongation and desaturation of ALA. After 4 weeks of consuming an ALA-enriched diet using flaxseed oil and a spread consisting of flaxseed oil and butter (2:1), ALA and EPA content in mononuclear phospholipids is increased [115]. Despite the fact that all of these studies agree on the fact
that humans can convert ALA into longer chain fatty acids, there are some concerns regarding the efficiency of ALA conversion [43,120], which in humans seems to be lower than in other mammals as baboons [43,120]. However, it is important to stress that if our hypothesis is correct, this is a difficult assessment as it does not address ALA conversion at the levels of tissue, but rather relies merely on analysis of blood components, leaving tissues in which ALA conversion is demonstrated in animal models unanalyzed. Hence, the point regarding conversion in humans of ALA to longer chain n–3 fatty acids is still subject to significant debate [121,122].

3. A kinetic perspective on ALA conversion to DHA

The most elusive, yet perhaps the most important concept, in the field of n–3 fatty acid metabolism is whether ALA is efficiently converted to DHA and in which tissue(s) does this occur in mammals. In previous sections, we examined several key points. First, that if ALA is converted, it is done in a tissue-selective manner. This is based upon our own studies as well as those by others, demonstrating an accretion of DHA in brains in ALA-fed rats combined with studies showing a tissue-selective expression of desaturases and elongases. Expression of both of these classes of enzymes is equally important for the formation of DHA from ALA. Second, that despite all of the controversy, rodents are a reasonable model for higher mammals with regards to the study of ALA conversion. Nonetheless, ALA-deficient diets have been traditionally used to reduce brain DHA, despite the fact that ALA, as other fatty acids, undergoes β-oxidation and that the metabolic rates in rodents for many processes are considerably faster than in humans, fact that points to the relevance of these models to model n–3 deprivation in humans. Whether or not true n–3 deprivation occurs in humans to this same extent is questionable. In the end, the major questions plaguing the field remains, how much of the body’s DHA comes from dietary ALA? In other words, can a terrestrial animal (humans) that is an omnivore truly requires dietary DHA in order to have optimal physiological performance despite the true rarity of DHA in the world’s food web, but a web where ALA exists in abundance?

Previous studies in humans, rodents, and baboons have focused on using stable isotope methods to address the question of ALA conversion to DHA [24–26,113,120,123–127]. While in rodents and non-human primates these studies permit post-mortem analysis of tissues such as brain, this analysis is precluded in humans. This makes non-human primate studies particularly important and these studies do tell us a number of key points about ALA metabolism. First, that [13C]ALA is not accumulated to nearly the same extent as tissue labeled [13C]DHA as is dietary [13C]DHA [24]. Second, as such, less dietary DHA is required to increase tissue DHA levels as compared to dietary ALA [26]. This study also demonstrates that ALA only needs to comprise 0.45% energy of the diet to meet the requirements of the growing fetal brain. Third, that 0.6% of ALA (10:1 LNA to ALA) is elongated [25], indicating that consumption of the recommended daily dose of ALA (1200 mg/d) would account for nearly 7 mg/d of DHA in the fetal brain. What is important to note about this study is that the accumulation of ALA-derived DHA in the brain was constant after 3 d post-injection, however earlier time points were not measured. This is problematic as the liver and brain immediately metabolize fatty acids [55,60], suggesting that ALA is rapidly converted by liver and brain to longer chain n–3 fatty acids, including DHA (see below). Hence, this study in fetal baboons is limited in that it has a small sample size and lacks early measurements of ALA and its products in tissues. Despite the above limitations, these three studies from the same laboratory demonstrate the conversion of [13C]ALA to [13C]DHA in non-human primate brains, with the percentage of the dose converted to DHA ranging from 0.23% to 0.57% [24–26,127].

In rat pups, a recent study demonstrate that d5-ALA is converted into d5-DHA and found in all of the organ systems assayed [123]. More importantly, this study demonstrates that inclusion of dietary DHA dramatically reduces the accumulation of d5-DHA, including a 40% reduction in brain and in liver. This indicates a profound down-regulation of ALA to DHA conversion in the presence of dietary DHA. Although tissue DHA levels are increased in the group fed dietary unlabeled DHA as compared to the dietary ALA-only groups, this is not unexpected based upon the literature (see previous sections). Nonetheless, these studies collectively demonstrate that elongation and desaturation of labeled ALA occurs in vivo, however they do not take into account the rapidity of fatty acid metabolism in vivo nor do these studies address the tissue compartment accounting for the majority of this metabolism.

Human studies using stable isotopes have also been done. Pawlosky and colleagues developed a model using dietary d-5-ALA to measure the kinetics of longer chain n–3 fatty acids formation in human plasma [120]. Only 0.2% of the d-5 mass is found as plasma d-5-EPA, thereby the authors assume that ALA is only converted in the liver and that ALA entry into other tissue compartments and its subsequent elongation and desaturation to longer chain n–3 fatty acid is not considered. This suggests, as Pawlosky and colleagues concluded, that there is a flow of d5-ALA through the elongation and desaturation pathway to the level of d5-EPA, but that there is a restriction in the conversion of d5-EPA to d5-DPAn–3, accounting for the reduced formation of d5-DPAn–3 and d5-DHA. In another study, this same group concluded that less than 1% of dietary ALA is used to form longer chain n–3 fatty acids [125], but that inclusion of DHA in the diet reduces this conversion, similar to what is reported in rats [123]. This is important as it has long been thought that the ALA to LNA ratio is an important factor in the conversion of ALA. However, the impact of the ALA to LNA ratio on formation of longer chain n–3 fatty acids from [13C]ALA demonstrates that in humans there is minimal effect of this ratio on ALA conversion [113]. Similar to what Pawlosky and colleagues reported, less than 0.1% of the dietary ALA is converted to DHA, but interestingly increased ALA mass results in less EPA, but more DHA formation. These authors conclude that the ALA is a viable alternative source of long chain n–3 fatty acids in the absence of the ability to consume dietary DHA [113].

Apparently the smoking of tobacco increases the conversion of ALA to DHA in both men and women [126] and chronic alcohol consumption increases the utilization of ALA for formation of longer chain n–3 fatty acids [124]. Again, using the well documented and validated model system, Pawlosky and colleagues demonstrate a precursor to product relationship and the addition of liver biopsies reveals that there is an increased conversion of EPA to DHA in the livers of chronic alcohol consumers [124]. Collectively, these studies demonstrate the kinetics of the appearance and disappearance of stable isotope labeled ALA or longer chain n–3 fatty acids, but are limited in that only the plasma compartment is analyzed and the assumption by the authors that ALA is only converted to EPA, DPAn–3, and DHA in the liver. The obvious limitation of access to tissue compartments in human studies is certainly a major problem that is overcome using rodent and non-human primate models. Although stable isotopes offer the ability to use mass spectrometry to analyze the deposition of fatty acids and their metabolites into lipid pools, unstable isotopes offer an additional means to address the question of ALA conversion to DHA. As previously noted, we have clearly shown that dietary ALA (7%) increases brain DHA levels, but not heart and liver DHA levels, suggesting the ability of the brain to elongate ALA and accumulate DHA over a period
of time [22]. This is not without precedence as fetal rat brain elongates and desaturates ALA to DPA–n and DHA in vivo [128]. The elongation and desaturation of ALA is similar to that of LNA, but the authors conclude that in the fetal system the brain itself, not the liver, accounts for this increase. Repletion of brain DHA in n–3 deprived rats further substantiates the capacity of dietary ALA to restore brain DHA levels [112]. Thus, there is a disconnect between the studies using stable isotopes and long term dietary studies showing elevation in brain DHA levels in ALA-fed rats.

Perhaps the most significant advances in the field have arisen from the work over the last several years from the Rapoport laboratory. This group has applied its well-validated steady-state radiotracer modeling to address several important questions. First, does DHA in the diet appreciably alter the conversion of ALA to DHA? Second, does n–3 deprivation appreciably alter this conversion? Third, in what organ system(s) does this elongation occur? Fourth, is it possible for the body to only utilize ALA to meet the DHA needs of the body? These questions are each addressed below.

First, does DHA in the diet appreciably alter the conversion of ALA to DHA? Stable isotope studies in humans and rats demonstrate that inclusion of dietary DHA results in depressed conversion of ALA to DHA [123,125]. In rats fed a diet enriched in DHA (2.3%), the kinetics of converting [1-14C]ALA to longer chain n–3 fatty acids was determined following a 5 min infusion (i.v.) period [16]. Under these conditions, no radioactive long chain n–3 fatty acids are found in the plasma, indicating that any DHA found in the brain is from plasma-derived [1-14C]ALA. In these rats, 86% of the [1-14C]ALA entering the brain is β-oxidized, while about 0.3% of the ALA coming into the brain is found as [1-14C]DHA and the half-life of the [1-14C]ALA is 1.3 h. This short half-life coupled with a dilution coefficient, λ, of 0.77 indicates that the majority (77%) of the ALA is coming into the brain from the plasma, but that it is rapidlyturning over and converted into other products. This is further substantiated by the presence of EPA-CoA (0.2% of the tracer), DPA–3-CoA (0.2% of the tracer), and DHA-CoA (0.3% of the tracer) in these brains, indicating the elongation and desaturation in the presence of a high DHA-containing diet. In liver, [1-14C]ALA is rapidly converted to DHA at a rate of 15.8 nmol g–1 s–1 over the same 5 min infusion period [70]. The majority of the tracer (52%) is found in triacylglycerol (TAG), presumably for export via the VLDL pathway. In liver, saturated fatty acyl-CoA are present as well as radioactive cholesterol, indicating efficient, rapid carbon recycling in the liver, similar to what has been observed by our group with erucic acid [60]. However, a block in the conversion of 1[14C]22:5n–3 to 1[14C]24:5n–3 is also seen in this study, demonstrating that the Elo2 step is perhaps the rate-limiting step in the conversion ALA to DHA.

Second, does n–3 deprivation appreciably alter this conversion? Again, the Rapoport laboratory addresses this question by feeding rats for 15 weeks either a 0.2% ALA-containing diet or a 4.6% ALA-containing diet [17,18]. The conversion of [1-14C]ALA by liver and brain was examined after a 5 min infusion period using the tissue from the same rats. In brain, [1-14C]ALA undergoes extensive β-oxidation (77%), but about 0.2% of the tracer is found as [1-14C]DHA in either group and the rate of conversion to DHA is the same between groups [18]. Although the incorporation rate into DHA did not change, in deprived groups there is a profound and significant reduction in the individual n–3 acyl-CoA mass (50–80%), with a corresponding increase in individual n–6 acyl-CoA mass (1.3–to 3-fold). This change may influence the trafficking and conversion of ALA, although this point is not discussed by the authors. Hence, the dietary status of the rats does not impact the conversion of ALA to DHA in the brain.

In contrast, in the liver there is a large 2.7-fold increase in the secretion rate of [1-14C]DHA from [1-14C]ALA in the n–3 adequate rats as compared to the deprived rats [17]. Again, the tracer and its products are predominantly targeted to TAG, although this targeting is similar between n–3 deprived and n–3 adequate rats. Unlike in brain, significantly less [1-14C]ALA is subjected to β-oxidation, (about 30%), although this estimate may be low due to the much higher levels of radioactivity found in liver saturated fatty acids and cholesterol. Again, this demonstrates the rapidity by which carbons are recycled in the liver. However, the rates of [1-14C]DHA synthesis from [1-14C]ALA are increased 7– to 8-fold depending upon the lipid compartment in the inadequate n–3 dietary groups (0.2% ALA), clearly demonstrating that in liver n–3 deprivation causes the liver to up regulate its capacity for the conversion of ALA to DHA, while the brain has a much more limited capacity to respond to altered dietary conditions.

Third, in what organ system(s) does this elongation occur? Clearly the plasticity of the liver to respond to dietary deprivation suggests that it is the major organ system in which conversion of dietary ALA to DHA occurs. Brenna and colleagues hypothesized this point in their stable isotope studies in baboons [25]. However, the 6– to 10-fold greater rates of ALA conversion in liver as compared to brain is indicative of liver’s central role in providing DHA from dietary ALA [17] as well as longer chain n–3 intermediates such as EPA and DPA–3. The 1.4-fold increase in the conversion of ALA to DHA by liver under low n–3 fatty acid dietary conditions and the lack of altered changes by the brain under similar conditions, suggests that indeed the liver is the major source of DHA produced endogenously through the conversion of ALA [21]. This is further supported by evidence that the heart has no capacity to convert plasma-derived ALA to DHA due to the lack of Elo2, the apparent rate-limiting step in the conversion of ALA to DHA [54]. However, this evidence also supports the concept of a tissue-selective process by which ALA is elongated and desaturated at the individual tissue level following entry of plasma-derived dietary ALA into the tissue. As such, the brain can and does convert ALA to DHA. However, the primary organ system for ALA conversion to longer chain n–3 fatty acids is the liver.

Fourth, is it possible for the body to utilize only ALA to meet the DHA needs of the body? If the brain converts 0.2–0.3% of the plasma-derived ALA to DHA within 5 min, not counting the nearly 1% of the tracer located in either EPA and DPA–3 pools either as free acid or CoA thioesters, a 1200 mg per day consumption of ALA by a human would result in the brain itself producing 2.4–3.6 mg of DHA per day. If the various intermediates are taken into account, as the brain contains very little EPA, this might be up to 5–8 mg of DHA per day produced by the brain. This is important as the human adult brain is reported to use only 4.6 mg of DHA per day [21,129]. In contrast, the human brain requires 17.8 mg of ARA per day [21], which is significantly greater than its requirement for DHA. In addition, the half-life of DHA in the human brain is 773 d as estimated using positron emission tomography (PET) to assess brain DHA uptake parameters [21,129]. Again, this is in stark contrast to ARA which has an estimated half-life of 147 d using the same technique [21]. Thus, the low daily requirement of the human brain for DHA and the capacity of the brain to retain DHA as demonstrated by its long half-life, suggests that the 2.4–3.6 mg of DHA produced by the rodent [16] and non-human primate [25,127] brain is indicative of the brain’s capacity to make an adequate supply of DHA in the presence of sufficient dietary ALA (1200 mg of ALA per day).

That said, the liver itself will also more than adequately convert ALA to DHA at a rate that is 6–10 times that of the brain, indicating that the liver is the primary source of non-dietary DHA in mammals. Recent whole-body synthesis experiments using [U-13C]ALA further substantiate this point as the total daily synthesis of longer chain n–3 fatty acids is 8.4 μmol of EPA per day, 6.3 μmol of DPA–3 per day, and 9.8 μmol of DHA per day [130]. This production of DHA is greater than 30 times the required needs of the
brain, again indicating that the liver can provide more than adequate DHA to meet the needs of the brain and the other organ systems for DHA. In summary, the work by Rapoport and colleagues agrees with many of the earlier studies using stable isotope methods and demonstrates that the liver is the primary tissue in which dietary ALA is converted to DHA. The rapid conversion and packaging into TAG destined for export by the liver as well as its plasticity to increase the conversion of ALA to DHA under low n-3 conditions, indicates that this organ system is fully capable of providing ample DHA when adequate ALA is consumed. While the brain is not the major source of DHA, it has the capacity to nearly meet its daily needs for DHA through the conversion of plasma-derived ALA. This coupled with the long half-life for DHA in human brain provides the brain a degree of protection when liver function may be limited or when n-3 deprivation is on-going.

4. ALA health benefits

Chronologically, the studies on the health benefits of DHA alone or together with EPA preceded the interest in the potential beneficial effects of ALA. Recently, there has been an increased interest in this topic as we expand our understanding of ALA metabolism. As is the case of DHA and EPA, it is still not clear the mechanism by which ALA may exert its beneficial effects. First, ALA could be beneficial by simply acting as the precursor of EPA and DHA. As demonstrated in previous sections, an increase of ALA consumption elevates tissue ALA, EPA and DPA-n-3 content and, in some cases, DHA content. Second, because ALA competes for the same metabolic enzymes as does LNA, ALA consumption may be a good strategy to decrease elongation of n-6 fatty acids leading to reduced ARA content (Fig. 2). The n-6 family fatty acids are present at very high levels in the so called Western diet [131], which is thought to promote an unhealthy balance between the n-3 and n-6 fatty acid families. Third, ALA may produce a beneficial by its direct interaction with ion channels [132] or nuclear receptors such as PPAR or RXR [133]. Thus, similar to EPA and DHA, ALA may have numerous beneficial effects to promote human health.

4.1. Preterm and neonates

DHA is the most abundant PUFA in the central nervous system, being particularly concentrated in synaptic plasma membranes [134–136] and in photoreceptor cells [137]. For this reason, it is critical that the correct acquisition of n-3 and n-6 fatty acids occurs during embryogenesis and early postnatal stages of development. In mammals, the period for ARA and DHA accumulation differs depending on the species. Thus, while rats accumulate DHA during the embryonic period and the first three postnatal weeks of life [9], in humans this process takes place during the last trimester and first 6–10 months after birth [138,139]. The rapid accumulation of DHA is directly related to its crucial need for normal neurological and visual development [140–143]. While the exact role of DHA is unknown, it modulates important membrane functions such as ion or solute transport, receptor activity, and adenylate cyclase activity [77,144–147].

The accretion of long chain PUFA during the intrauterine period occurs from maternal sources and postnatal sources are either through maternal milk or artificial formula. Maternal breast milk fatty acid composition varies according to the diet [148–151], but on average contains DHA (0.3–0.6%), ARA (0.4–0.7%), LNA (8–17%) and ALA (0.5–1%) [152,153]. All of these fatty acids are well absorbed and readily used by infants. However, the supply of long chain PUFA may be hampered in certain situations as in preterm and formula-fed infants. Thus, infants born prematurely miss the period of peak accumulation of ARA and DHA from maternal sources during the last trimester of pregnancy [138,139,154]. Because infant formulas provide LNA and ALA in contrast to breast milk, this led to the inclusion of DHA and ARA in a number of newer infant formulas. Several studies have demonstrated that the lack of DHA in infant formula leads to lower DHA levels in infants brain [155,156], RBC and plasma [157,158].

Several reasons may explain why lower DHA levels are found in these infants. First, the enzymes for the synthesis of long chain PUFA derivatives may be low in activity at birth [157,158]. A second possibility is that, despite the fact that ARA and DHA formation occurs during first days of life, including in very immature preterm neonates [159], it is possible that these fatty acids are not fully available to growing neural tissues [138]. However, regardless of the origin of this n-3 fatty acid deficiency, large reductions in brain and retinal DHA proportions are associated with impaired cognitive and visual function across species including: primates [160,161], rats [162], guinea pigs [163], and infants [140,164–167]. As previously discussed, n-3 deficiency is associated with biochemical changes in brain and retina including decreased DHA and increased DPA-n-6 content [134,142,168,169], altered enzymatic activities [53,101,170], depressed learning ability [53,171,172], and disturbed behavior and water intake [173,174]. In n-3 deficient rhesus monkeys, a diet containing ALA as the only n-3 fatty acid restores plasma, RBC, and brain n-3 levels, but retinal DHA content is not fully restored, more than likely contributing to the incomplete recovery of visual acuity caused by the n-3 restriction [93]. This point is important in that it demonstrates two very important concepts. First, that ALA is more than adequate to restore DHA levels in a young primate. Second, that the visual system may have a special need for DHA itself and that dietary ALA may not be converted in the eye to form tissue-derived DHA.

Because of the importance of DHA in development, there has been a great effort to establish the adequate fatty acid composition for infant formula. This issue is critical because the brain’s fatty acid composition is very sensitive to changes in the diet [23,134,168]. However the impact of the diet on the fatty acid composition will also depend on the developmental stage of the embryo and on the brain cell type. Hence, glia and neurons responded differently to changes in diet, as glial fatty acid composition is more affected than is neuronal fatty acid composition [175]. The reason for this difference might be that neurogenesis is almost complete at prenatal stage [176], while gliogenesis and myelination occurs after birth [177,178]. Therefore, the composition of the infant formula has to fulfill two requirements to provide enough DHA, but also ARA to the infant since both are critical for brain and nervous system development. The first strategy taken was to include ALA and LNA in the infant formula. In fact, brain conversion of ALA into DHA was shown in developing rodent brain [128,179] and preterm and appropriate-for-gestational-age infants [159]. Therefore, although it is clear that neonatal brain is able to elongate ALA into DHA, it has been a controversial and highly debated issue whether the efficiency of this process is sufficient to fulfill the high requirements existing during brain and central nervous system development.

To address this question, several studies were done in animal models to evaluate the inclusion of ALA into neonate formulas. In newborn rats receiving ALA (0.12 g/100 ml milk) or ALA + DHA (0.12 + 0.24 g/100 ml milk respectively), rats in the ALA group did not reach dam-reared brain DHA levels. Although the DHA content was 5.5% of total fatty acids in the ALA group, it was less than the 6.7% that was found in the ALA + DHA and dam-reared group. Nevertheless, this study confirmed that dietary ALA was elongated to DHA in the newborn pups [180]. Similar conclusions were made after examining glial cells isolated from 2 week old rat pups fed a DHA-containing diet where DHA is more effective in increasing
DHA levels in EtnGpl and in PtdSer of glial cells than those isolated from pups given a diet containing low or high levels of ALA [181]. In another experiment examining various LNA to ALA ratios (as low as 1–12), brain DHA levels in ALA-fed pups failed to equal DHA levels in dam-reared rats [182].

However, rats may be not the best model to mimic the situation in newborn humans. A more suitable model may be piglets, because the timing of perinatal brain growth spurt [183] and brain and milk lipid fatty acids [184] are similar to that found in humans. Sow’s milk fatty acid composition is very sensitive to dietary change, such that a sow receiving a high ALA diet for 10 d before parturition and for 14 d postpartum has increased milk ALA (141%) and DHA (86%) content. In piglets from these sows weaned 14 d after birth, liver DHA and ALA were increased 333% and 54% respectively, while brain DHA content was increased 24% [71]. In a similar study, piglets receiving a high ALA formula (3.9% of total fatty acids, LNA to ALA ratio 4:1) for 15 d after birth, have an adequate deposition of DHA in piglet retina and synaptic plasma membrane EtnGpl compared to sow-milk-fed piglets [185]. Collectively, these studies demonstrate that dietary ALA can support the synthesis and deposition of adequate DHA levels in piglets. However, dietary ALA content and brain DHA accumulation present a rather complex relationship and very high ALA dietary content does not imply a corresponding high increase in brain DHA. In fact, LNA to ALA ratios above 4 to 1 lead to smaller increases in piglet brain DHA content [92], suggesting that to obtain an adequate brain level ratios above 4 to 1 lead to smaller increases in piglet brain DHA content [92], suggesting that to obtain an adequate brain level of DHA at high LNA to ALA ratios, it may be necessary to resort to DHA-enriched sources [92].

Finally, studies in infants show that plasma and RBC DHA levels are related to breast milk DHA levels in a curvilinear manner, releasing a plateau when breast milk contains about 0.8% of total fatty acid as DHA [186]. Results obtained in animal models regarding ALA conversion into DHA were confirmed in infants. In 3-week-old infants receiving infant formula containing a fixed percentage of LNA (16%) and increasing amounts of ALA (receiving 0.4%, 1.0%, or 3.2% of total fatty acids), ARA and DHA were synthesized [187]. It is estimated that increasing ALA consumption from 0.4% to 3.2% of total fat results in roughly a 2.5-fold greater rate of incorporation of DHA into the plasma phospholipid fraction [187]. Moreover, the capability of neonates to synthesize DHA from ALA has been confirmed in additional studies [188,189]. Similar to animal models, the optimal LNA to ALA ratio for human infants appear to be within the range of 3:1–4:1 [45,190,191]. In fact, studies suggest that ratios below 4:1 would have little further impact on DHA levels. Nonetheless, DHA levels in ALA-containing formula-fed infants do not achieve the proportions of plasma DHA seen in breast-fed infants or the proportions achieved with DHA supplements [190,191]. Additionally, there is the risk of reducing plasma ARA levels if very high ALA levels are included, which is considered an undesirable outcome [190,192].

All together, the evidence provided by studies in human infants indicate that despite the fact that the ALA-supplemented infant formula contribute efficiently to the maintenance of the n−3 status in premature newborns, they have a modest impact on DHA levels and that these levels do not reach those observed in breastfed infants [190,192]. Because many traditional infant formulas do not contain ARA or DHA [192,193], the addition of ARA and DHA into infant formulas has been recommended [194–197]. For this reason, many manufacturers around the world now include ARA and DHA in infant formulae [92].

### 4.2. Cardiovascular disease

Cardiovascular disease (CVD) in general and coronary heart disease (CHD) in particular, is the leading cause of death in industrialized countries. The numbers of factors contributing to this disease are numerous and among them, diet plays a critical role. For this reason, health policy authorities have issued different programs trying to change the detrimental dietary habits of at risk populations. Current recommendations are to increase n−3 fatty acid consumption, in particular EPA and DHA (Table 1) [198,199]. The relationship between dietary n−3 fatty acids, particularly EPA and DHA, and risk of developing CVD began to emerge in the late 1970s [200,201]. Since then, most of the attention has been focused on the effects of these two fatty acids and it is now widely accepted that EPA and DHA have beneficial effects on CVD [202–209]. Some of the potential mechanisms for the cardioprotective effect of n−3 fatty acids include antiarrhythmic, anti-inflammatory, hypotensive, and hypotriglyceridemic effects [210]. Nonetheless, it is still not clear if all n−3 fatty acids might have beneficial effects in CVD or if the benefit is exclusively related to EPA and DHA.

#### 4.2.1. Effect of dietary ALA on plasma lipid levels

There is considerable interest in understanding how different dietary lipids influence the concentration of lipoprotein in plasma because the risk of developing atherosclerosis and CHD is strongly correlated with LDL concentration in plasma [86]. So far, studies using relatively high doses of fish oil indicated that the most consistent effect on plasma markers of CVD risk is a decrease in triacylglycerol (TAG) levels [210,211]. This effect is dose-dependent and is influenced by the baseline plasma TAG level of the subjects [210]. In addition, the modest decreases in TAG levels are frequently accompanied by increases in LDL-cholesterol (LDL-C) levels. Additionally, there is some evidence that n−3 fatty acids may act in a similar manner to fibrate drugs, which are known to increase the conversion rate of VLDL to LDL [212]. Although, the link between these effects and circulating levels of n−3 fatty acids is still not clear, it seems that the latter event plays an important role.

As we have previously delineated, both fish and vegetable oil consumption increases EPA and DPA to −3 content in serum, platelet, and RBC. Hence, there has been a great interest to investigate whether ALA-enriched sources would have similar effects to DHA-enriched sources on CVD. However, results of these studies are not as consistent as studies with fish oil diets. Thus, although TAG and CE fatty acid composition is clearly affected by ALA-enriched sources on CVD. However, results of these studies are not as consistent as studies with fish oil diets. Thus, although TAG and CE fatty acid composition is clearly affected by ALA-enriched sources on CVD, it is still not clear if all ALA-enriched sources have the same effects on plasma lipid levels. In rats fed perilla oil for 4 weeks, hepatic TAG and total cholesterol levels were reduced to the levels observed in the rat group fed fish oil group [85]. Moreover, plasma TAG levels in the perilla oil group were also reduced although not to the same extent as in the fish oil group. Interestingly, the effects on plasma total cholesterol and HDL-cholesterol (HDL-C) were identical in both fish oil and perilla oil groups, which is important because increased HDL levels are considered cardioprotective. All these changes were accompanied by an increase in n−3 fatty acid content in hepatic microsomal membranes [85]. However, other studies demonstrate a more moderate effect on plasma TAG, cholesterol, or lipoprotein levels when using ALA-enriched diets [86,117].

Studies in humans demonstrate that ALA is rapidly incorporated into lipoproteins within 3 h after ALA consumption [213,214] and consequently plasma ALA, EPA and DPA concentrations are elevated after consumption of ALA-enriched sources. Even so, no changes in TAG, HDL-C and LDL-C were observed in either high (3 g/d) [215] or in lower doses trials (35 mg/d) [119]. In a randomized, double-blind trial, 56 participants were given 3 g ALA/d from flaxseed oil capsules or olive oil placebo capsules. Within 12 weeks, EPA and DPA plasma levels increased 60% and 25% respectively, but no changes were observed in TAG, HDL-C and LDL-C concentrations or LDL, HDL or IDL particle size [215]. Similar
effects on lipoproteins were observed when the daily dietary supplement contained only 35 mg of ALA [119]. Finally, healthy subjects consuming a low-fat ALA-enriched diet, showed decreased LDL-C levels and increased ALA content in CE from LDL, but no additional changes were observed in other plasma markers [214]. Some authors suggest that the reason for these limited effects may reflect the limited capacity of humans to elongate and desaturate ALA [199], but this supposition is not supported by growing data demonstrating accretion of DPA–3 in subjects receiving as little as 2.4 g/d of flax oil [33]. Interestingly, a recent study evaluated individually the different metabolic effects of isolated dietary ALA, EPA and DHA in normallipidemic nonobese volunteers [213]. In this study, the volunteers received for 6 weeks margarines containing 4.4 g/d of ALA, 2.2 g/d of EPA or 2.3 g/d of DHA in the form of fatty acid ethyl esters. The results showed not only an increase in the content of ALA and EPA in LDL, but also a reduction in fasting serum TAG in the ALA group. Importantly, as the authors pointed out, this beneficial effect was observed after consumption of a relatively low dose of 4.4 g of ALA per day, a dose that is perfectly achievable without dietary supplements, but through the regular consumption of ALA-rich sources (Table 2) [213].

4.2.2. ALA antiarrhythmic properties

The antiarrhythmic properties of fish oils were first shown in rats [88,216], non-human primates [217] and dogs [218]. Interestingly, in the latter study ALA presented highly cardioprotective properties as well [218], but this important point is often overlooked. However, this effect has been recently confirmed in a study where the effects of ALA and DHA on heart rate were compared [88]. Thus, the study clearly shows that although the ALA-rich diet has a delayed effect compared to the DHA group, the heart rate in the ALA group is decreased to the same value as in the DHA group [88]. Despite these results, the antiarrhythmic properties of ALA have been studied less when compared to studies using EPA and DHA [91,103,218]. The mechanism for the antiarrhythmic effects probably involves the modification ion channels currents by the incorporation of these polyunsaturated fatty acids into the cardiomyocytes membrane phospholipid [219–221]. Among all ion channels, Kv1.5 channels are considered as good pharmacological targets for antiarrhythmic drugs because they are atria-specific [222]. It is important to note that along with EPA and DHA, ALA is also able to block the atrium-specific Kv1.5 channels at physiological concentrations [223], suggesting that ALA might share the antiarrhythmic properties of marine n–3 PUFA [132], supporting earlier reports [218]. Unfortunately, a study in humans has not confirmed antiarrhythmic effects of ALA on heart rate variability [224].

The beneficial effects of ALA-enriched diets on preventing CVD are less consistent although the number of studies dealing with this topic is lower than with EPA and DHA-enriched diets. Nonetheless, there are a number of clinical trials and systematic reviews which ascribe beneficial effects to ALA diets because they lower the risk of myocardial infarction and fatal ischemic heart disease in women and in men [203,207,225,226]. In addition, the accumulation of ALA in adipose tissue was positively associated with heart rate variability in women suspected to suffer from coronary artery disease, with a stronger association in women who smoked [224]. The favorable reduction in heart rate variability, which is an electrophysiological predictor of arrhythmia, leaves these women less likely to develop ventricular arrhythmias. These results showing the antiarrhythmic effect associated with ALA consumption [224,227] are corroborated by prospective cohort studies which provided evidence that dietary ALA is beneficial to CVD [205,206,208]. However, a recent meta-analysis concluded that increased ALA intake produces modest, if any, cardioprotective changes [228]. In addition, more recent prospective and cross-sectional studies conclude that there is either no benefit of dietary ALA, or that dietary ALA is associated with less carotid and coronary atherosclerosis [229,230]. Finally, some case-control studies have also been inconclusive with respect to the relationship between ALA and CVD [231,232]. Thus, the impact of ALA consumption on CVD are not supportive of any claims towards the dietary consumption producing positive effects on CVD, indicating that additional studies are needed to clarify this potential.

4.2.3. ALA anti-inflammatory properties

Inflammation is the cell physiological response to exposure to certain substances released, predominantly, by activated leukocytes. This response usually consists in the reddening and swelling of the targeted area and it is mediated by at least two different groups of biomolecules, the n–6 eicosanoids and the cytokines. The n–6 eicosanoids are biosynthetically derived from ARA, via cyclooxygenases (COX1 and COX2) or lipoxygenases, and within this

### Table 2

Most recent dietary recommendations on n–3 fatty acid consumption issued by different organizations.\(^a\)

<table>
<thead>
<tr>
<th>Organization</th>
<th>Date</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eurodiet Conference, University of Crete</td>
<td>2000*</td>
<td>200 mg n–3 fatty long chain PUFA/d</td>
</tr>
<tr>
<td>France: AFFSA/CNERSA and CNRS</td>
<td>2001*</td>
<td>500 mg n–3 fatty long chain PUFA/d; DHA 120 mg minimum</td>
</tr>
<tr>
<td>American Heart Association</td>
<td>2002*</td>
<td>Patients without documented coronary heart disease (CHD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eat a variety of (preferably fatty) fish at least twice a week. Include oils and</td>
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<tr>
<td></td>
<td></td>
<td>foods rich in alpha-linolenic acid (flaxseed, canola and soybean oils;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>flaxseed and walnuts)</td>
</tr>
<tr>
<td>UK Scientific Advisory Committee</td>
<td>2004*</td>
<td>Patients with documented CHD</td>
</tr>
<tr>
<td>Australia and New Zealand Government Recommendation</td>
<td>2005*</td>
<td>Consume about 1 g of EPA + DHA per day, preferably from fatty fish. EPA + DHA</td>
</tr>
<tr>
<td>Institute of Medicine of National Academies</td>
<td>2005*</td>
<td>in capsule form could be considered in consultation with the physician</td>
</tr>
<tr>
<td>Health Council of the Netherlands</td>
<td>2006*</td>
<td>Patients who need to lower triglycerides</td>
</tr>
<tr>
<td>Superior Health Council of Belgium</td>
<td>2006*</td>
<td>2–4 g of EPA + DHA per day provided as capsules under a physician’s care</td>
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<tr>
<td></td>
<td></td>
<td>500 mg n–3 fatty long chain PUFA/d</td>
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<tr>
<td></td>
<td></td>
<td>610 mg n–3 fatty long chain PUFA/d for men; 410 mg n–3 fatty long chain PUFA/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>for women; LNA intake 4–10% en; ALA 0.4–1.0% en</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6 g/d of ALA for men and 1.3 g/d of ALA for women</td>
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<tr>
<td></td>
<td></td>
<td>450 mg n–3 fatty long chain PUFA/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A minimum of 0.3% EPA + DHA for adults</td>
</tr>
</tbody>
</table>

\(^a\) Table modified from [305].
\(^b\) [305].
\(^c\) [198].
\(^d\) [306].
\(^e\) [307].
\(^f\) [308].
heterogeneous group, the most representative are prostaglandins (e.g., PG_{E2}), thromboxane (e.g. TBX_{2}) and leukotrienes (e.g. LTB_{4}) [233]. One of the most common pharmacological approaches to treat inflammation is to inhibit the biosynthesis of n−6 eicosanoids. This could be achieved by providing COX1/2 inhibitors, e.g. aspirin, or by increasing n−3 fatty acid content, particularly EPA and DHA [234]. In turn, n−3 enriched diets can also suppress production of cytokines [115,235,236]. This is important because several clinical studies have established a positive correlation between circulating cytokine levels and the increased risk for future vascular events [237,238] or for rheumatoid arthritis [239, 240]. Thus, consumption of fish oil capsules decreased TNF-α and IL-1β [115,235,236,241,242] or pro-inflammatory eicosanoids (TBX_{2} and PG_{E2}) [242] in healthy subjects and patients with rheumatoid arthritis [241]. Diseases such as atherosclerosis and obesity are currently catalogued as low-grade inflammatory disease [243,244] and consequently, it is plausible to reduce their effects by modulating the inflammatory response [237]. There are several plasma inflammatory markers that can be monitored to diagnose the risk of a patient suffering from atherosclerosis or obesity and then to follow the benefits of a treatment. Some of these markers are: high-sensitive C-reactive protein (CRP) [245], soluble intercellular cell-adhesion molecule-1 (sICAM-1) and soluble vascular cell-adhesion molecule-1 (sVCAM-1). Once again, the beneficial effects have been related to the increase in EPA and DHA circulating levels [234] which could be achieved by the consumption of ALA-enriched sources. However, the possibility that ALA may act differently than EPA and DHA should not be ruled out [246]. All together these data suggest that the anti-inflammatory effect of ALA-enriched diets may provide a possible additional mechanism for its beneficial effect in primary and secondary prevention of coronary artery disease [247].

In fact, studies in vitro show that both ALA and DHA inhibit nuclear factor κB (NF-κB) DNA binding activity in THP-1 cells, which is associated with activation of PPAR-γ [248]. Because NF-κB binding is also critical for induction of genes encoding many pro-inflammatory cytokines [249], these data are suggestive that both ALA and DHA can suppress expression of pro-inflammatory cytokines, e.g. IL-6, IL-1, and TNF-α via a reduction in NF-κB induced gene expression. In addition, dietary ALA could elicit its anti-inflammatory effects via activation of PPAR-γ [114]. Experiments using mononuclear cells obtained from subjects who consumed flaxseed oil-based diet for 4 weeks produced 30% less TNF-α and IL-1β and 20% less TBX_{2} and PG_{E2} when they were stimulated with LPS [115], supporting this hypothesis. However, some studies show a decrease in inflammatory markers after ALA-enriched diets [114,247], while other studies show no differences [250]. Thus, dyslipidemic patients consuming 15 ml of flaxseed oil per day (~7 g ALA/d) for 3 months have decreased CRP (38%), SAA (23%) and IL-6 (10%) levels compared to patients consuming 15 ml of safflower oil (rich in LNA) [247]. Similar reductions in CRP levels are observed in hypercholesterolemic patients consuming of 6–8 g/d of ALA for 2 years [251]. However, in this study no other changes were observed in markers of atherosclerosis such as oxidized LDL lipoprotein, sICAM, IL-6 and IL-10 [251]. In turn, shorter treatments were sufficient to observe differences in inflammatory marker levels. Thus, cultured peripheral blood mononuclear cells obtained from hypercholesterolemic patients consuming ALA diet (6.5% energy) produced lower levels of IL-6, IL-1β, and TNF-α compared to patients consuming a LNA-enriched diet (12.6% energy). Additionally, serum TNF-α level was decreased as well in the ALA diet group. These changes are accompanied by an increase in plasma ALA, EPA and DPA/Ar-3 levels in subjects receiving the ALA diet [114].

So far, all these studies have been done in subjects with either hypercholesterolemia or dyslipidemia. In healthy patients though, the effects of ALA on inflammatory markers are more subtle and in some case no differences are observed. In healthy males consuming a low-fat ALA-enriched diet (based on walnuts) for 28 d, show a decrease in sVCAM-1 levels, although there were no changes in sICAM [214]. In healthy firefighters, consumption of up to 3.6 g/d of flax oil did not reduce levels of pro-inflammatory cytokines, but in this same study no reduction was seen in subjects consuming up to 1.2 g/d of fish oil [33]. These studies indicate the difficulty in assessing the ability of either DHA or ALA to reduce indicators of inflammation in healthy human subjects.

Because obesity is currently regarded as low-grade chronic inflammatory disease [244], n−3 enriched diets may be a useful strategy to decrease inflammatory markers levels associated with obesity. A study in a group of obese subjects receiving 5 g ALA/d for 2 weeks supports this point, as obese subjects showed elevated CRP and SAA levels that were reduced after ALA supplementation [252]. However, no alterations in IL-6, CRP, SAA and TNF-α plasma levels are observed in a study where healthy subjects with large waist circumferences increased their ALA consumption by including flaxseed oil capsules in their diet during 8 weeks [253]. Thus, the importance of ALA consumption on reducing obesity induced inflammation is complex and additional studies are required to further elucidate the important of ALA intervention in reducing the morbidity associated with obesity.

4.3. Neuroprotective properties of ALA

The phospholipids in brain have a very high DHA content and it is clear that DHA is critical for adequate brain development, however it is still not fully understood which unique feature makes DHA so essential for brain functioning. Because DHA levels are reduced in some neurodegenerative diseases such as Alzheimer’s disease (AD) [254,255], there has been an increased emphasis on studying the impact of DHA on mitigating the course of this disease. In animal models of AD, administration of low levels of DHA (0.6%) reverses changes in synaptic architecture and downstream cognitive decline in mice expressing mutant human APP (Tg2576 mice) [256,257]. Mechanistically, this improvement is the result of the ability of dietary DHA to reverse the activation of caspases, the reduced phosphatidylinositol-3–kinase activity, and the decreased N-methyl-d-aspartic acid (NMDA) receptor numbers associated with a reduced brain n−3 fatty acid levels [256]. In another mouse model of AD, dietary DHA reverses memory impairment associated with amyloid-beta infusion in rats, via a reversal of increased cholesterol and saturated fatty acid accumulation in lipid rafts [258,259]. Thus, there is increasing evidence that dietary DHA is capable of reversing not only biochemical markers associated with AD, but also the cognitive decline associated with these models. However, the ability of ALA to have a similar effect is unknown, although we and others have demonstrated a marked increase in brain DHA levels in ALA-fed rats [22,23], suggesting that ALA itself would have this potential to reverse or limit the effects of disease progression in animal models.

Similar to the explanation for the ability of ALA to elicit an anti-arrhythmic effect in heart by altering the function of heart ionic channels [132,221], an equivalent potential for ALA may occur in the brain. This possibility was evaluated in a model of cerebral global ischemia in vivo where ALA was injected into the brain 30 min prior to the onset of either cerebral, spinal cord ischemia, or kainite-induced seizures [260,261]. Under these conditions, neuronal loss is significantly reduced and seizures are prevented, presumably due to the positive effects on ion channels function, promoting a significant degree of neuroprotection. In traumatic spinal cord injury (SCI), ALA (250 mmol/kg) treatment for 30 min after spinal cord hemisection reduces lesion size (40–45%), reduces apoptotic cell death, while increasing neuronal and oligodendroglial survival,
collectively resulting in improved locomotor function measured one week after injury [262]. Thus, while there are a limited number of studies demonstrating any impact of ALA as a neuroprotective agent, these limited studies suggest that ALA itself may have a positive impact on limiting central nervous system injury.

5. ALA sources

One major contributor to reduced ALA consumption was increased consumption of high LNA containing grains, such as corn, in the Western diet. In Western Europe and North America, this has dramatically shifted the ratio \( n-6 \) to \( n-3 \) fatty acids from 8:1 to as high as 20:1 [66,263–265]. This very high ratio is far from what is considered optimal, that is, a ratio LNA to ALA of 4:1 [23,67]. This led to an ever increasing awareness on the part of governments to educate the public as they issued recommendations for increased consumption of ALA and EPA/DHA (Table 2) [266,267]. The recommendations for daily intake of long chain \( n-3 \) PUFA, such as DHA and EPA, but surprisingly not DPA–3, is 200–400 mg/d [131,268,269], while the recommendation for ALA intakes ranges from 1.35 to 2.2 g/d. However, it is important to note that these recommendations are made under the assumption that very little dietary ALA is elongated to EPA and DHA, which certainly is not consistent with the kinetics of elongation presented earlier.

Regardless, a number of factors contribute to the reasons why initiating a higher intake of \( n-3 \) fatty acids is difficult for the general population as well as the ability of the general population to sustain these habits over a long enough period of time to make them part of their normal diet. The natural sources containing high levels of EPA and DHA are basically limited to oily fish and to a certain number of other types of seafood [268]. However, there is a certain resistance to consume oily fish and indeed poor access to many alternative routes to enhance EPA and DHA consumption [118,270]. For example, in the United States, the average annual per capita fish intake is only 7.4 kg [271], with only some of this intake of species containing high levels of \( n-3 \) FA [272]. Because of extensive use of the world’s fisheries, fish sources containing high levels of EPA and DHA are rapidly diminishing and equally important are the increasing concerns that levels of various toxins are increasing in the fish, most notably methyl mercury [273,274]. While consumption of capsules containing EPA and DHA is an alternative, the bioavailability of these fatty acids is less than that from consuming food containing EPA and DHA [275]. Again, a problem is that in the long run, there are issues with sustainability as compliance is low over long periods of time, in part due to issues with palatability.

A good dietary source useful for increasing \( n-3 \) fatty acid consumption is a greater consumption of vegetable derived \( n-3 \) fatty acids (Table 1). Unfortunately, the bulk of the common crops are high LNA producers, e.g. corn, soybean, while of the most common vegetable oils such as soybean (~7% ALA) and canola (9% ALA), do not contain ALA as the majority of the fatty acids. Thus, the vegetable oils most widely consumed contain highly percentage of LNA, such as corn oil (57%) or sunflower oil (71%), which competes directly with ALA for enzymes involved in elongation and desaturation (see Fig. 1). Some calculations estimate that in the typical Western diet, 20–25 fold more \( n-6 \) fatty acids are consumed relative to \( n-3 \) fatty acids [234] and LNA consumption represents a 7–8% of fatty acids consumed in countries such as USA and Australia [234]. In addition, the high consumption of \( n-6 \) fatty acids affects not only humans, but also livestock and aquaculture as cattle, hogs, poultry, and fishes are fed grains containing high LNA. This not only affects the health of the animals, but it also results in a significantly lower \( n-3 \) fatty acid content as compared to free-range animals [276,277]. Therefore, it is necessary to find alternative \( n-3 \) fatty acid sources that are readily available in large quantities and more importantly are renewable. A good alternative would be plant-derived sources that contain high levels of ALA, e.g. flax, while another route for increasing these fatty acids in the diet is to obtain foods naturally enriched in \( n-3 \) PUFA.

5.1. Natural sources

There are only three major commercial sources that have significant amounts of ALA: Linum usitatissimum, linseed or flaxseed oil (53% ALA); Brassica spp. Canola or rapeseed oil (9% ALA) and Glycine max, soybean oil (7% ALA) [278]. Perilla oil, Perilla frutescens, also contains a high percentage in ALA (approx. 60%) although its consumption is restricted to Asia, while consumption of camelina oil, Camelina sativa (38% ALA) provides a high ALA-containing specialty oil consumed in Nordic countries. However, variable amounts of ALA are found in plants, animal zooplankton, phytoplankton, and marine species. In plants, ALA is found in leaves, mainly in glycolipids, and as TAG in certain seed oils (rapeseed, flaxseed, perilla seed, chia seed), beans (soybeans, navy beans) and nuts (walnuts).

5.2. ALA-enriched sources

As it was noted, it is not always an easy goal to increase \( n-3 \) fatty acid intake because of the scarcity of \( n-3 \) natural sources and their limited availability. Because of the problems associated with \( n-3 \) fatty acid-enriched capsules, it is necessary to use a functional foods approach to provide \( n-3 \) fatty acids to the general population. Several studies show that it is possible to increase \( n-3 \) fatty acid levels by selecting \( n-3 \) natural or enriched sources and substituting in the diet for common food items [46,270]. In Australia, healthy male volunteers incorporated foods enriched in ALA (cooking oil, margarine, salad dressing and mayonnaise) or in EPA plus DHA (sausage and savory dip) or naturally enriched in ALA (flaxseed) and EPA and DHA (fish) into their diets. The incorporation of these nutrients meant a daily intake of approximately 1.8 g/d of EPA plus DHA and 9.0 g/d of ALA. At the end of the study, these intakes led to an average 3-fold increase in EPA in plasma, platelet, and mononuclear cell phospholipids and a decrease in TBX (36%), PGE (26%), and IL-1β (20%) [270]. However, the consumption in this study is well above the current recommendations and certainly not sustainable. In fact, inclusion of ALA-containing sources such as flaxseed, walnuts and other ALA sources in baked products and cereals, flaxseed oil in salad dressings, as well as \( n-3 \)-enhanced dairy products [279–281], is a more functional and sustainable mean of introducing ALA into the diet. In addition, ALA-rich cooking oils and spreads have a considerable potential for altering the balance of \( n-3 \) to \( n-6 \) because they are a major source of dietary fat feed and can be used in food preparation [46,115].

One important constituent of the Western diet the meat. Unfortunately, because cattle and lambs are often fed diets high in grain that is rich in LNA and poor in ALA, the meat produced from these animals is low in \( n-3 \) fatty acids and consequently considered less healthy [82,105,282–284]. For this reason, a great deal of research has been focused in finding a commercially sustainable means to increase \( n-3 \) fatty acid content in meat. One approach is to provide cattle with a concentrated \( n-3 \) fatty acid source such as flaxseed, but bacteria in the rumen will reduce the PUFA as the double bonds of 20-carbon fatty acids are hydrogenated [285], necessitating examining a number of different approaches to protect these fatty acids from hydrogenation [107,286]. Despite this difficulty, several studies demonstrate that cattle consuming forages or feeds containing \( n-3 \) fatty acids can produce meat with higher levels of \( n-3 \) fatty acids and lower levels of \( n-6 \) fatty acids [39,40,69,287,288]. Thus, experiments feeding cattle 907 g/d of flax protected with lignosulfate for a 71-d period led to a significant increase in \( n-3 \) fatty acids (4-fold ALA, 1.4-fold
EPA, and 1.4-fold DPAn-3) in *longissimus dorsi* muscle [39]. While others suggest cooking effects [82,289–291], in our study we grilled the meat with no adverse consequences observed in the n–3 fatty acids when meat is cooked [39]. We have also examined the impact of flaxseed processing on n–3 fatty acid deposition in the meat and compared cattle fed with 8% ration (dry matter basis) of either whole flaxseed, ground flaxseed (700 µm), or rolled flaxseed (1300 µm) and performance data were compared to control diet. Results indicate that inclusion of flax in cattle finishing diets increased phospholipid and non-polar lipid proportions of n–3 fatty acids, and flax processing (rolled or ground) further elevated intramuscular ALA acid content [40]. In this study, heifers fed flax had greater marbling scores than those heifers fed the control diet and inclusion of flax produced steaks that have a lower shear force but are also rated as less juicy by a trained taste panel. While there is a range of n–3 fatty acid content in beef, a person who consumes 227 g/d (8 oz serving), would obtain 352 mg of n–3 fatty acids (ALA, EPA, DPAn-3, and DHA) [288]. All together, these studies suggest that flax or other n–3 fatty acid-enriched sources can be successfully used in beef feedlot rations to increase n–3 fatty acids content in meat. Similar studies demonstrate that feeding hogs with ALA leads to n–3 fatty acid-enriched meat as well. Unlike in cattle, hogs fed ALA can show DHA accumulation when hogs are fed flaxseed (4 g ALA/kg feed) [292] or rapeseed oil (3% and 6%) [293]. Conversely, others have shown no accumulation of DHA in meat following ALA finishing diets [294]. Similar to our study in humans [33], longer finishing times do not translate into higher n–3 fatty acid content in the meat. In Duroc-cross gilts (type of hog) fed with ration containing 6% whole crushed flaxseed for 20, 60, or 100 d, indicates that the lowest n–6 to n–3 fatty acid ratio in plasma, adipose tissue and *longissimus* muscle fatty acid composition is obtained after 60 d treatment [295]. As in the case of cattle fed flaxseed, the inclusion of flaxseed in swine diets does not impact the organoleptic characteristics of the meat [295,296]. Analogous studies have been done in lamb [288,297,298], and poultry [299,300] obtaining similar results.

Therefore, foods that are strategically or naturally enriched in n–3 PUFA can be used to achieve desired biochemical effects without the ingestion of supplements or dramatic changes in dietary habits. A wide range of n–3 enriched foods can be developed to support large-scale programs on the basis of the therapeutic and disease-preventive effects of n–3 FA [270,301]. Because evidence indicates that dietary ALA is sufficiently elongated and desaturated into longer chain n–3 fatty acids such as EPA, DPAn-3, and DHA, increased consumption of ALA will provide the general population with sufficient n–3 fatty acids to meet daily needs.

### 6. Conclusion

Thus, in this review we demonstrate significant evidence to conclude that dietary ALA is rapidly accumulated in different compartments despite the fact that a certain percentage of this fatty acid, is as the case of many fatty acids, is subjected to β-oxidation. Upon entering tissues, ALA is the substrate of the elongation and desaturation enzymatic machinery in a tissue-dependent manner, leading to the synthesis of longer chain fatty acids as EPA, DPA and, in certain tissues, of DHA. Taking into account the estimated values for DHA daily requirement and considering the kinetic values of ALA and DHA half-life, we propose that dietary ALA is able to fulfill the requirement for DHA. In addition, there are a relatively high number of *in vitro* and animal studies, which have been generally underappreciated, showing that ALA exerts identical effects as does DHA in a number of different physiological paradigms, although in most cases, longer treatments or higher concentrations of ALA are needed as compared to when dietary DHA is consumed. Hence, these results suggest that ALA may in many cases use an identical mechanism as DHA to exert its action, while in other cases its effect is through its conversion to DHA. In the end, based upon kinetic evidence, dietary studies with ALA, and human studies, the conversion of ALA to DHA by the liver and other specific DHA requiring tissues such as the brain, will provide ample DHA when sufficient ALA (>1200 mg) is consumed.

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