

## Metabolism and Health effects of Alpha Linolenic Acid

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### *Summary*

Essential fatty acids (EFAs) are required in the diet as they can not be synthesized by humans from the shorter chain fatty acid, oleic acid (C18:1). The two established EFAs are linoleic acid (C18:2, LA) which is converted in the body into longer chain omega-6 fatty acids, and the omega-3 fatty acid alpha-linolenic, ALA (18:3, n-3). Flaxseed is the highest plant based source of ALA. ALA is converted to the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the long chain fatty acids found in fish oil. The conversion of ALA to EPA has been described as limited and somewhat slow in humans. There is great variability in the conversion rate reported by researchers, with one estimate as high as 6% converted to EPA and one as low as 0.2% converted.

ALA conversion to its longer-chain metabolites is significantly affected by dietary intakes of LA. A diet rich in LA can reduce ALA conversion by as much as 40%, and a high intake of LA by pregnant women lowers EPA and DHA levels in umbilical plasma. The absolute amounts of ALA and LA in the diet effects ALA conversion - decreasing the intake of LA has been found to increase the proportion of dietary ALA converted into EPA, while increasing ALA intake can increase the absolute amount of DHA synthesized. Other factors that interfere with ALA conversion include the intake of dietary cholesterol, saturated fat, oleic acid and *trans* fatty acids and the ratio of polyunsaturated to saturated fats in the diet. More recent studies in which dietary parameters have been more appropriately controlled show conversion of ALA to EPA and somewhat to DHA in both men and women with or without cardiovascular disease.

Research has also identified ALA as a regulator of LA and AA metabolism. ALA acts to competitively inhibit the conversion of LA to AA resulting in decreased amounts of substrate available for the production of proinflammatory eicosanoids. Clinical studies as well as small and large-scale population studies indicate that the consumption of ALA-rich diets lower the risk of cardiovascular disease. In epidemiological research, overall cardioprotective effects of ALA were seen despite differences in study populations, length of follow-up, outcomes and method of analyzing the study data statistically. Diets high in ALA elicit heart health effects through numerous mechanisms and evidence suggests that total intakes of approximately 1.5 to 3 g/d ALA are very beneficial.

### ***Alpha linolenic acid – an Essential fatty acid***

Essential fatty acids (EFAs) are required in the diet as they can not be synthesized by humans from the shorter chain fatty acid, oleic acid (C18:1). The two established EFAs are linoleic acid (C18:2, LA) which is converted in the body into longer chain omega-6 fatty acids, and the omega-3 fatty acid alpha-linolenic, ALA (18:3, n-3). LA and ALA are components of cellular membranes and act to increase membrane fluidity. These fatty acids are necessary for cell membrane function, as well as for the proper functioning of the brain and nervous system<sup>1,2</sup>. Flaxseed is the richest dietary source of ALA. Oil constitutes 32-45% of the composition of flaxseed, of which 51-55% is ALA.

ALA plays an important role in growth and development, reproduction and vision; in maintaining healthy skin and cell structure; in the metabolism of cholesterol and in gene regulation. ALA has also been linked to the prevention and/or amelioration of several chronic conditions including cardiovascular disease, certain cancers, rheumatoid arthritis and autoimmune disorders<sup>3</sup>. ALA is the most commonly consumed omega-3 fatty acid in the typical Western diet<sup>4</sup>.

The EFAs serve as the starting point for the production of a number of important, very active, hormone-like compounds called “eicosanoids”. The omega-6 fatty acid LA and the omega-3 fatty acid ALA form different eicosanoids with different activities. LA and ALA also compete with one another for the enzymes responsible for the synthesis of various eicosanoids. Thus, an excess of one family of fatty acids can interfere with the metabolism of the other, reducing its incorporation into tissue lipids and altering their biological effects<sup>2</sup>. A proper balance of the EFAs in the diet is important for the maintenance of good health.

ALA is converted by a series of alternating desaturations and elongations to the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the long chain fatty acids found in fish oil. Fish oil supplementation has been studied extensively and there is no doubt that EPA and DHA have very significant and well supported positive effects in numerous chronic conditions and diseases.

LA is converted to long-chain omega-6 fatty acids also by a series of desaturations and elongations to arachidonic acid (AA), the precursor of eicosanoids, several of which promote the clumping (aggregation) of blood platelets, the clotting of blood within blood vessels (thrombosis) and inflammatory reactions. When diets are high in omega-6 fatty acids, AA and its potent

eicosanoids are produced in abundance, resulting in an over-active immune system that may contribute to chronic diseases like cancer, stroke, diabetes and coronary heart disease.

The metabolic pathways of the omega-6 and omega-3 fatty acids and EFA conversion to the n-6 and n-3 derived eicosanoids is outlined in Figure 1. Table 1 outlines some consequences of eating a diet rich in omega-6 fats versus the benefits of eating a diet rich in omega-3 fats.

### ***ALA Conversion to EPA and DHA***

The conversion of ALA to EPA has been described as limited and somewhat slow in humans<sup>5</sup>. In truth, there is great variability in the conversion rate reported by researchers, with one estimate as high as 6% converted to EPA<sup>6</sup> and one as low as 0.2% converted<sup>7</sup>. The 30-fold difference between these conversion rates reflects key differences in the study protocols.

ALA conversion to its longer-chain metabolites is significantly affected by dietary intakes of LA. A diet rich in LA can reduce ALA conversion by as much as 40%<sup>8</sup>, and a high intake of LA by pregnant women lowers EPA and DHA levels in umbilical plasma, suggesting reduced ALA conversion and availability of omega-3 fatty acids for the developing fetus<sup>9</sup>. In a study of 22 healthy men, an LA-rich diet (10.5% energy) reduced the EPA content of plasma phospholipids significantly after four weeks compared with a low-LA diet (3.8% energy), even though both diets contained the same amount of ALA (1.1% energy)<sup>10</sup>. The absolute amounts of ALA and LA in the diet also effect ALA conversion - decreasing the intake of LA has been found to increase the proportion of dietary ALA converted into EPA, while increasing ALA intake can increase the absolute amount of DHA synthesized<sup>11</sup>.

High intakes of EPA and DHA can also block ALA conversion, possibly by signaling that tissue levels of omega-3 fats are adequate. Furthermore, a diet containing more than 12 g of ALA per day can reduce ALA conversion<sup>12</sup>.

Other factors that interfere with ALA conversion include the intake of dietary cholesterol<sup>13,14</sup>, saturated fat, oleic acid<sup>15,16</sup> and *trans* fatty acids<sup>17,18</sup> and the ratio of polyunsaturated to saturated fats in the diet<sup>19</sup>.

More recent studies in which dietary parameters have been more appropriately controlled show conversion in both men and women with or without cardiovascular disease. A summary of

seventeen human clinical trials which assessed conversion under various experimental designs is shown in Table 1.

ALA has three main biologic effects, which together contribute to many positive health effects.

1. ALA functions as the precursor of EPA and DHA. Its effect on blood clot formation may differ from those of EPA and DHA<sup>20,21</sup>, and its presence in colostrum and breast milk suggests a role for ALA in the growth and development of infants<sup>22,23</sup>. ALA plays an important role in maintaining the health of the skin.<sup>24</sup>

2. ALA-rich diets increase the ALA, EPA and total omega-3 fatty acid content of cell membrane phospholipids. In one study of 20 healthy men and women taking 6 flax oil capsules a day (providing 3.5 g of ALA/day) for 8 weeks, the ALA content of red blood cell membranes increased 100%, the EPA content increased 33%, and the DPA (docosapentaenoic acid) content increased 20%; the DHA content was unchanged<sup>25</sup>. In another study, diets containing more than 4.5 g of ALA/day (contained in about ½ tbsp of flax oil or 2 heaping tbsp of milled flax daily) increased the EPA content of plasma phospholipids between 33% and 370% and the DPA content between 5% and 50%<sup>26</sup>. (The large ranges in the response of study volunteers to dietary ALA reflect differences in the amount of LA in their diets, among other factors). Increasing the omega-3 fatty acid content of membrane phospholipids increases the flexibility of membranes and alters the way they behave in beneficial ways<sup>27</sup>.

3. ALA dampens inflammatory reactions through effects on:

- **EICOSANOIDS.** ALA affects eicosanoids in two ways. First, ALA is a precursor of EPA, which is itself a precursor of eicosanoids. Eicosanoids control inflammatory reactions. Their release is a normal response to injury, and their actions are required to help repair damaged tissue. A diet rich in omega-3 fatty acids produces more beneficial eicosanoids and less inflammation and decreases the risk of chronic diseases compared with diets rich in omega-6 fatty acids.

Secondly, ALA interferes with the conversion of LA to AA and blocks the conversion of AA to its pro-inflammatory eicosanoids. Pro-inflammatory eicosanoids such as thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) are derived from AA. TXA<sub>2</sub> is one of the most potent promoters of platelet aggregation known. LTB<sub>4</sub> increases the release of

reactive oxygen species and cytokines like tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin 1 $\beta$  (IL-1 $\beta$ ), IL-6 and IL-8.

Early research identified ALA as the regulator of LA and AA metabolism. ALA acts to competitively inhibit the conversion of LA to AA resulting in decreased amounts of substrate available for the production of proinflammatory eicosanoids<sup>28</sup>. In a clinical study of healthy men who consumed 1 $\frac{3}{4}$  tbsp of flax oil daily for 4 weeks, the TXB<sub>2</sub> concentration in immune cells decreased 30%<sup>29</sup>. (TXB<sub>2</sub> is an inactive metabolite of TXA<sub>2</sub>.) A study of 64 patients with chronic obstructive pulmonary disease (COPD) found that levels of LTB<sub>4</sub> in serum decreased 32% and in sputum 41% in patients who received an ALA-rich nutritional support (1.4% ALA) daily for 24 months compared with those who received a low-ALA nutritional support (0.18% ALA) during the same period<sup>30</sup>. Diets rich in ALA decreased significantly the concentration of AA in neutrophils<sup>31</sup>, and in serum<sup>32,33</sup>.

- CYTOKINES are proteins liberated from immune cells in response to injury, infection or exposure to foreign substances. The cytokines that contribute to inflammation are tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 and -8 (IL-6 and IL-8). Serum levels of IL-6 decreased 25% in men who consumed 1 tbsp of flax oil daily for 12 weeks<sup>34</sup>, whereas the concentrations of TNF- $\alpha$  and IL-1 $\beta$  in immune cells decreased 26% and 28%, respectively, when healthy men consumed flax oil for 4 weeks<sup>29</sup>. The serum level of TNF- $\alpha$  decreased by 43% and the production by immune cells of TNF- $\alpha$  and two other cytokines, IL-6 and IL-1 $\beta$ , decreased between 18% and 22% when adults with hypercholesterolemia consumed a diet rich in ALA compared with the average American diet<sup>33</sup>. In the COPD study mentioned previously, sputum TNF- $\alpha$  and IL-8 concentrations decreased 48% and 55%, respectively, in the patients who received the high-ALA nutritional support, whereas the sputum cytokine concentrations did not change in the patients who received the low-ALA nutritional support for 2 years<sup>30</sup>. A reduction of 18-55% in the concentrations of these pro-inflammatory compounds is a significant clinical outcome.

- C-REACTIVE PROTEIN (CRP) is produced early in the body's response to inflammation or infection and is also an indicator of systemic inflammation. High blood levels of C-reactive protein (CRP) indicate the presence of systemic inflammation or infection<sup>35</sup>. Half of all heart attacks and strokes in Canada and the United States occur in people with normal

cholesterol levels, and 20% of all events occur in people with no major risk factors. It has been demonstrated that CRP levels, when added to the traditional ways of measuring risk, provide a better way than assessment of serum lipids alone, of detecting who is a high-risk patient.

In a clinical trial, serum CRP levels decreased 75% when men and women ate a high-ALA diet for 6 weeks<sup>32</sup>. In randomized, controlled, crossover studies conducted in hypercholesterolemic men and women, a diet high ALA dramatically decreased CRP<sup>36</sup>. Cross-sectional data from women involved in the Nurses Health Study demonstrated an inverse association between ALA intake and plasma concentrations of CRP<sup>37</sup>.

SERUM AMYLOID A (SAA): During inflammation the liver also releases SAA in response to acute injury, infection, malignancy, hypersensitivity reactions and trauma. CRP and SAA are markers of systemic inflammation, and they are present in the lesions of atherosclerosis. Consuming flax oil reduced CRP and SAA levels in a study of 50 Greek men with high blood cholesterol levels who consumed 1 tbsp of flax oil daily for 12 weeks. Serum CRP decreased 48% and serum SAA decreased 32% after 12 weeks<sup>34</sup>.

Support for an anti-inflammatory effect of ALA comes from a community-based study in two small towns in Tuscany, Italy<sup>38</sup>. The researchers examined the relationship between the concentration of fatty acids in plasma and the level of inflammatory markers in 1,123 persons aged 20-98 years. A low plasma ALA concentration was associated with higher levels of CRP and interleukin 1 receptor antagonist (IL-1ra). IL-1ra is considered an acute-phase protein and a reliable measure of the pro-inflammatory state. Thus, ALA-rich diets containing flax oil have substantial effects on systemic markers of inflammation.

### ***ALA and Heart Health***

Four case-control studies, one cross-sectional study, three prevention trials and three cohort studies found a benefit of ALA-rich diets in lowering the risk of CHD, ischemic heart disease (IHD), nonfatal myocardial infarction (MI) and stroke. One prevention trial found no change in the estimated 10-year IHD risk but reported a significant decrease in fibrinogen and CRP levels on ALA-rich diets<sup>36,39</sup>. The number of participants in the studies ranged from 233 to 76,283, as shown in Table 3.

Numerous intervention studies have established the beneficial effects of ALA on reducing the risk of adverse cardiac events. The Health Professional Follow-up Study, which began in 1986 with a cohort of 51,529 health professionals, demonstrated that a 1% increase in ALA intake was associated with a 40% reduction in the risk of non-fatal CHD<sup>20,40</sup>. The Lyon Diet Heart Study included participants who had previously survived a myocardial infarction compared to an experimental group who consumed a typical Mediterranean style diet rich in ALA. The control group consumed a typical Western-type diet low in ALA. The results were impressive with a 75% reduction in non-fatal myocardial infarctions, and a 70% reduction in total death noted amongst the ALA group in comparison to the control group<sup>21,41</sup>.

In the Nurse's Health Study, which involved a 10-year follow-up of 76,283 women with no previously diagnosed CVD, a higher intake of ALA was associated with a lower relative risk of fatal and non-fatal myocardial infarction<sup>42,43</sup>. ALA can reduce ventricular fibrillation (rapid and irregular heartbeat), and may be more effective in this regard than EPA<sup>4</sup>. The cardio-protective effects of ALA have also been attributed to improvements in arrhythmia (abnormal heart rhythms) and to reductions in platelet aggregation (blood platelet stickiness)<sup>44</sup>.

#### ***ALA and stroke risk***

Two population studies found a benefit of ALA in reducing stroke risk. In the Edinburgh Artery Study, significantly lower levels of ALA were found in the red blood cell phospholipids of men and women who had had a stroke compared with participants who had no evidence of disease<sup>45</sup>. In the Multiple Risk Factor Intervention Trial (MRFIT), 96 men who had had a stroke were compared with 96 men without stroke who were matched for age. In the multivariate model, each increase of 0.13% in the serum ALA level was associated with a 37% decrease in risk of stroke<sup>46</sup>. After controlling for risk factors of stroke like smoking and blood pressure, ALA emerged as an independent predictor of stroke risk – that is, men with higher levels of ALA in their serum phospholipids had a lower stroke risk.

#### ***ALA and cardiac rhythm (arrhythmia)***

The rhythmic pumping action of the heart is controlled by the heart's electrical system. Arrhythmias are abnormal rhythms of the heart muscle and are a risk factor for MI. In humans, the ALA content of adipose tissue was inversely related to risk of MI in one case-control study conducted in Europe and Israel<sup>47</sup> and with nonfatal acute MI in another study carried out in Costa Rica<sup>48</sup>. In the Nurses' Health Study, a prospective cohort study involving 76,763 women, the

dietary intake of ALA was inversely associated with the risk of sudden cardiac death, but not with other fatal CHD or nonfatal MI<sup>42</sup>.

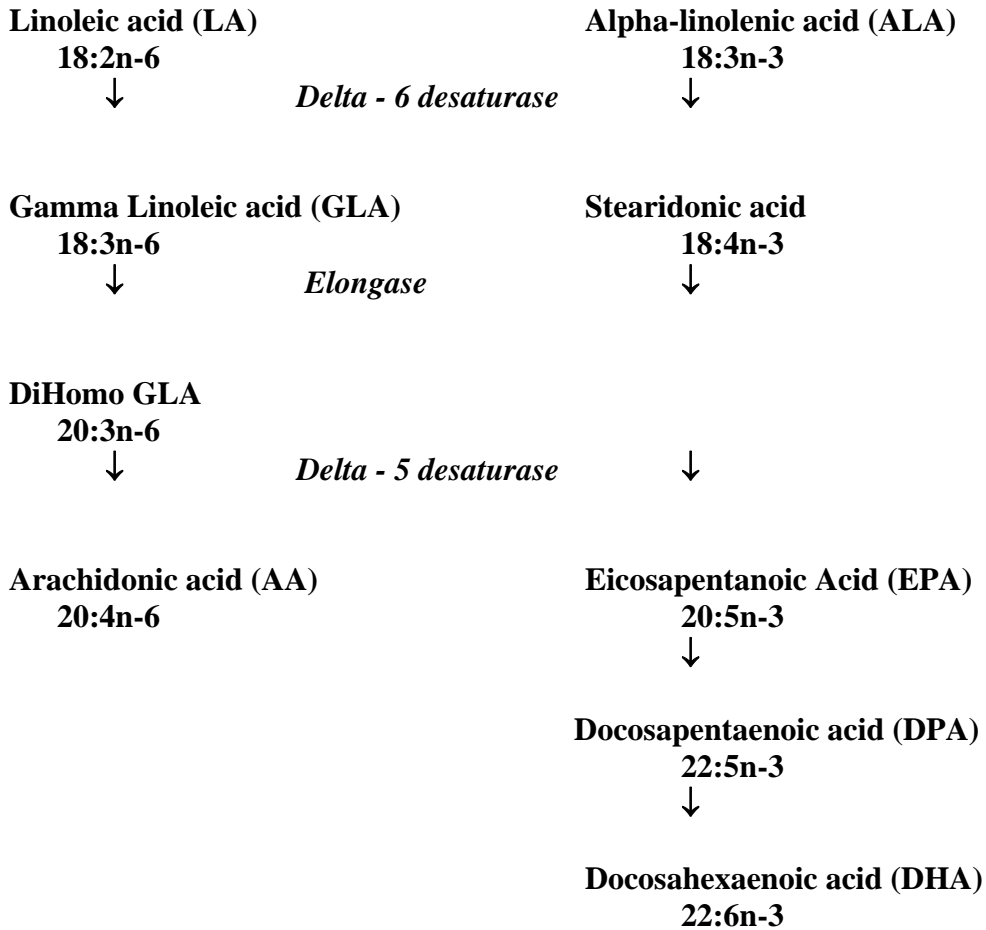
The mechanism by which ALA helps lower the risk of fatal or nonfatal MI appears to involve its effect on cardiac rhythm. In the Family Heart Study, Djoussé and colleagues<sup>49</sup> found that the higher the dietary ALA intake, the lower the risk of abnormally prolonged repolarization of the heart muscle – an indicator of cardiac arrhythmia. In a clinical study among women referred for elective coronary angiography<sup>50</sup>, the ALA content of adipose tissue was positively correlated with 24-hour heart rate variability. That is, women with a higher content of ALA in their adipose tissue had better heart rate variability scores which made them less likely to develop ventricular arrhythmias. Taken together, these findings suggest that ALA helps maintain the heart's normal rhythm, thus partly explaining how ALA helps reduce CVD risk.

Clinical studies as well as small and large-scale population studies indicate that the consumption of ALA-rich diets lower the risk of CVD<sup>4,51</sup>. In epidemiological research, overall cardioprotective effects of ALA were seen despite differences in study populations, length of follow-up, outcomes and method of analyzing the study data statistically. Diets high in ALA elicit heart health effects through numerous mechanisms and evidence suggests that total intakes of approximately 1.5 to 3 g/d ALA are very beneficial<sup>52</sup>. In fact, the *American Heart Association Dietary Guidelines* recommends the inclusion flaxseed and canola, and food sources like flaxseeds, high in ALA in a healthy diet for the general population.

## FIGURE 1

### Metabolic pathways of the omega-3 and omega-6 fatty acids

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## Sources and actions of eicosanoids

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### *Omega 6 EFA*

Linoleic (LA)



Gamma-Linolenic Acid (GLA)



DiHomo GLA



Arachidonic acid (AA)



PGE1

15(OH)DGLA

PGE2

TXA2

LTB4



*Vasodilator*  
*Anti-Inflammatory*

*Pro-inflammatory*  
*Vasoconstrictor*

*Vasoconstrictor*  
*Pro-Thrombotic*   *Pro-Inflammatory*

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### *Omega 3 EFA*

Alpha-Linolenic Acid (ALA)



Eicosapentanoic Acid (EPA)



PGI3

TXA3

LTB5



*Vasodilator*  
*Anti-Thrombotic*

*Weak Vasoconstrictor*  
*Weakly Pro-Thrombotic*

*Weakly*  
*Pro-inflammatory*

**TABLE 1**

**Comparison of health consequences of diets rich in omega-6 versus omega-3 fats<sup>53</sup>**

<p><b>Consequences of eating a diet rich in omega-6 fats</b></p>	<p><b>Benefits of eating a diet rich in omega-3 fats</b></p>
<p>↑ n-6/n-3 in cell membrane phospholipids</p> <p>↑ production of arachidonic acid</p> <p>↑ release of pro-inflammatory eicosanoids derived from arachidonic acid</p> <p>↑ production of pro-inflammatory cytokines</p> <p>↑ expression (activation) of pro-inflammatory genes</p> <p>↑ biomarkers of inflammation such as C-reactive protein</p> <p>↑ blood viscosity</p> <p>↑ constriction of blood vessels</p> <p>↑ oxidative modification of low-density-lipoprotein (LDL) cholesterol</p> <p style="text-align: center;">↓</p> <p style="text-align: center;"><b>Increased risk of chronic diseases</b></p>	<p>↓ n-6 fatty acids in cell membranes</p> <p>↓ n-6/n-3 in cell membrane phospholipids</p> <p>↓ levels of pro-inflammatory compounds like eicosanoids and cytokines</p> <p>↓ clumping (aggregation) of blood platelets</p> <p>↓ expression (activation) of pro-inflammatory genes</p> <p>↓ biomarkers of inflammation such as C-reactive protein</p> <p>↑ production of interleukin-10, an anti-inflammatory cytokine</p> <p style="text-align: center;">↓</p> <p style="text-align: center;"><b>Decreased risk of chronic diseases</b></p>

Adapted from: Morris, D. 2007. Flax: A Nutrition Primer. Flax Council of Canada. Winnipeg, Canada.

**TABLE 2**  
**Conversion Studies of ALA to EPA/DHA**  
**Research Summary**

Subjects	Amount	Study Design	# Subjects	Endpoint	Length of Study	Result	Reference
Healthy men and women	Control diet (7% of energy from LA, 0.4% of energy from ALA, ALA-to-LA ratio 1:19). For the next 6 wk, a control diet, a low-LA diet (3% of energy from LA, 0.4% of energy from ALA, ratio 1:7), or a high-ALA diet (7% of energy from LA, 1.1% of energy from ALA, ratio 1:7)	Crossover metabolic feeding trial	29	Extent of conversion of ALA to EPA and DHA, reductions in LA and AA	4 weeks on control; 6 weeks on test diets	Phospholipid ALA increased 3.6% in the low-LA group; decreased by 8.0% in the high-ALA group. Nearly all ALA from the plasma phospholipid pool was converted into EPA. Decreasing LA increased ALA conversion into EPA.	Goyens et al (2006) (11)
Healthy men	Diets with LA:ALA ratio of 3:1 or 9:1	Crossover metabolic feeding trial	22	Extent of conversion of ALA to EPA and DHA, reductions in LA and AA	4 weeks	LA:ALA diet ratio of 3:1 - significant increase in plasma phospholipid EPA, lower LA and AA/EPA ratio (linear relationship with dietary ratio)	Liou et al (2006) (10)
Healthy men and women	flaxseed oil (3510 mg alpha-linolenic acid + 900 mg linoleic acid/day)	Randomized single blind	20	Extent of conversion of ALA to EPA and DHA	8 weeks	Flax oil - erythrocyte membrane EPA increased to 133%; DPA to 120%, no change in DHA. EPA, DPA, and DHA - slight insignificant increase in plasma phospholipids	Cao et al (2006) (25)
African-American men and women with	3g ALA from 5.2 g flaxseed oil or 5.3 g Olive oil per day	Randomized, double-blind trial	56	Extent of conversion of ALA to EPA, DPA and DHA	12 weeks	Flax - Plasma levels of EPA increased 60%, (24.09 ± 16.71 to 38.56 ± 28.92 μmol/L), DPA increased 25% (19.94 ± 9.22 to	Harper, C., (2006) (54)

vascular disease						27.03 ± 17.17 μmol/L). Plasma DHA levels did not change in either group	
Healthy women (aged 28 +/- 4 yrs)	700mg of U13C-ALA (consumed once as a drink (150ml) and accompanied by a test meal) Subjects then resumed habitual diet	Clinical Trial	6	Extent of conversion of ALA to EPA, DPA, and DHA	21d	Estimated net fractional ALA inter-conversion to EPA=21%, DPA=6%, DHA=9% Approx. 22% administered 13C-ALA recovered as 13CO2 on breath over the 1 <sup>st</sup> 24hrs of study	Burdge, G.C., Wootton, S.A. 2002 (55)
Healthy males (aged 27-40yrs)	700mg of U13C-ALA	Clinical Trial	6	Capacity for conversion of ALA to EPA, DPA, and DHA	21d	Approx. 33% 13C-ALA recovered as 13CO2 on breath over 1 <sup>st</sup> 24 hrs	Burdge, G.C., et al. 2002 (56)
Healthy men/women	1g oral dose of isotope tracer ALA (dose of 18:3n-3 ethyl ester)	Clinical trial	8	To investigate ALA metabolism in healthy adults	21day (beef based diet for 21d period – ALA administered in final week of dietary period)	Approx. 0.2% of plasma ALA was destined for synthesis of EPA (% isotope transferred through n3 fatty acid compartments calculated for each intermediate & used to determine efficiency of biosynthetic process)	Pawlosky, R.J., et al. 2001 (7)
Healthy adults	Administration of 45mg C13 labeled ALA (following 6wk consumption of either ALA (8.3g/d) or OA rich diet)	Clinical trial	12 (OA rich diet; n=5 or ALA rich diet; n=7)	Effect of diet rich in ALA vs. diet rich in OA on oxidation of labeled C13 ALA and its conversion to LC n3 PUFAs	8 wk (6 wk feeding then administration of labeled ALA – followed by 2 additional weeks on experimental diet - blood samples - 0, 5, 11, 24, 96, 336 hr)	Mean proportion of labeled ALA recovered as 13CO2 in breath after 12h = 20.4% in ALA group and 15.7% in OA group. Conversion of 13C-ALA to LC n3 PUFA may be decreased on diets rich in ALA Oxidation of 13C-ALA negatively correlated w/conversion to LC n3 PUFA	Vermunt, S.H.F., et al. 2000 (57)

Men	6.5g DHA or <0.1g DHA fed in diet for 90d period in metabolic unit. At end of dietary period – mixture of TG's containing deuterated OA, LA, and ALA administered and blood samples drawn over 72 hrs.	Clinical Trial	6	Effect of dietary DHA on metabolism of deuterated TGs containing OA, LA, and ALA	93 d	Accumulation of n-3 LCPUFAs synthesized from ALA in typical American diet was reduced from approx 120 to 30 mg/d by supplementation w/6.5g DHA  Accumulation of n-6 LCPUFAs synthesized from LA in US diet estimated to be reduced from 800mg/d to 180mg/d by supplementation w/6.5g DHA. Supports hypothesis that DHA supplementation reduces accretion of n-6 LCPUFAs and increases n-3 LCPUFA levels in tissue lipids	Emken, E.A., et al. 1999 (58)
Men and Women	Diet 1 – FSO (2.2 energy % ALA) Diet 2 – SO (0.3% energy % ALA) (both diets = similar proportion SAT, MUFA, PUFA – differ only ALA content)	Randomized blind cross-over	40	Ability of ALA in FSO to compensate for restricted fish intake on plasma fatty acid composition	2 x 6wk periods	Proportion of ALA in TG and CE decreased on SO diet and increased on FSO diet.  Proportion of EPA in all plasma fractions decreased on SO diet but not on FSO diet.  Proportion of DHA decreased on both diets  ALA is metabolized to EPA in humans to a degree equivalent to a weekly portion (50-100g) of fatty fish.	Valsta, LM., et al. 1996 (59)
Men	40g FSO (N=5) or 40g SO	Randomized controlled	11	Supplement low fat diet ALA or LA platelet composition	23d	Change of platelet FA: ALA 0.2 +/- 0.1 to 0.4 +/- 0.1 EPA 0.5 +/- 0.1 to 1.2 +/- 0.2 DPA 1.8 +/- 0.2 to 2.8 +/- 0.5 DHA 1.9 +/- 0.4 to 1.7 +/- 0.5	Allman, M.A., et al. 1995 (60)

Healthy men/women	50g FS/d	Clinical Trial	8	To determine the influence of consuming FS	4 weeks	Changes in plasma PL: ALA: 0.6 +/- 0.2 to 0.8+/-0.2 EPA: 0.9 +/- 0.4 to 1.3+/-0.2 DHA: 3.6 +/-1.0 to 3.5+/-1.0	Cunnane, S., et al. 1995 (61)
Young adult males	Triacylglycerol mixture of deuterated LA and ALA (single dose administered following 12 d baseline diet containing 2 levels of LA: 15g/d or 30g/d )	Clinical trial	7	Investigate conversion of LA and ALA to respective longer chain metabolites	12d baseline diet followed by single administration of deuterated mixture of LA and ALA –for 48hrs	Total % conversion of deuterated ALA to longer chain n3 metabolites = 11-18.5% Conversion of deuterated LA to long chain n6 metabolites = 1.0-2.2% Conversion of ALA reduced by 40-54% when dietary LA increased 15g/d to 30g/d	Emken, EA., et al., 1994 (6)
Men	SO (20g/d LA + 1 g/d ALA)  FSO (8g/d LA + 13g/d ALA) Both diets followed by 4wk supplementation with FO (2.7g/d EPA + DHA)	Randomized controlled	30	To compare effects of diet high in ALA, LA, EPA and DHA on phospholipid composition	4 weeks experimental diet + 4 weeks fish oil supplementation	Change in plasma PL following supplementation with FSO: ALA 0.1 +/- 0.1 to 1.1 +/- 0.8 EPA 0.8 +/- 0.3 to 1.9 +/- 0.7 DHA 2.9 +/- 0.6 to 3.3 +/- 0.9 Following FO supplementation: ALA 0.1 +/- 0.1 to 0.7 +/- 0.5 EPA 0.8 +/- 0.3 to 5.4 +/- 1.9 DHA 2.9 +/- 0.6 to 7.0 +/- 1.2	Manzioris, E., et al. 1994 (62)
Men	Basal diet: SO (11g/day – wt% ALA=1.1, LA=25.6) Experimental diet: FSO (4.3 g/day – wt % ALA=21.2, LA=5.2)	Randomized, double-blind crossover	10	Effect of dietary ALA on indices of lipid and coagulation status, and on PBMNC lipids	56d x 2	Serum: ALA increased 0.2 +/- 0.0 to 3.2 +/- 0.4; EPA remained at 0.6 +/- 0.0; DHA at 1.1 +/- 0.1 PBMNC FA: ALA increased 0.2 +/- 0.0 to 0.6 +/- 0.1; EPA increased 1.1 +/- 0.1 to 1.6 +/- 0.3; DHA decreased 1.4 +/- 0.1 to 0.9 +/- 0.2	Kelley, D.S., et al. 1993 (63)
Mildly hypercholesterolemia men	SFO (LA = 14.3g/d) FSO (ALA = 9.2 g/d) FO (EPA + DHA= 3.4g/d)	Randomized double blind	33	Investigation of the effect of n3 fatty acids on BP, TC and TG values	6 weeks	As % total fatty acids: FO = sevenfold increase in EPA and twofold increase in DHA FSO = doubled EPA and minimal effect on DHA.	Kestin, M., et al. 1990 (64)

Abbreviations: ALA=alpha-linolenic acid, CE=cholesteryl esters,, DHA=docosahexaenoic acid, DPA=docosapentaenoic acid, EPA=eicosapentaenoic acid, FO=fish oil, FS=flaxseed, FSO=flaxseed oil, LA=linoleic acid, OA=oleic acid, PL=phospholipids, PBMN=peripheral blood mononuclear cells, SFO=safflower oil, SO=sunflower oil, TG=triglyceride; BP=blood pressure.

**TABLE 3**

**Epidemiologic studies of ALA intake or tissue levels and CVD risk<sup>a</sup>**

Studies showing a benefit of ALA on CVD risk	Number of study participants	Average intake of ALA g/day	Main findings
<b>Case-control and cross-sectional studies</b>			
Cardiovascular Health Study (65)	179 cases, 54 controls	NR	A higher intake of ALA was associated with a lower risk of fatal ischemic heart disease.
Costa Rica study (48,66)	482 cases, 482 controls	NR	A higher concentration of ALA in adipose tissue was associated with a lower risk of nonfatal acute MI. The greatest protection against MI was seen in those with high levels of ALA and low levels of <i>trans</i> fatty acids in adipose tissue.
EURAMIC study (47)	639 cases, 700 controls in eight European countries and Israel	NR	A higher concentration of ALA in adipose tissue was associated with a lower risk of MI.
India study (67)	340 cases, 700 controls	NR	Consumption of mustard oil, which is rich in ALA, was associated with a lower risk of ischemic heart disease.
National University of Singapore Heart Study (68)	145 Asian Indian men and 147 Chinese men	NR	In this cross-sectional study, Asian Indian men had significantly lower plasma concentrations of ALA, DHA and total omega-3 fatty acids compared with Chinese men.

<b>Prevention trials</b>			
Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (69)	21,930 men who smoked cigarettes	0.9-2.5 (median = 1.5)	A higher ALA intake was associated with a lower relative risk of coronary death.
Lyon Diet Heart Study (21,41)	605 men and women who had survived a heart attack	1.74 – 1.8	An ALA-rich diet was associated with a 70% reduction in heart attacks and cardiac deaths.
MARGARIN Study (36,39)	282 men and women	6.3	Although the ALA-rich diet did not reduce the estimated 10-year risk of ischemic heart disease, it significantly decreased two factors associated with increased risk: <ul style="list-style-type: none"> <li>• fibrinogen levels (283)</li> <li>• C-reactive protein (284)</li> </ul>
MRFIT (70)	6,250 men in the usual care group	1.69	A higher intake of ALA was associated with a lower risk of CHD and all-cause mortality.
<b>Population-based studies</b>			
Family Heart Study (71-74)	1,575 – 4,584 men and women	Men = 0.81 Women = 0.68	In both men and women, a higher ALA intake was associated with... <ul style="list-style-type: none"> <li>• a lower risk of CHD (275)</li> <li>• lower blood levels of triglycerides (276)</li> <li>• a lower prevalence of carotid artery plaques (277).</li> <li>• a lower prevalence of calcified athero- sclerotic plaque (278)<sup>b</sup></li> </ul> <p>The reduction in CHD risk appeared to be independent of fish consumption (275).</p>

Health Professionals Follow-up Study (20,40)	43,757 men (1996), 45,722 men (2005)	0.8 – 1.5	A higher ALA intake was associated with a lower risk of non-fatal heart attack and total CHD.
Nurses' Health Study (42,43)	76,283 women	1.1 g <sup>c</sup> (median range = 0.66–1.39 g)	A higher ALA intake was associated with a lower risk of fatal ischemic heart disease (1999) and sudden cardiac death (282).

<sup>a</sup>Abbreviations = ALA, alpha-linolenic acid; CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction (heart attack); NR, not reported.

<sup>b</sup>Calcified plaques occur when calcium is deposited in the walls of arteries, forming a lesion; the presence of calcified plaques can be used to predict heart attacks and coronary death.

<sup>c</sup>Mean ALA intake in 1984 when a 116-item food questionnaire was completed by the cohort of women assessed for risk of ischemic heart disease.

<sup>d</sup>Mean intake.

<sup>e</sup>The association between ALA intake and CHD risk in this study was not statistically significant ( $p = .17$ ) after adjusting for age, body mass index, smoking, intakes of alcohol, energy, fiber, *trans* fatty acids and other factors.

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