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Review

Metabolism and functional effects of plant-derived omega-3 fatty acids in humans

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ABSTRACT

Alpha-linolenic acid (ALA) is an essential fatty acid and the substrate for the synthesis of longer-chain, more unsaturated ω-3 fatty acids, eicosapentaenoic acid (EPA), docosapentaenoic acid and docosahexaenoic acid (DHA). EPA and DHA are associated with human health benefits. The primary source of EPA and DHA is seafood. There is a need for sustainable sources of biologically active ω-3 fatty acids. Certain plants contain high concentrations of ALA and stearidonic acid (SDA). Here we review the literature on the metabolism of ALA and SDA in humans, the impact of increased ALA and SDA consumption on concentrations of EPA and DHA in blood and cell lipid pools, and the extent to which ALA and SDA might have health benefits. Although it is generally considered that humans have limited capacity for conversion of ALA to EPA and DHA, sex differences in conversion to DHA have been identified. If conversion of ALA to EPA and DHA is limited, then ALA may have a smaller health benefit than EPA and DHA. SDA is more readily converted to EPA and appears to offer better potential for health improvement than ALA. However, conversion of both ALA and SDA to DHA is limited in most humans.

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Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; CHD, coronary heart disease; CHF, congestive heart failure; CRP, C-reactive protein; CVD, cardiovascular disease; D5D, delta-5-desaturase; D6D, delta-6 desaturase; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GM, genetically modified; IL-6, interleukin-6; LA, linoleic acid; MUFA, monounsaturated fatty acid; NEFA, non-esterified fatty acid; PUFA, polyunsaturated fatty acid; RBC, red blood cell; RCT, randomised control trial; sICAM-1, soluble intercellular cell adhesion molecule-1; sVCAM-1, soluble vascular cell-adhesion molecule-1; SAA, serum amyloid A; SDA, stearidonic acid; SFA, saturated fatty acid; TAG, triacylglycerol; VLC, very long-chain; ω-3, omega 3; WHO, World Health Organisation.

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1. Introduction

Epidemiological studies and several randomised control trials (RCTs) demonstrate a positive relationship between consumption of very-long chain (VLC) ω-3 polyunsaturated fatty acids (PUFAs), specifically eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3), and long term health benefits [1], including a reduction in cardiovascular disease (CVD) morbidity and mortality [2–8], better visual and neurological development [9] and improvements in inflammatory conditions including arthritis [10] and asthma [11]. However, it is important to note that not all RCTs report reduced mortality in patients with existing CVD when they receive supplemental EPA and DHA [5,12,13]. Reasons for these inconsistencies have been discussed elsewhere [14].

The beneficial effects of EPA and DHA that have been reported involve modification of the biophysical properties of cell membranes [15–18], changes in specific cell signalling pathways and altered gene expression [19,20]. The primary source of EPA and DHA is seafood especially oily fish, although they are found in lower amounts in many other foods of animal origin. The World Health Organisation (WHO), as well as many other authorities, recommends consumption of oily fish once or twice a week in order to assure dietary intake of VLC ω-3 PUFAs with recognised health benefits [21]. However there are concerns about the sustainability of fish, and the current stocks of both farmed and wild fish are not likely to be sufficient to meet the needs of humans for VLC ω-3 PUFAs [22,23]. This has increased the interest in the metabolism, functional effects and health benefits of ω-3 PUFAs derived from plants, including alpha-linolenic acid (ALA; 18:3ω-3) and stearidonic acid (SDA; 18:4ω-3).

Sources of ALA include green plant tissues, some nuts (e.g. walnuts), rapeseed oil (also known as canola oil), soybean oil (in which ALA contributes 10% of total fatty acids), and flaxseeds and flaxseed oil (in which ALA contributes >50% of total fatty acids). ALA is the most abundant ω-3 PUFA in the diets of people who do not regularly consume oily fish or take concentrated VLC ω-3 PUFA supplements. Consumption of ALA in Europe, Australia and North America typically ranges from 0.6 to 2.3 g/d in adult men and 0.5 to 1.5 g/d in adult women [24–29]. Despite a higher dietary intake of ALA relative to EPA and DHA (approximately 25- and 15-fold greater [24]), concentrations of ALA within plasma and cell and tissue lipids are lower than those of EPA and DHA, apart from in adipose tissue stores. ALA is a metabolic precursor of EPA and DHA (Fig. 1). The biosynthetic pathway includes a series of desaturation, elongation and beta-oxidation reactions, with the rate-limiting enzyme considered to be that catalysed by delta-6 desaturase (D6D) (Fig. 1). However, it is also likely that there is regulation of other steps of the pathway, particularly the step involving translocation of 24:6ω-3 into the peroxisome. The observation that ALA levels in blood, cells and most tissues are much lower than the levels of EPA and DHA indicates that the primary biological role of ALA may be as a substrate for EPA and DHA synthesis. However, evidence suggests that conversion of

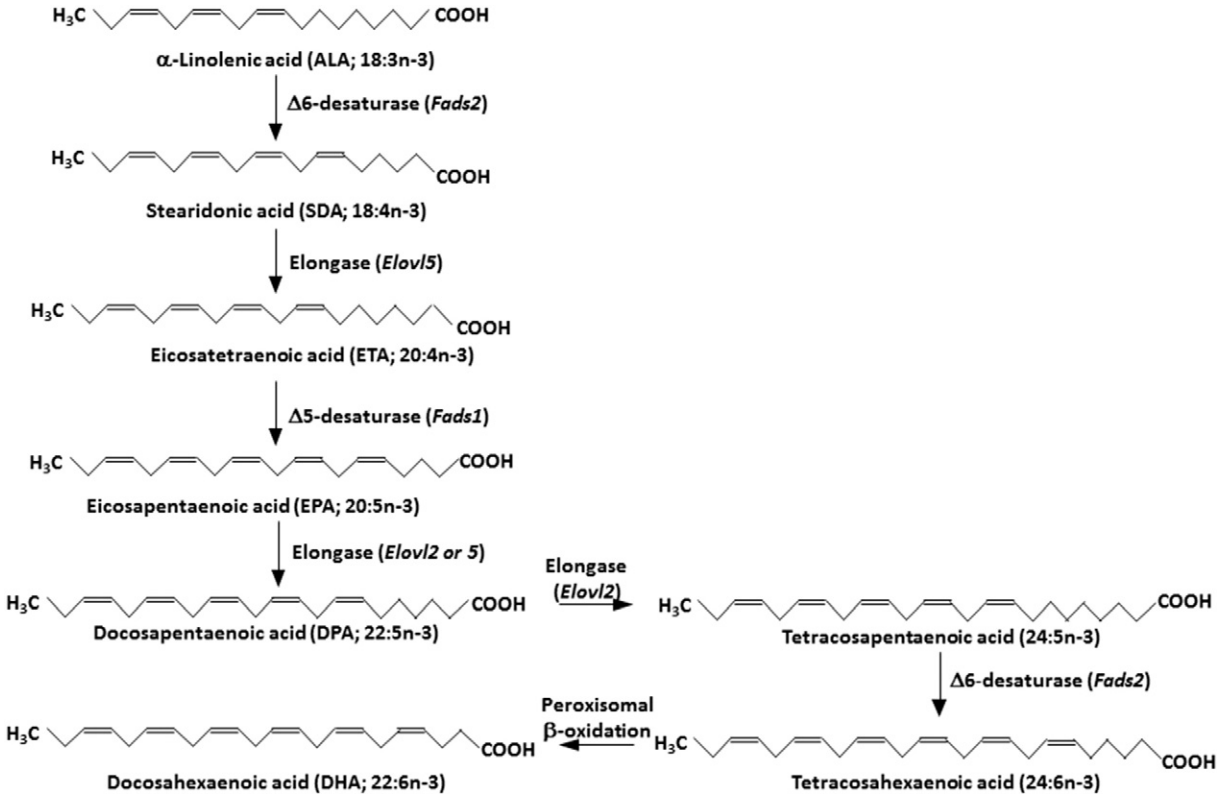


Fig. 1. The pathway of metabolic conversion of alpha-linolenic acid to longer-chain ω-3 polyunsaturated fatty acids. Genes encoding the various enzymes are shown in parentheses.

ALA to VLC ω-3 PUFAs may be poor [30,31], with conversion of ALA to EPA estimated at only 8 to 12% and to DHA much less at 1% [32]. This is discussed further in later sections.

ALA is one of the two essential fatty acids, the other being linoleic acid (LA; 18:2ω-6). Essential fatty acid deficiency is very rare in humans and evidence to support ALA essentiality comes mainly from patients who received parental (intravenous) feeds lacking ALA. Visual dysfunction was reported in 50% of children and 30% of adults receiving long-term total parenteral nutrition lacking ALA [33]. DHA in neural membrane phospholipids modulates the activities of several signalling pathways in the brain [34,35] and is critical for optimal retinal function [36,37]. The lack of ALA provision decreases availability of DHA for incorporation into neural and retinal membranes and may explain the impact of ALA deficiency on vision [33].

SDA is an intermediate in the pathway of EPA and DHA biosynthesis (Fig. 1), being the product of ALA desaturation by D6D. Since D6D is rate limiting for conversion of ALA to EPA, SDA is potentially a better substrate than ALA for the biosynthesis of VLC ω-3 PUFAs. There are few natural sources of SDA; it is found in Echium oil, where it contributes about 9–16% of fatty acids [38–40]. Levels of SDA have been substantially increased in soybean oil by genetic modification [41]. SDA levels in human blood, cells and tissues are normally very low.

This review discusses ALA and SDA as sources of ω-3 PUFAs to promote increased levels of VLC ω-3 PUFAs in human blood, cells and tissues and to provide the functional effects and health benefits of EPA and DHA. This review is based upon a previous article [42], but is broader in content and is updated.

2. Alpha-linolenic acid and stearidonic acid consumption in different countries

Typical intakes of ALA in adult Western populations are 0.5 to 2.3 g/d. Comparison between individual developed countries shows that average intake of ALA among adults has about a 3-fold variation [29,43,44] (Table 1). For example, French adults consume an average of about 0.8 g/d ALA, which is about one half to one third of the average intake of adults in Belgium, The Netherlands, Denmark, Finland and Germany (Table 1). The ratio of LA to ALA is important in determining relative rates of conversion of these precursor fatty acids to their long chain, more unsaturated derivatives (Fig. 1) as discussed in detail later. There is substantial variation in the dietary LA: ALA ratio among different countries (Table 1). The average ratio is 2- to 4-times higher among Spanish, Portuguese, Italian and Greek adults than among adults in other countries listed in Table 1.

Table 1
Reported mean daily intakes of alpha-linolenic acid (ALA) and linoleic acid (LA) in different countries for men and women [29,43,44].

Country	Alpha-linolenic acid (ALA) g/d		Linoleic acid (LA) g/d		LA:ALA ratio	
	Men	Women	Men	Women	Men	Women
Australia/New Zealand	1.4	0.9	12.8	9.1	9.1	10.1
Austria	1.5	1.3	13.6	12.4	9.1	9.5
Belgium	1.7	1.4	16.6	12.8	9.8	9.1
Denmark	2.2	2.1	14.3	10.9	6.5	5.2
Finland	1.8	1.3	8.1	5.8	4.5	4.5
France	0.9	0.7	8.3	6.3	9.2	9
Germany	1.59	1.32	9.3	8.0	5.8	6.1
Greece	0.6	0.7	9.3	9.9	15.5	14.1
Iceland	2.5	1.4	NA	NA	NA	NA
Italy	0.8[a]		14.5[a]		18.1[a]	
Netherlands	1.95	1.26	17.8	12.0	9.1	9.5
Norway	1.6	1.0	12.2	7.8	7.6	7.8
Portugal	0.7[a]		12.1[a]		17.3[a]	
Spain	0.8[a]		21.6[a]		27[a]	
Sweden	1.6	1.2	9.7	7.8	6.1	6.5
UK	1.4[a]		11.4[a]		8.1[a]	
USA	1.7	1.3	16.8	14	9.9	10.8

LA:ALA ratios are based on reported mean intakes.
[a] Data for men and women combined.

It is difficult to estimate the average dietary intake of SDA since there are few commonly consumed sources of this fatty acid and it is not usually included in nutrient databases. SDA is found in fish and other seafood, and some plants sources have been identified as being high in SDA. For example, seeds from members of the Boraginaceae family of plants, including the genera Borago (borage), Echium (e.g. Viper's bugloss) and Buglossoides (e.g. Corn gromwell) [45], contain SDA and the oils from those seeds contain approximately 15–22% of their fatty acids as SDA [45] (Table 2). A genetically modified (GM) soybean oil has been developed in which the SDA content has been enriched to 18–28% of total fatty acids [46,47]. Echium oil has a (naturally) lower content of SDA (approx. 12.5%) than this transgenic oil (Table 2), but it is commercially available [48] and has been used in intervention studies where the effects of increasing intakes of SDA have been investigated. More recently, Corn gromwell which is naturally rich in SDA, has been explored as a higher yielding commercial crop [48].

3. Bioavailability of alpha-linolenic acid and stearidonic acid from the diet

ALA absorption across the gut and its release into the bloodstream appear to be efficient in healthy humans. After ingestion of a single meal containing ^{2}H-labelled fatty acids, concentrations within the triacylglycerol (TAG) fraction of chylomicrons were measured. The results indicated a similar absorption and release of oleic acid (18:1ω-9), LA, and ALA [49], suggesting that bioavailability of ALA from a meal is comparable to that of other unsaturated fatty acids. In another study where patients with ileostomies were fed 100 g linseed oil, ALA was absorbed with 98% efficiency [50]. It is unlikely that SDA bioavailability would be limited in healthy people, although this has not yet been studied.

4. Alpha-linolenic acid and stearidonic acid metabolism in humans

4.1. Metabolic fates of alpha-linolenic acid

In common with other long chain fatty acids, ALA which has been absorbed from the gut passes into the circulation primarily esterified into TAG carried by chylomicron particles. Chylomicron TAG are hydrolysed by lipoprotein lipase expressed on the endothelium; adipose tissue lipoprotein lipase is upregulated in the post-prandial period which results in targeting of meal fatty acids for storage. As a result of this TAG hydrolysis, TAG-poor chylomicron remnants are formed. These are cleared from the circulation largely by hepatic receptor-mediated uptake. The fatty acids from the chylomicron remnants are processed in the liver and may re-appear in the bloodstream as components of TAG (in very low density lipoproteins) or phospholipids. Through these processes ALA from the diet can be made available to be incorporated into cell membranes and pools for storage (mainly in adipose tissue), energy production (many cell and tissue types) or conversion to longer-chain ω-3 PUFAs (this is believed to mainly occur in the liver). The metabolic fates of ALA are summarised in Fig. 2 and each fate is discussed in further detail below.

4.1.1. Incorporation of alpha-linolenic acid into adipose tissue

ALA is the most abundant ω-3 PUFA in human adipose tissue, accounting for approximately 1% of total fatty acids, while there are only trace amounts of DHA and EPA [51,52]. During the postprandial period there is metabolic drive to store fatty acids which is facilitated by a local increase in lipoprotein lipase activity and enhanced fatty acid uptake into adipose tissue and up-regulation of TAG synthesis. During the fasting state, hormone sensitive lipase hydrolyses adipose tissue TAG stores with the released fatty acids appearing in plasma as non-esterified fatty acids (NEFAs). When men consumed [U-^{13}C]ALA it was detected within the plasma NEFA pool after only 2 h and peaked at

Table 2
Typical fatty acid compositions of common vegetable derived food oils.

Fatty acid	Olive oil	Corn Oil	Sunflower oil	Safflower oil	Soybean oil	GM-soybean oil	Borage oil	Echium oil	Corn Gromwell oil	Flaxseed oil	Rapeseed oil
	% total fatty acids by weight										
16:0 (palmitic)	13	11	7	7	7–12	9–13	11.5	<1	11.4	9	4
18:0 (stearic)	3	2	5	2	2–5.5	2–5.5	4.9	3.7	8.3	9	2
18.1ω-9 (oleic)	71	28	19	13	19–30	10–20	19.5	15.9	27.1	18	62
18:2ω-6 (LA)	10	58	68	78	48–65	15–30	40.3	18.8	46.0	16	22
18:3ω-3 (ALA)	1	1	<1	<1	5–10	9–12	<1	28.4	7.3	57	10
18:3ω-6 (GLA)	NA	NA	NA	NA	NA	5–8	22.1	11.0	NA	NA	NA
18:4ω-3 (SDA)	NA	NA	NA	NA	NA	15–30	<1	12.5	NA	NA	NA

NA, not available, but likely to be very low if present at all. Data taken from [45–48].

6 h [30], indicating rapid release of non-esterified ALA possibly from chylomicron TAG. This could be due to lack of entrapment of the released ALA at the adipose tissue level, which could be a mechanism to facilitate supply of ALA to the liver. Heath et al. also demonstrated lack of entrapment of both EPA and DHA: they found that EPA in the NEFA pool was half of that in the meal given, and DHA was twice of that in the meal given, indicating less DHA is taken up into the adipose tissue compared to EPA [53].

Twenty one days after consuming the meal, the concentration of non-esterified [^{13}C]ALA in the fasting state was 2-fold greater in young women (mean age 28 years) than in men (age range 27 to 40 years) [30,31]. This suggests that there may be differences in ALA metabolism and storage between men and women, but this has not been explored across a range of ages so it is not clear if the sex differences seen in young adults are also apparent in children, adolescents and older adults.

Storage within adipose tissue is an important disposal route of dietary ALA [54]. The higher percentage of adipose tissue in women (average of 23% of body mass compared to 15% in men [42]) may suggest the storage of ALA in women is greater than in men. Table 3 provides an approximate calculation of total adipose tissue ALA content in normal weight men and women using an adipose tissue ALA proportion of 1% for both sexes. This results in an estimated ALA content of 97 g for men and 112 g for women (Table 3). This estimate would be affected by differences in dietary ALA intake, in body fatness, in the ALA content of different adipose depots, and in metabolism between individuals. However, our estimate indicates that there may be little difference in total adipose tissue stores of ALA between men and women, indicating that storage of dietary ALA in adipose tissue would not be a factor limiting hepatic conversion of ALA to EPA.

4.1.2. β-oxidation of alpha-linolenic acid

ALA has been shown to have the highest rate of β-oxidation among the unsaturated fatty acids tested [55]. Studies using tracers report that conversion of ALA to carbon dioxide via β-oxidation over the first hours following its consumption accounts for 15–35% of ALA consumed [30,31,54,56,57]. McCloy et al. followed ALA β-oxidation for 7 days following consumption of labelled ALA and showed a progressive increase in appearance of labelled CO_2 which accounted for ~70% of dietary ALA over a seven day period [54]. In comparison, LA oxidation at 9 hours after consumption accounted for 12% of the LA consumed and at 7 days after consumption ~40% of LA consumed [54].

4.1.3. Recycling of carbon from a-linolenic acid into saturated and monounsaturated fatty acids

During mitochondrial β-oxidation of ALA, carbon units generated in the form of acetyl-CoA can be recycled to synthesize saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), cholesterol and ketone bodies [56]. Burdge and Wootton measured concentrations of labelled saturated and monounsaturated fatty acids in men and women over 21 d after consumption of 700 mg [U-^{13}C]ALA. Labelled palmitic, stearic, palmitoleic and oleic acids were detected in plasma phosphatidylcholine (PC) and TAG [58]. There was greater incorporation of label from ALA into plasma PC than into TAG in both men and women. This difference in incorporation of label from ALA into plasma PC and TAG

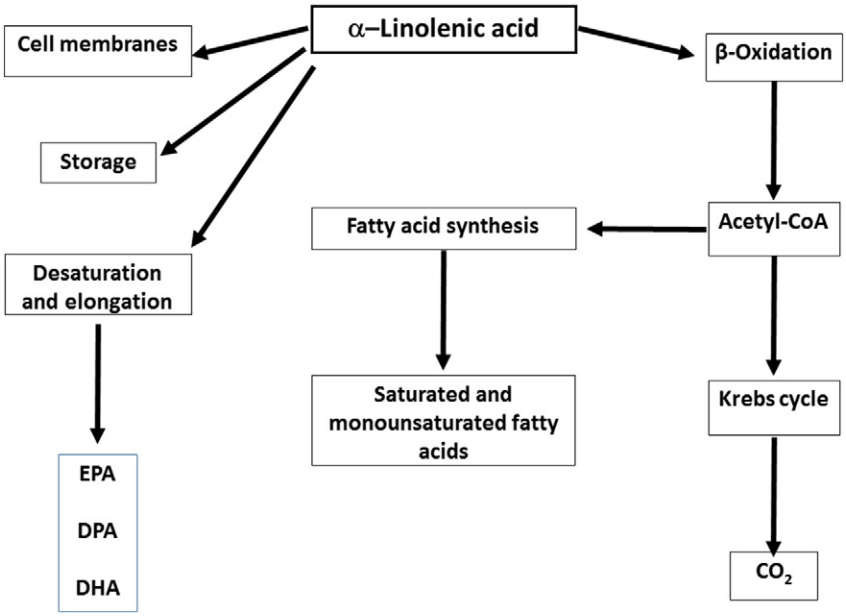


Fig. 2. Overview of the main metabolic fates of alpha-linolenic acid in humans. DHA docosahexaenoic acid; DPA, docosapentaenoic acid; EPA eicosapentaenoic acid.

Table 3
Estimated alpha-linolenic acid content of adipose tissue in men and women.

	Men	Women
Average body weight (kg)	80	60
Average body fat (% of body weight)	15	23
Total body fat (kg)	12	13.8
Adipose tissue TAG[a] (kg)	10.2	11.7
Adipose tissue stored fatty acid[b] (kg)	9.7	11.2
Adipose tissue ALA[c] (g)	97	112

[a] Based upon 85% of adipose tissue being TAG.
[b] Based upon 95% of TAG being fatty acid.
[c] Based upon ALA contributing 1% of adipose tissue TAG fatty acids.

was 6-fold in men and 25-fold in women. These findings suggest favourable channelling of SFA and MUFA synthesised from ALA by the recycling pathway into phospholipids by the liver in both men and women. This is in contrast to the molecular partitioning of the bulk of the hepatic SFA and MUFA pools which is towards TAG [42]. This recycling of carbon from ALA may be an important route for metabolism of ALA that is not required for EPA and DHA synthesis, or oxidised or stored in adipose tissue.

4.2. Metabolic fates of stearidonic acid

There are no tracer-based studies of SDA metabolism.

4.3. The pathway of conversion of alpha-linolenic acid and stearidonic acid to longer-chain polyunsaturated fatty acids

4.3.1. Overview of the pathway

ALA and SDA are converted to EPA and DHA through a series of elongation and desaturation processes occurring predominantly within the endoplasmic reticulum, excluding the last β-oxidation reaction which forms DHA and occurs in peroxisomes (Fig. 1). This pathway is believed to mainly occur within the liver [59], but there is some evidence that brain, retina and testis also have high expression of the genes encoding the relevant enzymes compared with other tissues [60,61]. Competition exists between the conversion of ω-6 and ω-3 fatty acids as both utilise this same metabolic pathway. The initial conversion of ALA to SDA is catalysed by D6D and is considered to be the rate limiting reaction in the pathway [62,63]. D6D is encoded by the gene fatty acid desaturase 2 (*Fads2*) and has a preference for ALA over LA: the Km of rat D6D for ALA is between 29 and 33 μM while the Km for LA is between 43 and 92 μM [64]. However, when there is much greater availability of LA than ALA the metabolism of the former exceeds that of the latter. The D6D reaction results in insertion of a double bond at the delta-6 position of ALA to form SDA. SDA is converted to 20:4ω-3 by the addition of 2 carbons by the enzyme elongase-5, encoded by fatty acid elongase 5 (*Elovl5*). 20:4ω-3 is then converted to EPA by insertion of a double bond at the delta-5 position by delta-5-desaturase (D5D), which is encoded by the gene fatty acid desaturase 1 (*Fads1*). EPA can be elongated by elongase 2 (encoded by *Elovl2*) or elongase 5 to form docosapentaenoic acid (DPA; 22:5ω-3) and then DPA is elongated by elongase 2 to yield 24:5ω-3. The subsequent desaturation of 24:5ω-3 to 24:6ω-3 again uses a D6D activity, that seems to involve the same D6D as in the first step of the pathway [59]. This is evidenced by the observations that skin fibroblasts from a patient with D6D deficiency were unable to desaturate either LA or 24:5ω-3 [65] and that rat D6D was able to desaturate both ALA and 24:5ω-3 [66]. 24:5ω-3 is then translocated to the peroxisome where it undergoes one round of β-oxidation to form DHA [63,67,68]. Recent mechanistic studies suggest conversion of EPA to DHA may be limited by the second product of *Elovl2* (24:5ω-3) [69]. This suggests that understanding the action and regulation of *Elovl2* may be critical in understanding DHA synthesis.

SDA is an intermediate in this metabolic pathway, being synthesised from ALA via the action of D6D (Fig. 1). EPA biosynthesis from SDA is considered to be more efficient than that from ALA due to it not requiring this first catalytic step, and SDA has been referred to as 'pro-EPA' because of this enhanced conversion [70].

4.3.2. Genetic and epigenetic influences on the conversion pathway

The activity of the PUFA biosynthesis pathway is differentially regulated by dietary fatty acids at the level of transcription of *Fads1* and *Fads2*, the genes which encode D5D and D6D, respectively [71]. Such regulation appears to involve activation of peroxisome proliferator activated receptors and sterol response element binding protein-1c [72]. Active response elements have been identified in *Fads2* for both of these transcription factors [72]. One implication of these findings is that capacity for conversion of ALA to longer chain metabolites may modified by genetic and/or epigenetic processes which influence the transcription of the *Fads* genes. In humans, *Fads* genes are located in head to head (*Fads 1* and *2*) and tail to tail (*Fads 2* and *3*) orientations on chromosome 11 [73]. This suggests the possibility of coordinated changes in gene expression by mutations or epigenetic changes in shared sequences.

4.3.2.1. Genetic influences on the conversion pathway. A number of single nucleotide polymorphisms (SNPs) have been identified in *Fads1* and *Fads2*, some of which have been associated with differences in ω-3 and/or ω-6 PUFA status [71–93]. In general, minor alleles of both *Fads1* and *Fads2* have been shown to be associated positively with essential fatty acid status, but negatively with longer chain PUFA status (Table 4). This suggests that these polymorphisms impair the activity of the gene or the encoded enzyme, although there is limited direct evidence to support such effects. A SNP (rs968567) in *Fads2*, which has been associated with lower concentrations of longer chain PUFAs [74] has been shown to create response elements for STAT 1 and 3, and ELK-1, and to increase ELK-1 binding to this region [75]. In addition, incubation of lymphocytes with simvastatin was associated with 20 to 40% greater expression of *Fads2* and *Fads1* compared to cells containing the minor alleles of SNPs associated with a SREBP response element to the major alleles [76]. Insertion-deletion mutations proximal to the SREBP-1c response element also modified response to simvastatin [76]. These findings provide proof-of-principle evidence that at least some SNPs in the 5′-regulatory region of *Fads2* can alter the level of transcription. SNPs in *Elovl2*, which encode elongases 2, have also been shown to alter longer chain PUFA status. Minor alleles of SNPs in *Elovl2* have been associated with lower DHA [77] and EPA [78] status. In addition, a SNP in *Elovl5* has been shown to be associated with lower capacity to convert EPA to DPA [91] . Overall, these findings show that minor alleles of SNPs in genes encoding fatty acid desaturase or elongase genes are associated with lower status of LC PUFAs. However, the absolute differences in proportions of individual fatty acids between individuals bearing different genotypes tend to be relatively small and there are no studies to date that have linked such differences to altered cell function. Such studies are important in order to know which polymorphisms, if any, need to be considered when making recommendations for consumption of ALA, EPA and DHA.

4.3.2.2. Epigenetic influences on the conversion pathway. There is emerging evidence that the expression of genes involved in PUFA biosynthesis can be modified by changes in the epigenetic regulation, although to date this evidence has been obtained largely from studies in animal models. Homocysteine concentration has been shown to be associated positively with the methylation of CpG loci in the *Fads2* promoter of mice [79]. Increasing the proportion of ALA to LA in the diet of pregnant mice increased the average DNA methylation of the *Fads2* promoter, but not intron 1, in the liver by 1% to 2% which was negatively associated with the level of the mRNA transcript [80]. Feeding female rats diets containing either 3.5% (low fat), 7% (adequate fat) or 21% (high fat) fat (w/w) enriched in either saturated and monounsaturated fatty acids derived from butter or in EPA and DHA derived from fish oil reduced

Table 4
Association studies of polymorphisms in *Fads* and *Elovl* genes and fatty acid outcomes.

Cohort	SNP	Main findings	Outcome	Reference
European adults	*Fads1* rs174544, rs174544, rs174546, rs174546, rs174556, rs174561. Intergenic FADS 1 - 2 rs174568, rs3834458, rs968567 *Fads2* rs99780, rs174570, rs2072114, rs174583, rs174589, rs174602, rs174620, rs482548	Haplotype explained up to 28% of the variation in the proportion of 20:4n − 6, up to 7% of the variation in 20:5n − 3 and up to 3% of the variation in 22:6n − 3	Proportions of fatty acids in blood serum	Schaeffer et al. (2006)[74]
Patients with cardiovascular disease	*Fads1* rs174545, rs174556, rs174561 Intergenic FADS 1 – 2 rs3834458 *Fads2* rs174570, rs2524299, rs174583, rs174589, rs498793, rs174611 Intergenic FADS 2 – 3 rs17831757, rs174627 *Fads3* rs1000778	All minor alleles except for rs498793 and rs17831757 were associated with higher proportions of essential fatty acids in erythrocytes compared to the major allele. The minor allele of rs498793 was associated with higher proportions of 20:4n − 6 and 20:5n − 3. All other minor alleles were associated with lower proportions of these fatty acids compared to the major allele. There were variable associations between the minor allele and the proportion of 22:6n − 3.	Proportions of fatty acids in erythrocytes	Malerba et al. (2008)[86]
Pregnant and lactating women	*Fads1 and 2* cluster rs174553, rs174561, rs174583, rs99780, rs498793, rs174575	Women homozygous for the minor alleles of rs174553, rs99780 and rs174583 had lower 20:4n − 6 and higher 18:2n − 6 in plasma and erythrocyte phospholipids Breast milk fatty acids were influenced by genotype, with significantly lower 14:0, 20:4n − 6, and 20:5n − 3 but higher 20:2n − 6 with the minor allele homozygotes of rs174553, rs99780 and rs174583 and lower 20:4n − 6, 20:5n − 3, 22:5n − 3, and 22:6n − 3 with the minor allele homozygotes of rs174575.	Proportions of fatty acids in plasma phospholipids, erythrocyte phospholipids or breast milk	Xie et al. (2008)[87]
InCHIANTI study cohort, adults over 65 years	*Fads* cluster rs2277324, rs16940765, rs17718324 (replaced with rs923838), rs174537 *Elovl2* rs953413	Carriers of the minor allele of *Fads1*–associated rs174537 had a lower proportion of 20:4n − 6 than those with the major allele, and genotype at this locus accounted for 18.6% of the variance in 20:4n − 6. rs174537 and rs953413 were associated with a lower proportion of 20:5n − 3. The minor allele of *Elovl2* rs953413 was associated with a higher proportion of 20:5n − 3 than the major allele.	Proportions of fatty acids in blood	Tanaka et al. (2009)[78]
KOALA and LISA Birth Cohort Studies, young children	*Fads1* and *2* cluster: rs174545, rs174546, rs174556, rs174561, rs3834458	All SNPs associated with several PUFAs.	Proportions of fatty acids in blood	Rzehak et al. (2010)[88]
Pregnant and lactating women (KOALA study)	*Fads1* rs174561 *Fads2* rs174575 Intergenic *Fads 1 – 2* rs3834458	The proportions of 18:2n − 6 and 18:3n − 3 were higher and their respective metabolites lower in women carrying the minor alleles for all three SNPs in plasma and breast milk, with the exception of 18:3n − 6 and 20:4n − 6 in breast milk which did not vary with genotype.	Proportions of fatty acids in plasma during pregnancy and in breast milk	Molto-Puigmartı et al. (2010)[89]
Lactating women in the Ulm Birth Cohort study	*Fads1* rs174547, rs174556, *Fads2* rs174602, rs498793, rs526126 Intergenic *Fads2 – 3* rs174626 *Fads3* rs1000778, rs174455	Significant associations of genotype with 20:4n − 6 at 1.5 and 6 months breast feeding, but not with change over time.	Fatty acid concentrations in breast milk	Lattka et al. (2011)[90]
Lactating women	*Fads* Cluster rs174537, rs412334, rs968567, rs174570, rs174575, rs2072114, rs2851682, rs174602, rs526126, rs174626, rs174627, rs472031 rs422249, rs7482316, rs174455, rs174464, rs528285, rs174468, rs13966 *Elovl2* rs3734397, rs953413, rs10498676, rs6936315, rs3798719, rs7744440, rs13204015 *Elovl5* rs17544159, rs2281274, rs2294859, rs761179, rs9395855, rs209494, rs11968589, rs209505, rs2397142, rs12207094	Minor alleles of rs174537, rs174570, rs2072114, rs174602, rs526126, rs174626, rs174464, rs174468 in the *Fads* cluster were associated with lower proportion of 20:4n − 6 and rs174602, and 174464 were associated with lower 22:6n − 3. The minor alleles of rs953413 and rs3798719 in *Elovl2* were associated with higher 20:5n − 3. None of the SNPs in *Elovl5* were associated with the proportions of any n − 6 or n − 3 PUFA.	Proportions of fatty acids in colostrum	Morales et al. (2011)[91]
Japanese adults	41 SNPs in the *Fads* cluster	rs2845573, rs2727270, rs2727271, rs2524299, rs2072114, rs2851682 in *Fads2* intron associated with higher *Fads1* expression.	Fads1 and 2 expression	Reardon et al. (2012)[76]

(continued on next page)

Table 4 (*continued*)

Cohort	SNP	Main findings	Outcome	Reference
		A 10-SNP haplotype in *Fads2* was associated with lower *Fads1* expression. Treatment with SRBP-1C or LXR agonists induced greater expression of Fads1 and Fads2 in lymphocytes carrying the minor alleles than those with the major alleles. Polymorphic insertion–deletion mutations were identified downstream of a putative SRBP response element.		
Healthy men and women	*Fads1* rs174537, rs174561 *Fads2* rs3834458	Minor allele haplotype was associated with higher proportions of 18:2n − 6 (2–3%) and 18:3n − 3 (5–9%), but lower proportions of 20:4n − 6 (18%). 20:5n − 3 (13-–16%) and 22:6n − 3 (6–10%).	Proportions of fatty acids in plasma and erythrocyte phospholipids	Al-Hilal et al. (2013)[92]
Caucasian and East Asian adults	*Fads1* rs174547, rs412334, rs695867,rs740006 *Fads2* rs174570, rs174576, rs174579, rs174593, rs174602, rs174611, rs174626, rs174627, rs17831757, rs2072114, rs2845573, rs2851682, rs482548, rs498793, rs526126, rs968567	Caucasians: Men, negative associations between n − 6 ADI and minor alleles of *Fads1* rs174547 and *Fads2* rs174576, and rs174611. Women, negative associations between the minor alleles of *Fads1* rs174547 and *Fads2* rs174570, rs174576, rs174679, rs174611, rs174593, rs174626 rs2072114, rs2845573 and rs2851682 and n − 6 ADI. In East Asian women, the minor alleles of *Fads2* rs174602, rs174626, rs2072114, rs2845573, and rs2851682 were negatively associated with n − 6 ADI.	Aggregate desaturase index (ADI: 20:4n − 6/18:2n − 6; 20:5n − 3/18:3n − 3) in plasma.	Abdelmagid et al. (2015)[93]

the proportion of AA in the liver of the adult offspring irrespective of the type of fat consumed [81]. There was no effect of maternal fat intake on *Fads1* promoter methylation or its mRNA expression. However, there was a positive relationship between maternal dietary fat content and the methylation of specific CpG loci in the 5′regulatory region of *Fads2* in the liver of the adult offspring irrespective of the type of fat consumed. This was associated negatively with *Fads2* mRNA expression. Mutation of a CpG located -394 bp from the *Fads2* transcription start site within a putative estrogen response element induced lower transcriptional response to estrogen compared to the wild type promoter. This suggests that this CpG is involved in the regulation of *Fads2* expression [82]. Feeding pregnant rats diets containing 7% or 21% (w/w) safflower oil, hydrogenated soybean oil, fish oil or soybean oil also induced increased *Fads2* methylation and decreased its expression in aorta from the adult offspring. However, the magnitude of change was related to the type of oil consumed by the dam [82]. Thus, induction of differential changes in methylation by different fatty acids may be tissue-dependent. Feeding adult rats a fish oil-enriched diet for 9 weeks induced reduced *Fads2* mRNA expression and increased methylation of CpG loci in the 5′regulatory region of *Fads2* compared to those fed soybean oil [81]. However, these changes were reversed in rats that were switched from fish oil to soybean oil. These findings suggest that that persistence of changes in DNA methylation induced by dietary fat is contingent on the period of exposure during the life course.

There is some evidence that DNA methylation also modifies fatty acid desaturase activity in humans. Higher methylation of CpG loci in the intergenic region between the *Fads1* and *2* genes was associated with lower expression of D5D and, to a lesser extent, D6D in human liver [83]. Furthermore, patients who had attempted suicide showed a trend toward lower CpG methylation within a region of *Fads2* upstream of the transcription start site and higher methylation in a region upstream of the *Elovl5* transcription start site compared to controls [94]. Such differences were not associated with statistically significant variation in plasma EPA and DHA concentrations after correction for age and sex. However, since astocytes and glial cells, but not neurones, are able to synthesise LC PUFAs [95], it is possible that these polymorphisms may have had a significant effect on PUFA concentrations locally within the brain. Methylation of one CpG locus in *Fads2* in blood has been shown to be related negatively to delayed memory performance in young children [84]. Furthermore, moderate intake of EPA and DHA ethyl esters has been shown to induce altered methylation of CpG loci within the 5′-regulatory regions of *Fads2* and *Elovl5*, but not *Fads1* and lovl*2*, in peripheral blood mononuclear cells from patients with mild renal impairment compared to individuals who consumed the same amount of olive oil [85]. However, the magnitude, direction and loci affected differed between men and women. These findings were replicated for the omega-3 ethyl ester supplement in healthy volunteers. This suggests that induced changes in DNA methylation may be one mechanism by which omega-3 dietary supplements can alter immune function. This is supported by the observation that immune response is impaired in *Fads2* null mice [96].

4.4. Conversion of alpha-linolenic acid and stearidonic acid to longer-chain PUFAs in humans

Knowledge of the extent to which humans can convert ALA and SDA to longer-chain PUFAs is based on short term studies with stable isotopes and longer term studies involving increased consumption of the fatty acids. As explained above, the biosynthesis of longer-chain ω-3 PUFAs from SDA is considered to be more efficient than from ALA due to it not requiring the rate limiting step catalysed by D6D and studies which have directly compared consumption of SDA to that of ALA have shown a greater increase of EPA in blood lipids and cells than seen for ALA consuming subjects, although no significant changes in plasma or red blood cell (RBC) DHA levels were observed [97–100].

The conversion of ALA to both EPA and DHA appears to be more efficient in women than in men. In men, conversion of ALA to EPA has been reported to be between 0.3% and 8%, and conversion to DHA < 1%, whereas in women up to 21% conversion to EPA and up to 9% conversion to DHA have been reported [101]. It has been suggested that the higher rate of conversion in women may be because of their greater requirement to produce VLC ω-3 PUFAs during pregnancy and lactation [102].

In studies where humans have increased their intake of SDA through supplementation, incorporation of SDA itself into blood lipids and cells has been very small but significant increases in EPA have been reported [97–99]. For example, Harris et al. demonstrated that SDA modifies the ω-3 index (EPA + DHA in RBCs) with greater efficiency than ALA [103]. This effect is likely due to increased EPA rather than DHA, since other studies show no net appearance of DHA when SDA is consumed [97, 103–107]. Other studies comparing the effects of SDA, ALA or EPA supplementation on the increase in blood and blood cell VLC ω-3 PUFAs

Table 5
Estimated conversion of alpha-linolenic acid to ω-3 long-chain polyunsaturated fatty acids.

Subjects	Form in which α-linolenic acid provided	Dose	Outcomes	EPA	DPA	DHA	Reference
Adult M	^{2}H-labelled mixed TAG	3.5 g	Absolute and relative AUC concentrations in total plasma lipids	50 μg/ml (8%)	26 μg/ml (4%)	25 μg/ml (4%)	Emken et al. (1999)[108]
Adults[a]	^{2}H-labelled mixed ethyl ester	1 g	Concentrations in total plasma lipids	57 ng/ml	ND	<2 ng/ml	Salem et al. (1999)[109]
Adult M + F	[U^{13}C]methyl ester	45 mg	Peak amount in total plasma lipids adjusted for estimated total blood volume	120 μg	50 μg	~ 10 μg	Vermunt et al. (2000)[57]
Adult M + F	^{2}H-labelled mixed ethyl ester	1 g	Mathematical modelling of kinetic parameters following consumption of beef based diet: data expressed as conversion efficiency from ALA using total plasma lipids	0.2%	0.13%	0.05%	Pawlosky et al. (2001)[110]
Adult M	[U−^{13}C] free FA	0.7 g	Concentrations in plasma TAG, NEFA, CE and PC over 21 d. Fractional conversion estimated from time x concentration AUC	8%	8%	ND	Burdge et al. (2002)[30]
Adult F	[U−^{13}C]free FA	0.7 g	Concentrations in plasma TAG, NEFA, CE and PC over 21 d. Fractional conversion estimated from time x concentration AUC	21%	6%	9%	Burdge & Wootton (2002)[31]
Adult M	[U−^{13}C] free FA		Consumption of 17 g ALA/d and/or LA/d for 12 weeks followed by tracer study and measurement in total plasma lipids				Hussein et al. (2005)[111]
			-High ALA diet	0.29%	0.05%	<0.01%	
			-High LA diet	0.19%	0.02%	<0.01%	
			- High ALA and High LA diet	0.26%	0.04%	<0.01%	
Adults[a]	[U−^{13}C] free FA	30 mg bolus + 8 x 20 mg daily doses	Kinetic model, 7% of ingested ALA was incorporated into plasma PL 99.8% of this was converted to EPA and 1% each to DPA and DHA	6.98%	0.07%	0.07%	Goyens et al. (2005)[112]

DPA, docosapentaenoic acid; M, male; F, female; TAG, triacylglycerol; AUC, area under the curve; PC, phosphatidylcholine; ND, not detected; CE cholesteryl ester; PL, phospholipid.
[a] Sex not disclosed.

report that EPA supplementation resulted in higher EPA levels than SDA supplementation, which in turn was higher than ALA supplementation. However, neither ALA, nor SDA, nor EPA resulted in higher DHA levels [97–100].

4.4.1. Stable isotope studies

Several studies using ALA labelled with either ^{13}C or ^{2}H provide estimates for conversion rates to longer chain more unsaturated derivatives in humans [30,31,57,108–112] (Table 5). Although the limitations of such studies have been discussed by Emken et al. and by Burdge et al. who suggest there are unresolved issues regarding tracer studies including; standardisation, quantification, and which lipid pool should be used as a measurement of fatty acid metabolism [113,114]. However, there are currently few feasible alternatives to stable-isotope tracer techniques in studying the metabolism of fatty acids *in vivo*. These tracer studies generally conclude that the conversion of ALA to the longer-chain metabolites EPA and DPA occurs, but is limited, and is greater than conversion to DHA (Table 5). For example, based upon appearance of label in total plasma lipids Emken et al. reported the highest estimated fractional conversion of ALA to DHA of 4% [49], though most other studies have concluded much lower conversions than this (e.g. 0.05% or even less) [57,58,108–112,115,116] (see Table 5 for details including the lipid pools examined). Burdge et al. detected no significant incorporation of stable-isotope into DHA above background ^{13}C enrichment in men aged 27 to 40 years [30]. Vermunt et al. also described that increasing intake of ALA by a mixed group of healthy men and women actually decreased the synthesis of EPA, DPA and DHA [57], though this observation is in contrast to other studies [56].

Efficiency of conversion of individual steps within the metabolic pathway has been estimated from kinetic analysis based on concentrations of ^{2}H labelled fatty acids in plasma [110]. Overall conclusions from the study were in agreement with others and showed 0.2% conversion of ALA to EPA, 0.13% to DPA and 0.05% to DHA.

Burdge et al. have also identified significant differences in conversion of ALA between women and men, with women having a significantly greater capacity than men to synthesise EPA and DHA from ALA. The estimated mean net conversion rate of ALA to EPA in women (mean age 28 years) was 21% compared to 8% in men (age range 27 to 40 years) and the estimated mean net conversion rate of ALA to DHA was 9% in the women and 0% in the men [30,31]. Pawlosky et al. reported a decrease in the conversion of DPA to DHA in women, but not men,after consuming a fish based diet providing EPA + DHA [115]. Modification of the diet by increasing consumption of EPA in combination with DHA, or of DHA alone, may affect the extent of ALA conversion, as there is potential for feedback inhibition of the pathway. A small number of studies have examined the impact of modifying the diet on the extent of ALA conversion to EPA and DHA. Emken et al. investigated the effect of increased consumption of DHA. Subjects consumed either <0.1 g/d DHA or supplemented their diet with 6.5 g/d of purified DHA for 90 days [108]. The group receiving supplemental DHA showed 76% lower EPA synthesis from ALA and 88% lower DHA synthesis. Burdge et al. compared conversion of ALA within individuals after consumption of 1.6 g/d EPA plus DHA over an 8 week period and showed that synthesis of EPA and DPA was decreased, but synthesis of DHA was unaffected [56]. These studies indicate that an increase in intake of VLC ω-3 PUFAs downregulates their synthesis from ALA [42]. Tang et al. suggest this is due to activation of the peroxisome proliferator-activated receptor-alpha leading to inhibition of D6D transcription [117]. Differences between findings of the two studies may be due to the much lower intake of DHA in the Burdge et al. study, which may have been insufficient to inhibit the DPA to DHA conversion. Another study reported that chronic consumption of LA at 17 g/d inhibited the conversion of ALA and that, conversely chronic consumption of ALA at 17 g/d inhibited the conversion of the LA [111].

In a recent study, Lin et al. analysed measurements of fractional percentages of plasma $^{2}H_5$-ALA and ^{13}C-EPA directed toward the synthesis of labelled DHA in 11 newborn infants by using compartmental modelling procedures [118]. Approximately 0.04% of labelled ALA was converted to plasma EPA, which was efficiently converted to DPA and on to DHA. Comparison of findings using $^{2}H_5$-ALA and ^{13}C-EPA suggested

Table 6
Studies investigating the effect of increased alpha-linolenic acid consumption on the fatty acid composition of blood lipids in adult human subjects.

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Blood lipid fraction	Change in proportion of total fatty acids from baseline (%)			Reference
					EPA	DPA	DHA	
M + F	7.8	Flaxseed oil capsules	2	Plasma PC	108	NA	−14	Sanders and Younger (1981)[131]
M	14	Flaxseed oil capsules	6	Plasma PL	100	NA	NA	Chan et al. (1993)[169]
M	20[a]	Flaxseed oil	8	Total serum	0	20	38	Kelley et al. (1993)[128]
M	13.7	Flaxseed oil + spread	4	Plasma PL	140	NA	14	Mantzioris et al. (1994)[130]
				Plasma CE	150	NA	17	
				Plasma TAG	200	NA	20	
M	5.6	Flaxseed oil	6	Plasma CE	−27	NA	NA	Freese et al. (1994)[126]
M + F	9	Muffins incorporating flaxseeds	4	Plasma PL	33	36	0	Cunnane et al. (1995)[121]
				Plasma TAG	300	250	−33	
M	3.7	Flaxseed oil + spread	4	Plasma PL	80	30	11	Li et al. (1999)[129]
				Plasma TAG	50	25	0	
				Plasma PL				
	15.4	Flaxseed oil + spread	4	Plasma TAG	367	50	−5	
					300	50	0	
M + F	3	Perilla oil	42	Total serum	45	NA	21	Ezaki et al. (1999)[123]
M + F	6.3	Spread	52	Serum CE	40	NA	50	Bemelmans et al. (2002)[119]
F	10	Flaxseed oil capsules	4	Total plasma	129	60	−8	Francois et al. (2003)[125]
M	4.7	Flaxseed oil capsules	12	Plasma PL	60	NA	3	Wallace et al. (2003)[132]
M + F	4.5	Spread	24	Plasma PL	90	5	−3	Finnegan et al. (2003)[124]
	9	Spread	24	Plasma PL	133	33	6	
M + F	0.75	ALA ethyl ester in capsules	3	Plasma PL	15	0	−3	James et al. (2003)[97]
	1. 5	ALA ethyl ester in capsules	3	Plasma PL	23	5	−7	
F	2.8	Spread	26	Plasma PL	−26	NA	−22	De Groot et al. (2004)[122]
M + F	17.5[a] (6.5% energy)	Walnuts, walnut oil and flaxseed oil	6	Serum	160	25	−7	Zhao et al. (2004)[134]
M + F	3	Flaxseed oil capsules	26	Total plasma	53	NA	4	Harper et al. (2006)[127]
M + F	1.1% energy	Spread	6	Plasma PL	10	NA	0	Goyens et al. (2006)[32]
M + F	19.1	Walnuts, walnut oil and flaxseed oil	6	Total serum	160	33	−6	Zhao et al. (2007)[133]
F	9	Bread and grains incorporating flaxseeds	48	Total plasma	51	39	27	Dodin et al. (2008)[139]
M + F	4	Baked goods incorporating flaxseeds	10	Total plasma	13	11	−22	Bloedon et al. (2008)[137]
M + F	1	Flaxseed oil capsules	12	Total plasma	7.1	NA	−1.2	Kaul et al. (2008)[143]
M + F	4.29	Muffins incorporating						Austria et al. (2008)[135]
	6.50	– Whole flaxseeds	36	Total plasma	−11	NA	36	
	5.74	– Ground flaxseeds	36	Total plasma	14	NA	4	
		– Flaxseed oil	36	Total plasma	90	NA	20	
M	5.4	Flaxseed oil	4	Total plasma	46	20	−6	Barden et al. (2009)[136]
M + F aged 18–29 y	6	Muffins incorporating						Patenaude et al. (2009)[144]
		– Ground flaxseeds	4	Total plasma	22	NA	10	
		– Flaxseed oil	4	Total plasma	64	NA	−3	
M + F aged 45–69 y	6	Muffins incorporating						Patenaude et al. (2009)[144]
		– Ground flaxseeds	4	Total plasma	23	NA	101	
		– Flaxseed oil	4	Total plasma	−2	NA	9	
M + F	4.4	Spread	6	LDL	24	NA	−6.8	Egert et al. (2009)[140]
F	1.2	Flaxseed oil	12	Total plasma	14	−5	−9	De Spirt et al. (2009)[269]
M + F	15.3	Walnuts, walnut oil and flaxseed oil	6	Total plasma	50	17	6	West et al. (2010)[149]
M + F	7.4	Baked goods incorporating						Taylor et al. (2010)[148]
		– Milled flaxseeds	12	Total plasma	33	11	−9	
		– Flaxseed oil	12	Total plasma	44	20	−3	
M + F	4.7	Walnuts	4	Plasma PL	10	6	2	Chiang et al. (2012)[138]
M + F	2	Spread	160	Total plasma	17	NA	0	Geleijnse et al. (2012)[141]
M + F	2.9	Flaxseeds	12	Total serum	25	NA	0	Hutchins et al. (2013)[142]
	5.8	Flaxseeds	12	Total serum	20	NA	−9	
M + F	0.5	Cheese enriched with ALA	3	Total plasma	36	NA	11	Pintus et al. (2013)[145]
M + F	7	Baked goods incorporating flaxseeds	24	Total plasma	43	NA	5	Rodriguez et al. (2013)[147]
M + F	7	Flaxseed oil	10	Total plasma	31	14	−7.5	Dittrich et al. (2014)[106]
M + F	3	Sesame/pumpkin/flaxseed mix	12	Serum PL	22	11	9	Ristic-Medic et al. (2014)[146]

DPA, docosapentaenoic acid; M, male; F, female; PC, phosphatidylcholine; NA, data not available; CE cholesteryl ester; PL, phospholipid; PE, phosphatidylethanolamine; LDL, low density lipoprotein.
[a] Approximate intake.

different metabolic handling of endogenous EPA compared to exogenous EPA. However, preformed EPA was still 3.6 times more effective in terms of DHA synthesis than ALA [118].

4.4.2. Studies investigating longer term changes in intake of alpha-linolenic acid and stearidonic acid

Concentrations of ω-3 PUFAs within plasma or blood cell pools have been measured after long term (weeks to months) changes (usually increases) in intakes of ALA or SDA in humans. Although there are numerous studies reporting the results of increased ALA intake in humans [32,97,106,119–164] (see Tables 6 and 7), fewer studies have investigated the effects of increased SDA intake [38,97–100,104–107,165–167] (see Table 8).

4.4.2.1. Increased intake of alpha-linolenic acid. A number of studies have reported the effects of consuming increased amounts of ALA for a period of weeks to months on fatty acid composition within plasma or blood cell lipids (Tables 6 and 7). Such dietary modification has usually been

Table 7
Studies investigating effect of increased alpha-linolenic acid consumption on the fatty acid composition of circulating cells in adult human subjects.

Subjects	ALA intake (g/d)	ALA form	Duration (weeks)	Cell lipid fraction	Change in proportion of total fatty acids from baseline (%)			Reference
					EPA	DPA	DHA	
M + F	7.8	Flaxseed oil capsules	2	Platelet PL	100	NA	−3	Sanders and Younger (1981)[131]; Sanders and Roshanai (1983)[158]
M	8.5	Rapeseed oil based foods	2.5	Platelet PC	100	0	−27	Weaver et al. (1990)[159]
				Platelet PE	133	51	−5	
M	8% of fatty acids	Rapeseed oil based foods	8	Platelet PL	17	24	20	Kwon et al. (1991)[155]
M	2.1% of energy	Rapeseed oil based foods	3	Total platelet lipid	−8	NA	20	Mutanen et al. (1992)[156]
M	20[a]	Flaxseed oil	8	Total mononuclear cell lipid	0	45	−36	Kelley et al. (1993)[128]
M	13.7	Flaxseed oil + spread	4	Neutrophil PL	140	NA	0	Mantzioris et al. (1994)[130]
M + F	18 (8.5% energy)	Flaxseed oil	3	Platelet PL	140	45	−11	Allman et al. (1995)[150]
M	13.7	Flaxseed oil + spread	4	Mononuclear cell PL	133	NA	−4	Caughey et al. (1996)[152]
M	3.7	Flaxseed oil + spread	4	Platelet PL	50	21	0	Li et al. (1999)[129]
	15.4	Flaxseed oil + spread	4	Platelet PL	150	56	−10	
M	3.5	Flaxseed oil + foods	6	Total platelet lipid	200	46	−50	Allman-Farinelli et al. (1999)[151]
M	4.7	Flaxseed oil capsules	12	Neutrophil PL	30	NA	15	Healy et al. (2000)[153]
M + F	0.75	ALA ethyl ester in capsules	4	RBC PL	5	0	−5	James et al. (2003)[97]
	1.5	ALA ethyl ester in capsules	4	RBC PL	13	4	−7	
M + F	4.5	Spread	24	Mononuclear	0	6	−28	Kew et al. (2003)[154]
	9.5	Spread	24	cell PL	33	0	−20	
M	15	Flaxseed oil and spread	12	Total RBC lipid	153	0	−6	Wlkinson et al. (2005)[160]
M + F	11.9	Flaxseed oil capsules	8	RBC PL	1020	NA	41	Nelson et al. (2007)[157]
M + F	19.1	Walnuts, walnut oil and flaxseed oil	6	Total mononuclear cell lipid	300	10	0	Zhao et al. (2007)[133]
M + F	4.7	Walnuts	4	Total RBC lipid	14	NA	−1.3	Rajaram et al. (2009)[164]
M + F	2.2	Flaxseed oil	8	Total RBC lipid	31	NA	−8	Dewell et al. (2011)[161]
	6.6	Flaxseed oil	8		65	NA	−14	
M + F	4.4	Spread	6	Total RBC lipid	13	NA	−13	Egert et al. (2012)[162]
M + F	8.7	Organic flaxseed oil	6	RBC PL	47	17	0.6	Kontogianni et al. (2013)[163]
M + F	7	Flaxseed oil	10	Total RBC lipid	31	19	−2	Dittrich et al. (2014)[106]

DPA, docosapentaenoic acid; M, male; F, female; PC, phosphatidylcholine; RBC, red blood cell; NA, data not available; PL, phospholipid; PE, phosphatidylethanolamine.
[a] Approximate intake.

achieved via use of encapsulated oils or of foods fortified with ALA-rich oils. Most often the oil used has been flaxseed. The durations of these studies varied between 2 and 160 weeks and doses of ALA ranged from <1 to about 20 g/day (Tables 6 and 7). These studies describe the effects of the dietary intervention on plasma or serum total fatty acids, individual plasma or serum lipid classes, primarily phospholipids, and on platelets, RBCs and leukocytes, primarily peripheral blood mononuclear cells (see Tables 6 and 7). Only one study investigated the effect of decreasing consumption of ALA [168].

Almost all (47 out of 54) studies which increased ALA consumption report increased content of EPA and, where measured, DPA, in blood lipids and blood cells, while most report either little or no change or even a decrease in DHA content (Tables 6 and 7). As an example of data obtained from one of these studies [97], Fig. 3 shows the effect of ALA (0.75 g/day for 3 weeks and then 1.5 g/day for 3 weeks) on EPA, DPA and DHA content of plasma phospholipid and RBCs. It is evident that EPA and DPA show small increases, with the increase in EPA being greater (~23% and 15% increases from starting value for EPA in plasma phospholipid and RBCs, respectively and ~5% and 4% increases from starting value for DPA in plasma phospholipid and RBCs, respectively), while DHA shows a decrease (~7% decrease from starting value for DHA in both plasma phospholipid and RBCs).

Plasma phospholipids may be a mechanism to transport PUFAs to cells and tissues, and their fatty acid composition reflects that of the membranes of some cells [154]. The effect of increased ALA intake on fatty acids in plasma phospholipids has been reported in several studies. Therefore, we chose to evaluate the relationship between increased ALA intake (g/d) and change in the EPA, DPA and DHA content of this fraction (Fig. 4). Combining data from all studies ($n = 9$) examining increased ALA intakes ranging from ~2 to 15 g/d revealed a positive linear relationship ($p = 0.0002$) between ALA consumption and change in plasma phospholipid EPA content (Fig. 4A). The data suggest that each 1 g increase in ALA intake results in about a 10% relative increase in EPA content. Five studies investigating increased ALA intakes ranging from ~2 to 10 g/d revealed a dose-dependent relationship ($p = 0.0011$) between ALA consumption and change in plasma phospholipid DPA content (Fig. 4B). The data suggest that each 1 g increase in ALA intake results in about a 4% relative increase in DPA content. However, combined data from nine studies indicates no change ($p = 0.49$) in plasma phospholipid DHA after increased consumption of ALA (Fig. 4C). The relationship between increased ALA intake and cell phospholipid EPA is also likely to be linear but there are insufficient data for any single cell type on which to base any firm conclusions on this.

Metabolic competition exists between LA and ALA, and therefore levels of LA within the diet could influence the conversion of ALA to VLC ω-3 PUFAs. Chan et al. demonstrated this by changing dietary LA intakes in normolipidaemic men to either 21 g or 50 g/d for 18 days, whilst providing a constant ALA intake of 7 g/d. It is important to note that in this study intakes of both LA and ALA were far above those normally consumed in the human diet. EPA content of plasma phosphatidylcholine and phosphatidylethanolamine, and of platelet phosphatidylcholine increased to a greater extent in subjects consuming the lower amount of LA compared to the higher amount [169]. This finding supports the idea that LA decreases ALA conversion to EPA in humans. This interaction between LA and ALA was further explored by Goyens et al. [32]. They described incorporation of EPA into plasma phospholipids being greater in a low LA group (3% of energy from LA, 0.4% energy from ALA, 7:1 ratio of LA to ALA) than in a high ALA group (7% of energy from LA, 1.1% energy from ALA, 7:1 ratio of LA to ALA). The authors argued that exchanging dietary LA for ALA may be the optimal dietary approach to increase VLC ω-3 PUFA status [32]. Although these studies demonstrate that a high LA intake seems likely to inhibit conversion of ALA to EPA, there is little information on the extent to which variations in habitual LA intake affect conversion of ALA consumed at habitual intakes.

Table 8
Studies investigating the effect of increased stearidonic acid consumption on the fatty acid composition of blood lipids and blood cells in adult human subjects.

Subjects	SDA intake (g/d)	SDA form	Duration (Weeks)	Blood fraction	Change in proportion of total fatty acids from baseline (%)			Reference
					EPA	DPA	DHA	
M + F	0.75	Ethyl ester in capsules	3	Plasma	41	16	−4	James et al. (2003)[97]
	1.5		3	phospholipid	87	28	−4	
	0.75		3	Total RBC lipid	21	3.5	−3.5	
	1.5		3		50	9.5	−6	
M	1	Echium oil capsules	12	Total mononuclear cell lipid	125	NA	61	Miles et al. (2004)[99]
M	1	Echium oil capsules	12	Plasma phospholipid	33	NA	11.5	Miles et al. (2004)[167]
				Plasma cholesteryl ester	25	NA	47	
				Plasma triacylglycerol	27	NA	2	
M + F	1.9	Echium oil capsules	4	Total plasma	190	73	1	Surette et al. (2004)[38]
				Total neutrophil lipid	400	100	29	
M + F	3.7	SDA-enriched soybean oil	16	Total RBC lipid	188	NA	0	Harris et al. (2008)[166]
M + F	4.2	SDA-enriched soybean oil	12	Total RBC lipid	4	3	1	Lemke et al. (2010)[98]
M + F	0.44	Ethyl ester in capsules	12	Total RBC lipid	11	8	−5	Krul et al. (2011)[100]
	1.3				89	33	2	
	2.6				118	40	0.6	
	5.2				172	47	3	
M + F	0.25	Echium oil and Borage oil capsules	3	Total plasma	19	10	3	Arm et al.(2013)[165]
	0.5				56	20	−5	
	0.875				70	22	0	
	1.75				200	2	9	
M + F	7	SDA-enriched soybean oil in foods	12	Total RBC lipid	89	30	−5	Lemke et al. (2013)[104]
M + F	1.2	Echium oil capsules	6	Total RBC lipid	20	10	−3	Pieters et al. (2014)[105]
M + F	2	Echium oil capsules	10	Total plasma	115	66	−2	Dittrich et al. (2014)[106]
				Total RBC lipid	63	35	−11	
M + F	2	Echium oil capsules	8	Total plasma	183	74	0	Kuhnt et al. (2014)[107]
20–35 y								
49–69 y					165	64	−4	
MetS					123	53	−10	
20–35 y				Total mononuclear cell lipid	74	40	−23	
49–69 y					98	41	−21	
MetS					62	30	−26	

DPA, docosapentaenoic acid; M, male; F, female; y, years; RBC, red blood cell; MetS, metabolic syndrome ; NA, data not available

Although the focus of most studies has been on the effect of increased ALA intake, because of the interest in strategies to increase EPA and DHA status, Han et al. describe effects of decreasing ALA consumption via use of a low ALA-soybean oil [168]. In this study the low ALA diet provided 54% of the amount of ALA (0.68% energy) found in the reference soybean oil based diet (1.26% energy). The findings showed that lower ALA intake resulted in lower plasma EPA (−25%) and DHA (−5%) concentrations. This finding supports a close relationship between ALA intake and EPA content of plasma phospholipid (and probably other lipid pools too), such that increasing ALA intake increases EPA, while decreasing ALA intake decreases EPA content. However, as indicated above, the amount of LA and the LA to ALA ratio will also play a role in determining the precise extent of conversion.

Taken together, these studies show that the amount of ALA consumed in the diet is directly related to the amounts of EPA and DPA in blood lipids and blood cells. One implication is that increased ALA intake is a potential strategy to increase EPA, and also DPA, status. Furthermore, the effectiveness of this strategy may be enhanced if LA intake is decreased at the same time as ALA intake is increased. In contrast, these studies also demonstrate that increased consumption of ALA does not increase the amount of in DHA in blood lipids and cells. This conclusion based on dietary intervention studies lasting weeks to months is consistent with findings, described above, from several short term stable isotope studies. Thus increased intake of ALA, even at high doses, does not appear to be a viable strategy to increase DHA status.

The studies described in Tables 6 and 7 were conducted in adults. There are now a small number of similar studies conducted in infants and children examining the impact of increased intake of ALA [170–173]. Schwartz et al. demonstrated a small increase in

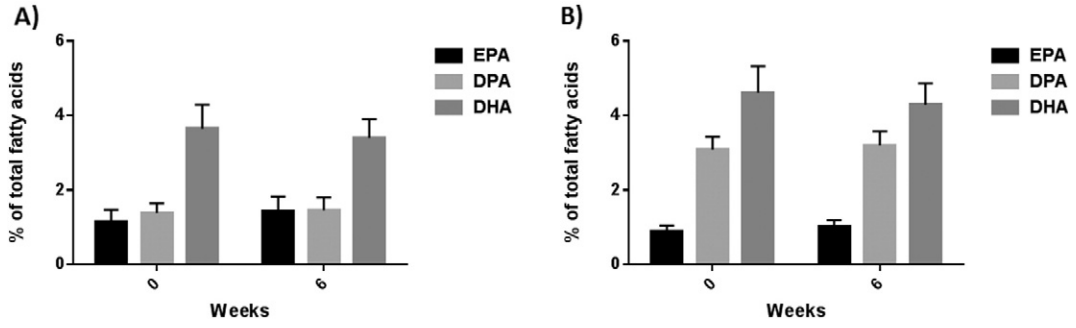


Fig. 3. EPA, DPA and DHA in plasma phospholipid (A) and RBC phospholipid (B) before and after 6 weeks of ALA treatment. Data are mean + SD and are taken from James et al. [97]

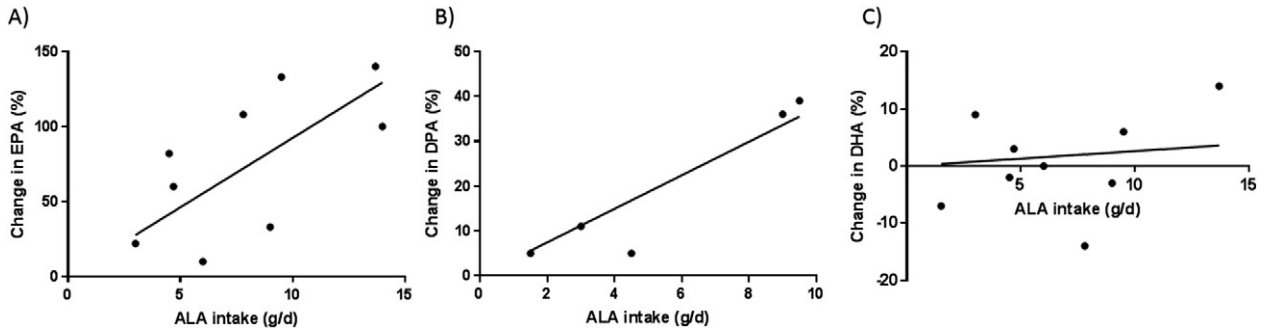


Fig. 4. Change in plasma phospholipid EPA (A), DPA (B) and DHA (C) as a result of increased ALA intake. Individual data are taken from references [32,97,121,124,130–132,146,169]. Linear regressions were forced to go through zero. Equations describing the linear regression are: a) y = 9.24x; b) y = 3.74x; c) y = 0.26x.

concentrations of EPA, DPA and DHA in plasma after increased ALA consumption via meals containing rapeseed oil in 4-10 month old infants [170]. Gracious et al. reported higher EPA and DPA concentrations, but no change in DHA concentration, in plasma after 16 weeks of stepped dose increases in ALA intake up to 6.6 g/d in children and adolescents aged 6–17 years [171]. This study is consistent with the observations in adults that an increase in ALA consumption increases EPA and DPA concentration, but not DHA concentration, in plasma and cell lipids. However, some older studies suggest that conversion of ALA to DHA may be more efficient in infants than seen in studies in adults. Sauerwald et al. fed 3 week old term infants a fixed LA diet, where formulas contained 16% of fat as LA and increasing amounts of ALA (0.4%, 1% or 3.2%). The authors estimated that there was a 2.5 fold greater rate of incorporation of DHA into the plasma phospholipid fraction with the highest amount of ALA given [172]. The ability of infants to synthesise DHA from ALA was also reported in two other studies that used ALA addition to formula that did not contain LC-PUFAs. Clark et al. observed increases in plasma total DHA when ALA was increased in formula [173]. Jensen et al. also reported increases in levels of DHA in both plasma and RBCs after increased ALA: infants were fed from birth to age 120 days with formula providing 16% of fats as LA and either 0.4, 0.95, 1.7 or 3.2% as ALA [174].

These studies may suggest that infants have a greater capacity to convert ALA to DHA than adults, perhaps due to it being a particularly critical development window (ω-3 LC PUFAs are required for rapid growth of neural tissues in the perinatal period). Brenna suggests a wide variability among human infants in the development of biosynthetic capability to convert ALA to DHA [175].

4.4.2.2. Sex differences in alpha-linolenic acid metabolism. There are few dietary intervention studies investigating effects of increased ALA consumption in men and women alone, with most reporting outcomes in mixed sex groups. However, studies where comparable consumption of ALA between sexes has been investigated indicate that ALA may have a sex-specific effect on EPA, DPA and DHA status in humans.

As mentioned earlier, Burdge et al. examined conversion of ALA to EPA and DHA in women (mean age 28 years) and men (age range 27 to 40 years) in comparable studies [30,31]. They showed that conversion of ALA to EPA and DHA in women was markedly greater, by 2.5 fold and >200 fold, respectively, than that in men. This is supported by kinetic analysis data, which demonstrates that the rate constant coefficient for the conversion of DPA to DHA is approximately 4-fold greater in women than men [116]. Burdge et al. suggest there may be a sex-related difference in the desaturation-elongation pathway [31].

Better conversion of ALA in women would suggest that higher concentrations of EPA, DPA and DHA would be seen in blood, cells and tissues in women compared with men. Lohner et al. evaluated by meta-analysis publications from 1975 to 2011 that compared LC PUFA concentrations between men and women [176]. Their analysis showed significantly lower DHA in total plasma lipids (33 studies) and in plasma phospholipids (23 studies) in men than in women. Primary analysis of the phospholipid fraction showed a mean difference in DHA of 0.37%. There was no sex difference in the concentrations of LA or ALA.

In an RCT where men and women where fed an ALA-rich diet (9.5 g/d ALA) for 6 months there were significant differences between sexes in the response to increased dietary ALA [177]. There was a greater increase in the EPA content of plasma phospholipids in women (absolute increase of 2%) compared to men (absolute increase of 0.7%). However, there was no sex specific enhancement of DPA or DHA content of plasma phospholipids after increased consumption of ALA. Childs et al. also report an inverse relationship between the age of the female participants and the change in EPA content after increased consumption of ALA, which may suggest a role for female sex hormones in regulating the endogenous synthesis of VLC ω-3 PUFAs. Indeed several studies support the idea that sex hormones may regulate the desaturation-elongation pathway, and so affect the metabolism of ALA differently in men and women. For example, Burdge et al. demonstrated that DHA synthesis was 3-fold greater in young adult women using an oral contraceptive pill containing 17-alpha-ethylnyloestradiol compared to those who did not [31]. They suggest that the action of oestrogen may therefore explain greater synthesis of EPA and DHA from ALA in young women due to increased activity of the pathway. Ottosson et al. reported that oestrogen-based hormone replacement therapy in postmenopausal women resulted in greater plasma di-homo-γ-linolenic and arachidonic acid (AA) concentrations than before treatment [178]. Giltay et al. describe greater DHA concentrations in the plasma cholesteryl ester fraction of women (0.53% of total fatty acids) compared with men (0.48% of total fatty acids) after consumption of increased ALA [179]. Furthermore, it has been shown that male to female transsexuals who were administered 17-alpha-ethylnyloestradiol had increased concentrations of DHA in plasma cholesteryl esters (42%) [31], whereas testosterone decreased the DHA concentrations by 22% in female to male transsexuals [179].

4.4.2.3. Increased intake of stearidonic acid. Studies which directly compared the effects of SDA with ALA or EPA supplementation on the concentrations of in EPA, DPA and DHA in humans reported that SDA resulted in higher plasma and cell EPA than ALA [97–100] (Table 8). None of these studies reported an increase in DHA concentrations after an increase in SDA intake, which is consistent with findings following increased intakes of ALA.

Although there are fewer studies describing effects of increased intake of SDA than of ALA, most conclude that chronically increased consumption of SDA increases plasma and cell concentrations of EPA and DPA, but decreases concentrations of DHA, with few exceptions (Table 8). It seems likely that the relationship between increased SDA intake and increased concentration of EPA and DPA in plasma lipids

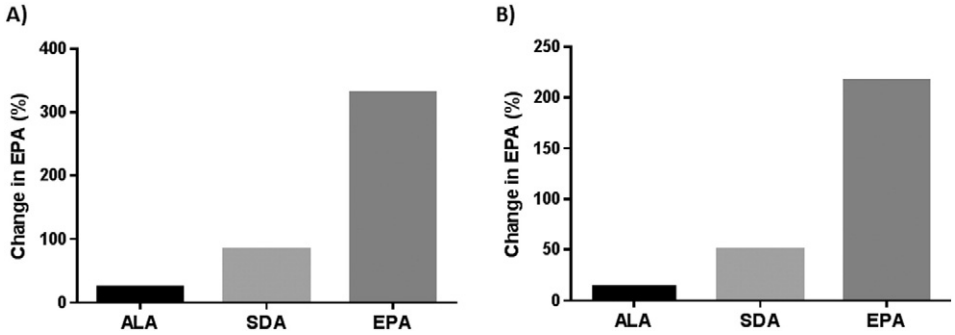


Fig. 5. Change in plasma phospholipid EPA (A) and RBC phospholipid EPA (B) concentration after 6 weeks intervention with either ALA or SDA or EPA. Data are calculated from James et al. [97]

and blood cells will be linear, as is the case for ALA (Fig. 3), but there are too few studies exploring this to be certain.

James et al. compared the metabolism of SDA and ALA: they describe the effect of increased intake of ALA or SDA or EPA for periods of 3 weeks at doses of 0.75 g/d and then 1.5 g/d [97]. The data obtained clearly demonstrate the superiority of SDA over ALA to increase EPA (and DPA) in plasma phospholipids and RBCs (Fig. 5), but there was no increase in DHA with either ALA or SDA (Fig. 5).

4.5. Alpha-linolenic acid and stearidonic acid - human health-related outcomes

Epidemiological studies in Europe, the USA and Japan indicate decreased risk of CVD [2,4,6,180,181] and inflammation [182–185] with increasing consumption of VLC ω-3 PUFAs. VLC ω-3 PUFA supplementation studies have demonstrated beneficial changes in a number of cardiovascular risk factors including fasting TAG concentrations [186, 187], postprandial lipaemia [188], blood pressure [189], platelet aggregation [131,190,191], blood coagulation and fibrinolysis [192], heart rate variability [193,194], cardiac arrhythmias [195], vascular reactivity [196–199] and inflammation [200–204]. However, some reports suggest that these fatty acids increase the susceptibility of LDL to oxidation *ex vivo* [124,205,206], which, if it occurred *in vivo*, could have a damaging pro-atherogenic effect. VLC ω-3 PUFAs may also modulate immune function [200,201,204]. It is possible that ALA and SDA can exert beneficial effects on human health as they act as the precursors to EPA and, to a more limited extent, DHA. ALA also competes with LA for metabolism and so may reduce the ARA content of cells and tissues, which might be important for controlling thrombosis, inflammation, bone loss, immunosuppression and cancer.

4.5.1. Alpha-linolenic acid and cardiovascular disease

4.5.1.1. Epidemiological associations. Reported effects of ALA-enriched diets on prevention of CVD are inconsistent. A population based case-control study in Costa Rica reported that a high ALA intake from the diet and a high ALA content in adipose tissue were associated with a lower risk of myocardial infarction (MI) [207,208]. Risk of MI decreased by 57% with median ALA intake of 1.79 g/d (0.65% energy) compared with 1.11 g/d (0.42% energy), but it did not decrease further with ALA intakes >1.79 g/d. Fish and EPA and DHA intakes, which were low, did not modify the observed association, suggesting that ALA confers cardiovascular protection in populations where intake of VLC ω-3 PUFAs is low [208].

The Multiple Risk Factor Intervention Trial showed that dietary ALA intake was significantly inversely associated with mortality from coronary heart disease (CHD) [209]. An ALA intake of 1.36 g/d (highest quintile of ALA intake) was associated with 45% fewer coronary deaths in women compared with an intake of 0.71 g/d (lowest quintile of ALA intake) [210]. Asherio et al. also reported that dietary ALA intake was inversely associated with risk of MI among men [211]. In another study of 4406 men and women, 1.1 g/d ALA was associated with 40% lower mortality from coronary artery disease compared with an intake of 0.5 g/d [212]. One prospective cohort study also concluded that a diet rich in ALA is beneficial in CVD [210] . In a meta-analysis of five prospective cohort studies, Brouwer et al. reported that a high intake of ALA was associated with a 20% decrease in CHD risk compared with a low intake [213]. This conclusion is further supported by a recent meta-analysis which showed that higher ALA intake was associated with a lower risk of CVD: the authors suggest that each increment of 1 g/d ALA intake is associated with 10% lower risk of CHD [214]. However, there is contradictory evidence from another meta-analysis, which identified only a very small, if any, cardioprotective benefit from higher ALA intake [215]. In older epidemiological studies, Oomen et al. and Simon et al. found no association between ALA intake and CHD risk [216,217]. More recently, Vedtofte et al. suggested that there is no association between ALA intake and risk of ischemic heart disease [218] and Lemtaire et al. concluded that ALA is not associated with incident congestive heart failure (CHF) in older adults [219], which is in agreement with findings from another prospective study by Gode et al. reporting no association between ALA intake and incident CHD [220].

Thus, data are inconsistent, and it is not clear whether ALA intake is associated with reduced incidence of CVD or not. It is possible that dietary intakes of ALA are easily miscalculated because of the self-report questionnaires used to assess fatty acid consumption, and this might contribute to inconsistencies in the literature. Harris et al. used fatty acid biomarkers instead of dietary recalls in a meta-analysis of studies and reported that ALA was inversely associated with non-fatal CHD events [221].

4.5.1.2. Intervention studies. The Lyon Heart Study reported a substantial reduction in coronary events and deaths among MI survivors following a Mediterranean diet which provided 1.5 g/d of ALA via an ALA-rich margarine as part of the intervention [222]. At the 27 month follow-up, the risk of cardiac death and non-fatal MI was lower (by >60%) in the intervention group compared with the control group. After 46 months, subjects adhering to the Mediterranean diet had 50–70% lower risk of recurrent heart disease [223]. ALA was the only fatty acid significantly associated with a lower risk of the composite primary end point of MI plus cardiovascular death. Fleming et al. suggest that ALA is responsible for the observed effects since EPA and DHA were not associated with lower risk of CHD [224]. However, it is difficult to attribute the findings of this study specifically to increased intake of ALA since other dietary changes were involved.

The Alpha Omega Trial was a double-blind, placebo-controlled trial in post MI patients [225]. Subjects consumed margarines with different amounts of ALA, EPA + DHA or the combination of ALA + EPA + DHA. Both groups consuming ALA had a daily intake of 2 g, which is not much greater than the amount consumed in many habitual diets (Table 1). There was a non-significant 9% reduction in the primary end

Table 9
Controlled intervention studies investigating effect of increased ALA consumption on blood lipid concentrations in adult human subjects.

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Outcomes investigated	Effect of ALA (% change where significant)	Reference
M + F	9.4	Flaxseed oil capsules	2	Total cholesterol	None	Sanders and Roshani (1983)[158]
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	38	Flaxseed oil	2	Total cholesterol	None	Singer et al. (1986)[233]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	−25	
M	9.2	Milkshake including flaxseed oil	9	Total cholesterol	None	Kestin et al. (1990)[229]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				VLDL-cholesterol	None	
				Triacylglycerol	None	
M	~7	Rapeseed oil or soybean oil	2.5	Total cholesterol	−18	Chan et al. (1991)[120]
				LDL-cholesterol	−22	
				VLDL-cholesterol	−41	
				Apo B	−19	
				Apo A1	−9	
M	~20	Flaxseed oil	8	Total cholesterol	None	Kelley et al. (1993)[128]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Apo B	None	
				Apo A1	None	
M	13.7	Flaxseed oil + spread	4	Total cholesterol	None	Mantazoris et al. (1994)[130]
				LDL-cholesterol	None	
				HDL-cholesterol	+20	
				Triacylglycerol	None	
M + F	9	Muffins incorporating flaxseeds	4	Total cholesterol	−6	Cunnane et al. (1995)[121]
				LDL-cholesterol	−9	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	2.5	Flaxseed oil capsules	12	Total cholesterol	None	Layne et al. (1996)[230]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	5.9	Flaxseed oil capsules	4	Total cholesterol	None	Freese and Mutanen (1997)[228]
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	20[a]	Spread + food made with flaxseed oil	4	Total cholesterol	None	Nestel et al. (1997)[231]
				LDL-cholesterol	None	
				HDL-cholesterol	−8	
				Triacylglycerol	None	
M	10.1	ALA rich foods	3 and 6	Total cholesterol	None	Pang et al. (1998)[232]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
F	8.5	Muffins and bread incorporating flaxseeds and flaxseed oil	6	Total cholesterol	−7	Arjmandi et al. (1998)[227]
				LDL-cholesterol	−16	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Lipoprotein (a)	−6	
M	3.7	Flaxseed oil + spread	4	Total cholesterol	None	Li et al.(1999)[129]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
	15.4	Flaxseed oil + spread	4	Total cholesterol	None	
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M	4.2	Perilla oil	40	Total cholesterol	None	Ezaki et al.(1999)[123]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				VLDL-cholesterol	None	
				Triacylglycerol	None	
				VLDL-Triacylglycerol	None	
M + F	10	Spread and foods made with rapeseed oil	4	Total cholesterol	−15	Junker et al. (2001)[235]
				LDL-cholesterol	−18	
				HDL-cholesterol	−12	
				Triacylglycerol	None	

(continued on next page)

Table 9 (*continued*)

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Outcomes investigated	Effect of ALA (% change where significant)	Reference
M + F	9.2	Spread, rapeseed oil and foods made with rapeseed oil	4	Total cholesterol	−11	Sodergren et al. (2001)[237]
				LDL-cholesterol	−11	
				HDL-cholesterol	−9	
				VLDL-cholesterol	None	
				Triacylglycerol	None	
				Apo A1	None	
				Apo B	None	
				Lipoprotein	None	
M + F	3	Rapeseed oil	6	Total cholesterol	−6	Karvonen et al. (2002)[236]
				LDL-cholesterol	−12	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	11.4	Camelina oil	6	Total cholesterol	−8	Karvonen et al. (2002)[236]
				LDL-cholesterol	−12	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	6.3	Spread	104	Total cholesterol	−12	Bemelmans et al. (2002)[119]
				LDL-cholesterol	+7	
				HDL-cholesterol	+11	
				Triacylglycerol	None	
M + F	4.5	Spread	24	Total cholesterol	None	Finnegan et al. (2003)[124]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Apo B	None	
	9	Spread	24	Total cholesterol	None	
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Apo B	None	
M	8	Flaxseed oil	12	Total cholesterol	None	Rallidis et al. (2003)[240]
				LDL-cholesterol	None	
				HDL-cholesterol	−4	
				Triacylglycerol	None	
M + F	17.5[a] (6.5% energy)	Walnuts, walnut oil and flaxseed oil	6	Total cholesterol	−11	Zhao et al.(2004)[134]
				LDL-cholesterol	−11	
				HDL-cholesterol	−6	
				Triacylglycerol	−18	
				Apo A	−5	
				Apo B	−10	
M	15	Flaxseed oil and spread	12	Total cholesterol	−21	Wilkinson et al. (2005)[160]
				LDL-cholesterol	None	
				HDL-cholesterol	−8	
				Triacylglycerol	−10	
M + F	1.1%E	Spread	6	Total cholesterol	None	Goyens et al. (2006)[245]
				LDL-cholesterol	−5	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Apo A	None	
				Apo B	None	
M + F	5	Flaxseed flour	2	Total cholesterol	None	Faintuch et al. (2007)[238]
				LDL-cholesterol	None	
				VLDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	8	Flaxseed oil	12	Total cholesterol	None	Paschos et al. (2007)[239]
				LDL-cholesterol	None	
				HDL-cholesterol	−5	
				Triacylglycerol	None	
M + F	4	Baked goods incorporating flaxseeds		Total cholesterol	None	Bloedon et al. (2008)[137]
				LDL-cholesterol	None	
				VLDL-cholesterol	None	
			10	HDL-cholesterol	−9	
				Male	None	
				HDL-cholesterol	None	
				Female	None	
				Triacylglycerol	None	
				Apo A	−12	
				Apo B		
				Lipoprotein (a)		

Table 9 (*continued*)

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Outcomes investigated	Effect of ALA (% change where significant)	Reference
F	9	Bread and grains incorporating flaxseeds	48	Apo A	None	Dodin et al. (2008)[139]
				Apo B	None	
				Lipoprotein	None	
M + F	1	Flaxseed oil capsules	12	Total cholesterol	None	Kaul et al. (2008)[143]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M	5.4	Flaxseed oil	4	Total cholesterol	None	Barden et al. (2009)[136]
				HDL-cholesterol	None	
M + F	4.4	Spread	6	Total cholesterol	None	Egert et al. (2009)[140]
				LDL-cholesterol	None	
				VLDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	−17	
M + F	6	Muffins incorporating	4			Patenaude et al. (2009)[144]
aged 18–29 y		– Ground flaxseed		Total cholesterol	None	
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
		– Flaxseed oil		Total cholesterol	None	
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	−20	
M + F aged 45–69 y		– Ground flaxseeds		Total cholesterol	None	
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
		– Flaxseed oil		Total cholesterol	None	
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	4.7	Walnuts	4	Total cholesterol	−5	Rajaram et al. (2009)[164]
				LDL-cholesterol	−9	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Apo A	None	
				Apo B	−9	
M	2.1	Walnuts	4	Total cholesterol	None	Din et al. (2011)[241]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M	3.6	Rapeseed oil and spread	3	Total cholesterol	None	Gladine et al. (2011)[242]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Apo A	+3	
				Apo B	None	
M + F	3.5	Rapeseed oil	72	Total cholesterol	−6	Baxheinrich et al. (2012)[243]
				LDL-cholesterol	−6	
				HDL-cholesterol	None	
				Triacylglycerol	−2	
				Apo A	None	
				Apo B	−8	
M + F	9.7[a]	Milled flaxseed added to foods	8	Total cholesterol	−15	Khalatbari Soltani et al. (2012)[244]
				LDL-cholesterol	−17	
				HDL-cholesterol	+16	
				Triacylglycerol	−31	
M + F	8.7	Organic flaxseed oil	6	Total cholesterol	−5	Kontogianni et al. (2013)[163]
				LDL-cholesterol	−7	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	0.5	Cheese enriched with ALA	3	Total cholesterol	−5	Pintus et al. (2013)[145]
				LDL-cholesterol	−7	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F	7	Flaxseed oil	10	Total cholesterol	−13	Dittrich et al. (2014)[106]
				LDL-cholesterol	−14	
				HDL-cholesterol	None	

(*continued on next page*)

Table 9 (*continued*)

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Outcomes investigated	Effect of ALA (% change where significant)	Reference
				Triacylglycerol	None	
				Lipoprotein	None	
M + F 20–35 y	5	Echium oil	8	Total cholesterol	−6.2	Kuhnt et al. (2014)[107]
				LDL-cholesterol	−9.6	
				HDL-cholesterol	−8.7	
				Triacylglycerol	−12	
				Total cholesterol	None	
M + F 49–69 y				LDL-cholesterol	−4.8	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F MetS				Total cholesterol	−8.2	
				LDL-cholesterol	−11	
				HDL-cholesterol	−6.8	
				Triacylglycerol	−11	
M + F	3	Sesame/pumpkin/flaxseed mix	12	Total cholesterol	−7	Ristic-Medic et al. (2014)[146]
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	−30	

M, male; F, female; Y, years; MetS, metabolic syndrome.
[a] Approximate intake.

point (any major cardiovascular event) with ALA supplementation compared to supplementation with EPA + DHA. There were no differences in cardiovascular events between the groups which received ALA and the groups which did not. However, there was a 27% reduction in major cardiovascular events among women in the ALA group. This may relate to better conversion of ALA to cardioprotective EPA in women than in men.

4.5.2. Alpha-linolenic and stearidonic acids and cardiovascular risk factors

4.5.2.1. Blood lipids. Increased intake of LA has been shown to lower blood total and LDL-cholesterol concentrations, particularly when LA is increased at the expense of SFAs [226]. Studies which investigated the effect of increased ALA consumption on blood cholesterol, TAG, LDL-cholesterol and HDL-cholesterol concentrations have shown inconsistent findings [32,106,107,119–121,123,128–130,134,136,137,139,140,143–146,158,160,163,164,227–244] (Table 9). Several studies have reported that ALA has an LDL-cholesterol lowering effect [106,107,120,121,134,145,163,164,227,235–237,243–245] (Table 9). Although Mantzioris et al. and Pang et al. both suggest that ALA is not as effective as LA at lowering LDL-cholesterol [130,232], several studies report that the effects of ALA are similar to those of LA. The reasons for these differing findings may relate to the nature of the fatty acid replaced by ALA: replacement of SFAs with ALA would lower LDL-cholesterol while replacement of LA with ALA may have no effect. Reports of the effect of ALA on HDL-cholesterol are also inconsistent with some studies reporting increases [119,130,244] and others decreases [107,134,137,160,231,235,237,239,240] (Table 9). Again, this may relate to the exact nature of the ALA intervention and what fatty acid ALA is replacing. When comparing ALA and LA intake, Bemelmans et al. reported that HDL-cholesterol concentrations were lower after increased ALA intake than after increased LA intake [119].

Some studies have investigated the independent effects of ALA and EPA + DHA on LDL-cholesterol concentration, although interpreting the results of such comparisons is made difficult by the fact that ALA can be converted to EPA, and in some sub-groups to DHA, *in vivo*. Goyens et al. [245] and Finnegan et al. [124] observed more favourable effects of ALA than EPA + DHA on LDL-cholesterol concentration. Both of these studies reported an increase in LDL-cholesterol in subjects consuming an EPA + DHA rich diet compared to those consuming an ALA rich diet. Thus, the effects of ALA and EPA + DHA on LDL-cholesterol are not comparable, and those of ALA are more favourable.

The reported effects of ALA on fasting TAG concentrations are also inconsistent; although a number of studies report a decrease [107,134,140,144,146,160,233,243,244], the majority (31 out of 40) report no effect (Table 9). There is consistent evidence that EPA and DHA lower blood TAG concentrations [186,246,247]. Egert et al. evaluated the effects of increased dietary intake of ALA, EPA and DHA on serum TAG in normolipidemic non-obese subjects [140]. The subjects consumed margarines providing either 4.4 g of ALA, or 2.2 g of EPA or 2.3 g of DHA ethyl ester daily for 6 weeks. The study confirmed the TAG lowering effect of EPA (15% lower) and DHA (30% lower) and reported a 15% reduction in fasting serum TAG in the ALA group [140]. Patenaude et al. compared the effect of increased consumption of ALA, from flaxseed oil (6 g/d for 4 weeks), in two age groups and reported a 20% decrease in circulating TAG concentration in younger subjects (18–29 y) compared to a 3.5% increase in older subjects (45-69 y) [144]. Some studies which directly compare the effects of ALA and LA on fasting TAG concentrations suggest that the effects of ALA are not markedly different from those of LA [134,229,231,232,240], while others describe higher TAG concentrations after increased ALA intake but not after a high-LA diet [124,227]. For example, Bemelmans et al. reported 12% higher plasma TAG concentrations after ALA than after LA [119]. Thus, whether ALA is seen to lower blood TAG concentrations may depend upon what ALA is replacing in the diet and the exact fatty acid comparisons being made.

Three studies investigated the effects of SDA-rich soybean oil on blood lipid concentrations in humans. Krul et al. report no effect of consuming different amounts of SDA (0.43, 1.3, 2.6 or 5.2 g/d) from this oil for 12 weeks on plasma cholesterol or TAG concentrations in healthy male and female subjects [100]. Harris et al. also found no change in serum cholesterol or TAG concentrations after consuming of 3.7 g/d SDA from SDA-rich soybean oil for 16 weeks in overweight or obese adults [166]. Another study reported no change in serum cholesterol concentration after consuming 4.2 g/d SDA for 12 weeks, although there was a non-significant trend (9% decrease) towards lower TAG concentration [98]. Furthermore, consuming 10 g Echium oil/d (providing 1.2 g SDA/d) did not alter serum total cholesterol, LDL-cholesterol, HDL-cholesterol or TAG concentrations in overweight and obese subjects [105]. It is possible that some of these studies were too small to detect a significant effect of SDA on blood lipids.

4.5.2.2. Postprandial lipaemia. Lichenstien et al. reported no differences in the postprandial TAG response to a test meal in subjects who

consumed diets rich in rapeseed, corn or olive oil and so differing in ALA content [248]. Finnegan et al. reported no effect of modified margarines providing 4.5 or 9 g/d of ALA for 6 months on the postprandial TAG response to a test meal, compared to starting response or to use of an LA-rich margarine [124].

One study which investigated the effect of SDA (4.2 g/day) on postprandial TAG in hypertriglyceridemic subjects reported no effect after 12 weeks intervention [98].

Overall, it appears that neither ALA nor SDA affect the post-prandial TAG response compared with other plant-derived unsaturated fatty acids.

4.5.2.3. Low-density lipoprotein oxidation. Compared to a monounsaturated fat-rich diet, consuming 20 g/d ALA for 4 weeks decreased the lag time for *ex vivo* Cu-induced oxidation of LDL by 14 min in overweight male and female subjects [231]. Additionally, the content of thiobarbituric acid reactive substances, which are the end product of PUFA oxidation, in the oxidised LDL was higher if the LDL came from the subjects on the ALA rich diet. These observations indicate increased susceptibility to oxidation of LDL which is related to increased cardiovascular risk and it may reflect a higher PUFA content of LDL. However, a more recent study reported no effect of consuming margarines providing 4.5 or 9 g/d ALA on lag time of LDL oxidation, compared with consuming an LA-rich margarine [124]. In addition, Ezakji et al. found that a diet which provided 4.2 g/d ALA did not alter plasma lipid peroxide or oxidised LDL concentrations in older Japanese adults [89]. Another trial saw no effect on serum oxidised LDL concentrations after 10 weeks intervention with 4 g/d ALA from flaxseed [103]. The difference in findings of the latter three studies compared with the study of Sodergren et al. [237] may relate to the lower ALA intake used in the latter studies. Kaul et al. did not detect a change in lag time of LDL oxidation after consumption of fish oil (352 mg/d EPA and 242 mg/d DHA), flaxseed oil (1 g/d ALA) or hempseed oil (400 mg/d ALA) for 12 weeks [143], perhaps reflecting the low intakes of PUFAs used. Overall, these studies suggest that ALA intakes up to 6 times greater than that found in the typical UK diet (~1.5/d) do not significantly alter markers of blood lipid peroxidation when consumed for relatively short periods of time. However, substantially higher intakes (for example 13-fold greater than the typical UK intake) may increase LDL oxidation, although the health implications of this are limited as such high intakes are unlikely to be achieved in most populations.

4.5.2.4. Blood pressure. It was reported that a 1% increase in adipose tissue ALA content (a marker of dietary ALA intake) was associated with a 5 mm Hg decrease in systolic and diastolic blood pressures [249], and in a large prospective study an inverse association between dietary ALA intake and blood pressure was observed [250]. Caligiuri et al. reported that participants with peripheral arterial disease, 75% of whom were hypertensive, who consumed 30 g milled flaxseed/d (providing 7 g ALA/d) for 6 months exhibited significant reductions in systolic (−10 mm Hg) and diastolic (−7 mm Hg) blood pressure [251]. However, other studies have not detected an effect of ALA intake or status on blood pressure. Increasing ALA consumption from habitual intake to 38 g/d for 2 weeks did not significantly alter systolic or diastolic blood pressure in either normotensive or hypotensive subjects [233]. Similarly, consuming 9.2 g/d ALA for 6 weeks did not significantly alter systolic or diastolic blood pressure in normotensive, hypercholesterolaemic subjects [229]. In addition, Finnegan et al. showed that increasing ALA intake from 1.5 g/d to 4.5 or 9 g/d for 6 months did not change blood pressure significantly in either group [124]. Similarly, Bemelmans et al. found that consuming 6.3 g/d ALA for 2 years did not significantly alter systolic or diastolic blood pressure in subjects with multiple cardiovascular risk factors [119]. Together, these studies show that high or prolonged increase in ALA intake has little effect on blood pressure. This is in contrast to the frequently reported hypotensive effect of EPA + DHA, confirmed in several meta-analyses, although most studies report that high dose EPA and DHA is needed to lower blood pressure [252–254].

4.5.2.5. Vascular reactivity. Consuming 20 g/d ALA for 4 weeks in exchange for oleic acid increased arterial compliance (aortic flow velocity and aortic root driving pressure) in obese subjects [231]. Further studies investigating whether ALA or SDA affect vascular reactivity are required.

4.5.2.6. Platelet aggregation and haemostasis. Increasing intake of ALA results in decreased levels of AA in platelets and increased levels of EPA [129,131,150,155,156,158,159] (Table 7). This may result in modulation of platelet aggregation, as this is partly regulated by AA and EPA-derived eicosanoids [255,256]. Consuming 18 g/d ALA for 23 days decreased collagen-induced platelet aggregation in healthy men compared to those consuming an LA-rich diet [150]. Another study reported decreased ADP-induced platelet aggregation in hyperlipidaemic participants who consumed an ALA-rich (2.1% energy as ALA) compared to a low-ALA diet (0.3% energy from ALA) [126]. However, some studies in which ALA intake was increased did not find a significant effect on collagen-induced platelet aggregation [129,228]. Kaul et al. also reported that flaxseed oil supplementation (providing 1 g/d ALA) induced no significant change in collagen or thrombin stimulated platelet aggregation [143], although this is a much lower dose of ALA than used in other studies looking at these outcomes. Overall, it is not clear whether high ALA intakes reduce platelet aggregation or not, due to the inconsistencies in findings from different studies. These inconsistencies seem not to be related to dose of ALA used.

Table 10 summarises the findings of intervention studies where haemostatic factors have been investigated after increased consumption of ALA [119,123,128,129,139,151,228,234,235,257]. Most studies (9 out of 10) reported no significant effect of increasing ALA intake on haemostatic factors (e.g., fibrinogen concentration, prothrombin time, PAI-1 activity or factor VII activity), even when high intakes (up to 20 g/day) [128,129,139,234,235] and long durations (up to 48 weeks) [123,139,234] were used (Table 10). Overall, these finding show that increasing ALA intake, even quite markedly, does not alter haemostasis significantly.

4.5.3. Alpha-linolenic acid, stearidonic acid and inflammation

Oxylipins produced from ω-6 and ω-3 PUFAs including octadecanoids, eicosanoids, and docosanoids are intimately involved in inflammatory processes [258] and therefore play an essential role in both the progress and intensity of a range of chronic diseases, although many derived from the VLC ω-3 PUFAs EPA, DPA and DHA, including resolvins and protectins, have been shown to resolve inflammatory processes [258], and so are considered to be protective. Increased consumption of ALA or SDA has been shown to increase the proportion of EPA and DPA in membranes of inflammatory cells such as neutrophils, monocytes and lymphocytes [38,99,107,128,130,133,152–154,163] (Tables 7 and 8), indicating the potential of plant derived ω-3 PUFAs to reduce inflammation and to protect against chronic inflammatory diseases.

Several plasma inflammatory markers are associated with increased risk of atherosclerosis including C-reactive protein (CRP), soluble intercellular cell adhesion molecule-1 (sICAM-1), and soluble vascular cell-adhesion molecule-1 (sVCAM-1) [259]. In the Nurses' Health Study 1 cohort there was a significant negative association between dietary ALA intake and plasma CRP, interleukin-6 (IL-6) and soluble E-selectin concentrations, after adjustment for body mass index, physical activity, smoking status and intakes of LA and SFAs [260]. This observation supports the idea that ALA may decrease inflammation.

Table 10
Studies investigating the effect of increased alpha-linolenic acid consumption on haemostatic factors in adult human subjects.

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Outcomes investigated	Effect of ALA	Reference
M	20[a]	Flaxseed oil	8	Prothrombin time	None	Kelley et al. (1993)[128]
				Partial prothrombin time	None	
M + F	5.9	Flaxseed oil	4	Factor VII activity	None	Freese & Mutanen (1997)[228]
				PAI-I activity	None	
				Fibrinogen concentration	None	
				Anti-thrombin III activity	None	
M	3.7, 15.4	Flaxseed oil + spread	4	Factor VII concentration	None	Li et al. (1999)[129]
				Fibrinogen concentration	None	
				Prothrombin time	None	
				Anti-thrombin III activity	None	
				Plasminogen concentration	None	
M	3.5	Flaxseed oil + flaxseed oil in foods	6	Factor VII concentration	None	Allman-Farinelli et al. (1999)[151]
				Factor VII activity	None	
				Fibrinogen concentration	None	
				t-PA concentration	None	
				t-PA activity	None	
				PAI-1 concentration	None	
				PAI-1 activity	None	
M	4.2	Perilla oil	40	Fibrinogen concentration	None	Ezaki et al (1999)[123]
				PAI-1 concentration	None	
				Prothrombin time	None	
M + F	10	Spread + foods made with rapeseed oil	10	Factor II concentration	None	Junker et al. (2001)[235]
				Factor VII concentration	None	
				Factor VII activity	None	
				Factor X concentration	None	
				Factor XI concentration	None	
				Factor XII concentration	None	
				Factor XII activity	None	
				t-PA activity	None	
				PAI-1 activity	None	
				D-dimer concentration	None	
				Fibrinogen concentration	None	
M + F	6.3	Spread	10	Fibrinogen concentration	Decrease by 0.18 g/l compared with control group	Bemelmans et al. (2002)[119]
M + F	4.5	Spread	24	Factor VII concentration	None	Finnegan et al. (2003)[234]
				Factor XII concentration	None	
	9	Spread	24	Fibrinogen concentration	None	
				t-PA concentration	None	
				t-PA activity	None	
				PAI-1 concentration	None	
				PAI-1 activity	None	
				Factor VII concentration	None	
				Factor XII concentration	None	
				Fibrinogen concentration	None	
				t-PA concentration	None	
				t-PA activity	None	
				PAI-1 concentration	None	
				PAI-1 activity	None	
F	9	Bread and grains incorporating flaxseeds	48	Fibrinogen concentration	None	Dodin et al. (2008)[139]
M + F	3.4	Rapeseed oil + spread made with rapeseed oil	24	Fibrinogen concentration	None	Egert et al. (2014)[257]

M, male; F, female; PAI, plasminogen activator inhibitor; t-PA, tissue plasminogen activator.
[a] Approximate intake.

Intervention studies that have investigated the effect of increased ALA on inflammatory responses in humans have measured either concentrations of inflammatory markers in blood or inflammatory cell responses *ex vivo* [97,132,134,139,143,146,152–154,161,163,235,240,244,257,261–263] (Table 11). Caughey et al. reported 30% less TNF-α and IL-1β production and 20% less TXB_2 and PGE_2 production by endotoxin-stimulated mononuclear cell cultures obtained from healthy subjects who consumed approximately 14 g/d ALA for 4 weeks compared to baseline and a control group [152]. Ralladis et al. reported lower CRP (38%), serum amyloid A (SAA) (23%), and IL-6 (10%) in dyslipidaemic patients after consuming 7 g ALA/d for 3 months, compared to subjects who consumed a LA rich diet [240]. Hypercholesterolaemic patients who consumed 6–8 g/d ALA for 2 years had a 12.5% decrease in blood CRP concentration, although there was no change in sICAM-1, IL-6 or IL-10 concentrations [261]. Egert et al. reported that consuming 0.9 g/d or 3.4 g/d ALA significantly decreased the serum concentrations of CRP, TNF-α, IL-6, sICAM-1 and sE-selectin in male and female subjects [257]. High ALA intake led to more a pronounced reduction in the serum concentration of YKL-40 compared to the lower intake of ALA [257]. Two recent studies which investigated the effect of increased ALA intake in patients receiving haemodialysis reported significant decreases in CRP concentrations after 8 weeks consuming 40 g/d ground flaxseed [244] or after consuming 30 g/d milled sesame, pumpkin and flaxseed mixture for 12 weeks [146]. TNF-α and IL-6 concentrations were also reduced in the latter study. Thus, 9 out of 19 studies have reported a decrease in inflammatory markers following increased intake of ALA for 4 weeks or more (Table 11). Differences in findings among studies may relate to the amount of ALA used, the duration of the study or other aspects of the diet or the subject group being studied.

Caligiuri et al. demonstrated a significant decrease in plasma concentrations of pro-inflammatory 5-HETE (derived from the ω-6 PUFA AA), 9,10,13-TriHOME and 9,12,13-TriHOME (both derived from the ω-6 PUFA LA) in older healthy subjects (aged 45–64 years) after 4 weeks of dietary intervention of one muffin/day containing 30 g of milled flaxseed (6 g/d ALA) [264]. However, although there was an increase in plasma ALA in these subjects, they showed no changes in ALA-derived oxylipins, and the authors suggested that high concentrations of ALA may decrease metabolism of long-chain PUFAs to oxylipins [264].

In the first study to examine the effect of SDA on inflammatory markers, James et al. reported that there was no effect on TNF-α or IL-1β production by endotoxin-stimulated mononuclear cells after supplementation with SDA-ethyl ester at 0.75 g/d for 3 weeks followed by a further 3 weeks at 1.5 g/d [97].

4.5.4. Alpha-linolenic and stearidonic acids and immune function

Few studies have investigated the effects of increased intake of ALA on immune functions other than inflammation [132,154,262,265–267] (Table 12). Increasing ALA intake to 20 g/d for 8 weeks decreased some, but not all, immune parameters [265]. For example, T cell proliferation was decreased in response to one mitogenic stimulant, but not to others. The observed decreased delayed type hypersensitivity response, which is the summation of the *in vivo* response to intradermal challenge with seven antigens, was in accordance with the decreased T cell function. However, Kew et al. reported no change from study entry in T cell proliferation or in a range of other neutrophil, monocyte and lymphocyte functions in healthy subjects who consumed either 4.5 or 9 g/d ALA for 24 weeks [154]. Other studies involving ALA intakes from 2 to 9 g/d also report no change in range of immune functions [132,262,266,267] (Table 12). Thus, although very high intakes of ALA may suppress some aspects of immune function [265], the overall conclusion from studies using ALA intakes of 2 to 9 g/day for a duration of at least 12 weeks is that ALA at achievable intakes does not influence immune function. Therefore, it is not likely that variations or achievable increases in ALA intake will affect host defences against microorganisms.

Miles et al. examined the effect of consuming 1g/d SDA (from Echium oil) on immune function in overweight and healthy weight subjects [99]. There was no effect of increased SDA intake on any immune parameter measured. Walker et al. suggest the low dose of SDA used in this study may be the reason for the lack of effect on immune function, since the amount of SDA given was too low to significantly enrich the EPA content of the cells involved in the immune response [268].

5. Summary and conclusions

ALA is an essential fatty acid. It is the substrate for the synthesis of the bioactive VLC ω-3 PUFAs EPA and DHA that are associated with health benefits in humans. SDA is the product of ALA metabolism and is also a substrate for synthesis of EPA and DHA. The primary source of EPA and DHA is seafood, especially fatty fish, which may not be sustainable. ALA and SDA are synthesised in plants and represent more sustainable sources of ω-3 PUFAs. There are a large number of studies investigating the effect of increasing intake of ALA on fatty acid composition of blood lipids and blood cells and on cardiovascular risk factors. These studies have used a wide range of ALA intakes and been of varying duration. Relatively few studies have investigated the effect of increasing intake of SDA. Most of these studies of ALA and SDA have been conducted in healthy subjects. Studies using stable isotopes to trace ALA metabolism have revealed that β-oxidation is the main fate of ingested ALA and that a relatively small proportion is metabolised to EPA and an even smaller proportion to DHA. However, there is evidence that ALA conversion to EPA and DHA is greater in young women than in young men, but comparisons between sexes have not been made for other age groups. ALA is stored in adipose tissue and humans have substantial ALA stores (probably around 100 g in normal weight individuals). Studies where ALA intake has been increased for a period of weeks or months show increased content of EPA, and also DPA, in blood lipids and blood cells, with clear dose-dependent increases being seen where there are sufficient data to explore these. In contrast, there appears to be no increase in the DHA content of blood lipids and blood cells with increased ALA intake. These observations are consistent with those from the stable isotope studies. Increased conversion of ALA to EPA has been reported with lowering of LA intake, as well as with increased ALA intake. EPA and DPA are increased more with SDA than with ALA, when given at the same intake levels, consistent with the position of SDA in the pathway of conversion being closer to EPA. Thus, both ALA and SDA may be useful alternatives to EPA, but they seem unlikely to provide an alternative to DHA, although this has not been fully explored in all subgroups of the population including pregnant and lactating women and infants.

Since EPA and DHA have a range of health benefits, there has been much interest in determining whether their plant-derived precursors share those benefits. ALA has been widely explored with regard to its effect on blood lipids and inflammation and there has been more limited examination of its effect on other risk factors for CVD. The effects reported are rather inconsistent, perhaps reflecting comparisons being made, the dose and duration of the study, and the health status of the subjects investigated. ALA may have a cholesterol and LDL-cholesterol lowering effect that is similar to that of the ω-6 PUFA LA, but effects on HDL-cholesterol and TAG are not clear. There are no reported effects of ALA on haemostatic factors and few reported effects on LDL oxidation, blood pressure, platelet aggregation or immune function. Where effects of high dose ALA on inflammation have been reported, these may relate to its conversion to EPA; this may also be the case where effects of ALA on platelet aggregation are reported. Some epidemiological and case-control studies report a protective effect of ALA on CVD morbidity and mortality; this is supported by some long-term intervention studies. This effect may relate to the cholesterol-lowering effect of ALA and/or

Table 11
Studies investigating the effect of increased alpha-linolenic acid consumption on inflammatory markers in adult human subjects.

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Outcomes investigated	Effect of ALA (% change where significant)	Reference
M	13.7[a]	Flaxseed oil + spread	4	Production of prostaglandin E_2 by PBMC Production of TNF-α by PBMC Production of IL-1β by PBMC	−33% −27% −30%	Caughey et al. (1996)[152]
M	4.7	Flaxseed oil capsules	12	Neutrophil chemotaxis Neutrophil respiratory burst	None None	Healy et al. (2000)[153]
M + F	10	Spread + foods made with rapeseed oil	4	Serum concentration of CRP Serum concentration of sL-selectin	None None	Junker et al. (2001)[235]
M + F	2	Flaxseed oil capsules	12	Neutrophil respiratory burst Monocyte respiratory burst Production of TNF-α by PBMC Production of IL-1β by PBMC Production of IL-6 by PBMC Plasma concentrations of sVCAM-1, sICAM-1, and sE-selectin	None None None None None None	Thies et al. (2001)[262]
M + F	4.5	Spread	24	Neutrophil respiratory burst Monocyte respiratory burst Production of TNF-α by PBMC Production of IL-1β by PBMC Production of IL-6 by PBMC	None None None None None None None None None	Kew et al. (2003)[154]
	9	Spread	24	Neutrophil respiratory burst Monocyte respiratory burst Production of TNF-α by PBMC Production of IL-1β by PBMC Production of IL-6 by PBMC	None	
M	4.7	Flaxseed oil capsules	12	Production of TNF-α by PBMC Production of IL-1β by PBMC Production of IL-6 by PBMC	None None None	Wallace et al. (2003)[132]
M + F	0.75	Ethyl ester in capsules	3	Production of TNF-α by PBMC Production of IL-1β by PBMC	None None	James et al. (2003)[97]
	1.5		3	Production of TNF-α by PBMC Production of IL-1β by PBMC	None None	
M	8	Flaxseed oil	12	Serum concentrations of CRP, SAA and IL-6 Serum concentration of sVCAM-1 Serum concentrations of sICAM-1 and sE-selectin	All −25% −15% None	Rallidis et al. (2003,2004)[240,263]
M + F	17.5	Walnuts, walnut oil + flaxseed oil	6	Serum concentration of CRP Serum concentration of sVCAM-1 Serum concentration of sICAM-1 Serum concentration of sE-selectin	−75% −20% −16% −15%	Zhao et al. (2004)[134]
M + F	6.3	Spread	52 and 104	Serum concentration of CRP Serum concentrations of sICAM-1, IL-6 and IL-10	−12.5% None	Bemelmans et al. (2004)[261]
F	9	Bread and grains incorporating flaxseeds	48	Plasma concentration of CRP	None	Dodin et al. (2008)[139]
M + F	1	Flaxseed oil capsules	12	Serum concentration of CRP Serum concentration of TNF-α	None +88%	Kaul et al. (2008)[143]
M + F	4	Baked goods incorporating flaxseeds	10	Serum concentration of CRP Plasma concentration of IL-6	None None	Bloedon et al. (2008)[137]
M + F	2.2	Flaxseed oil capsules	8	Plasma concentration of IL-6 Plasma concentration of sICAM-1	+19% None	Dewell et al. (2011)[161]
	6.6	Flaxseed oil capsules	8	Plasma concentration of IL-6 Plasma concentration of sICAM-1	+10% None	
M + F	9.7[a]	Milled flaxseed added to foods	8	Serum concentration of CRP	−38%	Khalatbari Soltani et al. (2012)[244]
M + F	8.7	Organic Flaxseed oil	6	Serum concentration of CRP Serum concentration of TNFα	−5% +53%	Kontogianni et al. (2012)[163]
M + F	3.4	Rapeseed oil + spread made with rapeseed oil	24	Serum concentration of CRP Serum concentration of TNFα Serum concentration of IL-6 Serum concentration of sVCAM-1 Serum concentration of sE-selectin	−28% −11% −13% +5% −21%	Egert et al. (2014)[257]
M + F 20–35 y	5	Echium oil	8	Serum concentration of CRP	−13%	Kuhnt et al. (2014)[107]
M + F 49–69 y				Serum concentration of CRP	+53%	
M + F MetS				Serum concentration of CRP	+40%	
M + F	3	Sesame/pumpkin/flaxseed mix	12	Serum concentration of CRP Serum concentration of TNFα Serum concentration of IL-6	−61% −79% −51%	Ristic-Medic et al. (2014)[146]

Table 12
Controlled intervention studies in human subjects investigating the effect of increased alpha-linolenic acid consumption on immune function, other than inflammation, in adult human subjects.

Subjects	ALA intake (g/d)	ALA form	Duration (Weeks)	Outcomes investigated	Effects of ALA	Reference
M	20[a]	Flaxseed oil	8	Serum concentrations of IgG, IgA and complement C3 and C4	None	Kelley et al. (1991)[265]
				Saliva concentration of SIgA	None	
				B cell proliferation	None	
				T cell proliferation	Decrease with some mitogens	
				Production of IL-2 and sIL-2 receptor by PBMC	Decrease by 23%	
				DTH response	None	
M + F	2	Flaxseed oil capsules	12	Neutrophil phagocytosis	None	Thies et al. (2001)[262,266,267]
				Monocyte phagocytosis	None	
				T cell proliferation	None	
				Production of IL-2, IFN-γ and IL-4 by PBMC	None	
				NK cell activity	None	
M + F	4.5	Spread	24	Neutrophil phagocytosis	None	Kew et al. (2003)[154]
				Monocyte phagocytosis	None	
				T cell proliferation	None	
				Production of IL-2, IFN-γ and IL-4 by PBMC	None	
	9	Spread	24	DTH response	None	
				Neutrophil phagocytosis	None	
				Monocyte phagocytosis	None	
				T cell proliferation	None	
				Production of IL-2, IFN-γ and IL-4 by PBMC	None	
				DTH response	None	
M	4.7	Flaxseed oil capsules	12	T cell proliferation	None	Wallace et al. (2003)[132]
				Production of IL-2, IFN-γ, IL-4 and IL-10 by PBMC	None	

M, male; F, female; PBMC, peripheral blood mononuclear cells; SIgA, secretory IgA; DTH, delayed type hypersensitivity; IFN, interferon; NK, natural killer.
[a] Approximate intake.

to effects on other risk factors which might be mediated by EPA rather than ALA itself. Thus, in the absence of significant intake of preformed EPA, there is a role for ALA in human health. Nevertheless, ALA seems unlikely to be an effective substitute for DHA. There are few studies of the effect of SDA on health-related outcomes in humans. However, because SDA is superior to ALA as a substrate for biosynthesis of EPA (and DPA), there is greater potential for health improvement with SDA than with ALA.

This review has identified several research gaps. First, the conversion of ALA to EPA, DPA and DHA should be explored across several subgroups of the population including children, adolescents, pregnant and lactating women, and the elderly and the influence of sex on this conversion more clearly identified. Secondly, regulation of the pathway of conversion of ALA to VLC ω-3 PUFAs needs to be better elucidated, including identification of the interactions among the enzymes acting at the different sites and of the genes encoding those enzymes and identification of endogenous and exogenous regulatory factors involved. Apart from age and sex, such factors will include the LA and micronutrient contents of the diet, sensitivity to insulin, and genotypic and epigenetic variation. Thirdly, the role of different tissues in synthesis of VLC ω-3 PUFAs from ALA in humans needs to be better described. Fourthly, due to the apparent inconsistency between the capacity for conversion of ALA to VLC ω-3 PUFAs (which is generally considered to be limited) and the reported associations between polymorphisms or epimutations in genes that encode the main enzymes in the PUFA biosynthesis pathway, there is a need for studies that integrate dietary analysis, assessment of ALA conversion, measurement of EPA, DPA and DHA status, and analysis of gene polymorphisms and epigenetic variations in carefully defined human cohorts. Finally, there should be further research on handling and metabolism of SDA in humans, on its biological effects and on its mechanisms of action.

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Notes to table:
M, male; F, female; PBMC, peripheral blood mononuclear cells; CRP, C-reactive protein; sVCAM, soluble vascular cell adhesion molecule; sICAM, soluble intercellular adhesion molecule, sE-selectin, soluble E-selectin; SAA, serum amyloid A.
[a] Approximate intake.

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