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Dietary α-linolenic acid and health-related outcomes: a metabolic perspective

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α-Linolenic acid (αLNA; 18 : 3*n*-3) is essential in the human diet, probably because it is the substrate for the synthesis of longer-chain, more unsaturated *n*-3 fatty acids, principally EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), which confer important biophysical properties on cell membranes and so are required for tissue function. The extent to which this molecular transformation occurs in man is controversial. The present paper reviews the recent literature on the metabolism of αLNA in man, including the use of dietary αLNA in β-oxidation, recycling of carbon by fatty acid synthesis *de novo* and conversion to longer-chain PUFA. Sex differences in αLNA metabolism and the possible biological consequences are discussed. Increased consumption of EPA and DHA in fish oil has a number of well-characterised beneficial effects on health. The present paper also reviews the efficacy of increased αLNA consumption in increasing the concentrations of EPA and DHA in blood and cell lipid pools, and the extent to which such dietary interventions might be protective against CVD and inflammation. Although the effects on CVD risk factors and inflammatory markers are variable, where beneficial effects have been reported these are weaker than have been achieved from increasing consumption of EPA + DHA or linoleic acid. Overall, the limited capacity for conversion to longer-chain *n*-3 fatty acids, and the lack of efficacy in ameliorating CVD risk factors and inflammatory markers in man suggests that increased consumption of αLNA may be of little benefit in altering EPA + DHA status or in improving health outcomes compared with other dietary interventions.

α-Linolenic acid: Human metabolism: Cardiovascular disease: Inflammation

Introduction

α-Linolenic acid (αLNA; 18: 3*n*-3) is an essential fatty acid in the human diet and is the most abundant *n*-3 PUFA in the Western diet. The present review will focus on two main areas of the biology of αLNA in human adults; its metabolism and its effect on CVD and inflammation.

The major dietary sources of αLNA are green leaves, and oils used in cooking such as rapeseed oil and soyabean oil where it accounts for up to 10 % of total fatty acids. Flaxseed (also known as linseed) oil, which is commonly consumed as a dietary supplement, is particularly rich in αLNA (> 50 % total fatty acids). Typical consumption of αLNA in Europe, Australia and North America ranges from 0·6 to 1·7 g/d in men and from 0·5 to 1·4 g/d in women (Ministry of Agriculture, Fisheries & Food, 1997; Hulshof *et al.* 1999; Ollis *et al.* 1999; Kris-Etherton *et al.* 2000; Innis & Elias, 2003).

αLNA can be converted to longer-chain *n*-3 PUFA such as EPA (20 : 5*n*-5) and DHA (22 : 6*n*-3), and it is difficult to distinguish any function which is innate to αLNA from those of its longer-chain metabolites (Sinclair *et al.* 2000). The concentration of αLNA in phospholipids in plasma and in cell membranes is typically less than 0·5 % of total fatty acids (Table 1). It thus seems unlikely that the amount of αLNA present in these pools would be able to exert a significant effect on the biophysical properties of the phospholipid membrane and so influence biological function. Typical consumption of αLNA in Western countries is about 25- and 15-fold greater than of EPA and DHA (Ministry of Agriculture, Fisheries & Food, 1997; Hulshof *et al.* 1999; Ollis *et al.* 1999; Astorg *et al.* 2004). Conversely, the concentrations of the longer-chain metabolites EPA, docosapentaenoic acid (DPA; 22 : 5*n*-3) and DHA exceed that of αLNA in most tissues (Table 1). This apparent mismatch between dietary intakes and concentrations in plasma and tissue lipids supports the view that the primary biological role of αLNA is as a substrate for EPA and DHA synthesis.

Abbreviations: DPA, docosapentaenoic acid; LA, linoleic acid; αLNA, α-linolenic acid; CRP, C-reactive protein; PC, phosphatidylcholine; SFA, saturated fatty acid; TAG, triacylglycerol.

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Table 1. Examples of proportions of α-linolenic acid (αLNA), eicosapentaenoic acid and docosahexaenoic acid in blood, cell and tissue lipid fractions from adult human subjects

	% Total fatty acids			
Lipid fraction	αLNA	EPA	DHA	Reference
Plasma phosphatidylcholine	0·1	0·8	2·9	Mantzioris *et al.* (1994)
Plasma cholesteryl ester	0·4	0·8	0·5	Mantzioris *et al.* (1994)
Plasma triacylglycerol	0·8	0·8	0·5	Mantzioris *et al.* (1994)
Platelet phosphatidylcholine	0·3	0·2	1·1	Weaver *et al.* (1990)
Platelet phosphatidylethanolamine	0·2	0·6	6·3	Weaver *et al.* (1990)
Mononuclear cell phospholipid	0·1	0·3	2·3	Caughey *et al.* (1996)
Neutrophil phospholipid	NA	0·6	1·3	Healy *et al.* (2000)
Erythrocyte phospholipid	NA	0·8	3·5	Burdge *et al.* (2005)
Liver phosphatidylethanolamine	0·2	1·6	7·7	Crawford *et al.* (1976)
Brain grey matter phosphatidylethanolamine	0·1	NA	24·3	Crawford *et al.* (1976)
Brain grey matter phosphatidylserine	NA	NA	36·6	O'Brien & Sampson (1965)
Brain grey matter phosphatidylcholine	NA	NA	3·1	O'Brien & Sampson (1965)
Brain white matter phosphatidylethanolamine	NA	NA	3·4	O'Brien & Sampson (1965)
Retina phosphatidylcholine	NA	NA	22·2	Anderson (1970)
Retina phosphatidylethanolamine	NA	NA	18·5	Anderson (1970)
Retina phosphatidylserine	NA	NA	4·6	Anderson (1970)
Testis total lipid extract	NA	NA	8·5	Bieri & Prival (1965)
Sperm phospholipid	NA	NA	35·2	Poulos *et al.* (1973); Tang *et al.* (1993)
White adipose tissue	0·7	NA	0·1	Kaminskas *et al.* (1999)

NA, not available.

Human essential fatty acid deficiency is very rare and the evidence to support the essentiality of αLNA in the human diet is largely restricted to patients who received parenteral feeds. Of patients receiving long-term total parenteral nutrition lacking αLNA, 50 % of children and 30 % of adults exhibited visual dysfunction (Vinton *et al.* 1990). The DHA content of neural membrane phospholipids modulates the activities of several signalling pathways in the brain (Sinclair *et al.* 2000; Astorg *et al.* 2004) and is critical for optimal retinal function (Jeffrey *et al.* 2001; Mitchell *et al.* 2003). Thus these findings are consistent with the suggestion that lack of αLNA intake decreased availability of DHA for incorporation into neural membranes (Vinton *et al.* 1990). This conclusion is supported by the observation that the offspring of monkeys fed an *n*-3 PUFA-deficient diet during pregnancy show visual impairments (Neuringer *et al.* 1986) and that supplementation of the infant monkeys with αLNA resulted in an increase in the concentration of DHA in neural tissues and an improvement in visual function (Connor & Neuringer, 1988).

Patterns of α-linolenic acid consumption in different nations

While typical intakes of αLNA are generally regarded as being approximately 1·5 g/d in Western populations, detailed comparison of the consumption of αLNA shows an almost 3-fold variation between individual nations (Fig. 1 (A)). For example, αLNA intake for French men is about one-third that of North American men (Fig. 1 (A)). Calculation of the linoleic acid (LA; 18 : 2*n*-6):αLNA ratio in the diet also shows marked variation between nations. For example, in men the LA:αLNA ratio differs between 5·5 in Denmark and 13·8 in France (Fig. 1 (B)). For women, this ratio varies between 4·3 in Denmark and 13·6 in France. However, the LA:αLNA ratio for Spanish men and women together is 27·0 (Fig. 1 (B)). The importance of these variations in the LA:αLNA ratio in the diet is that the relative consumption of LA and αLNA modulates the extent to which αLNA is converted to long-chain metabolites. This will be discussed in more detail later. One potential implication is that recommendations for the intakes of *n*-3 and *n*-6 PUFA may need to be devised for individual nations according to their habitual LA and αLNA consumption.

Bioavailability of α-linolenic acid from the diet

The bioavailability of αLNA from the diet is determined by the efficiency of absorption across the gastrointestinal tract. There is very little information regarding the absorption of αLNA by the human gut. Measurement of the concentrations of ^{2}H-labelled fatty acids in the triacylglycerol (TAG) fraction of chylomicrons following ingestion in a single meal showed that the absorption and secretion of oleic acid (18 : 1*n*-9), LA and αLNA were similar (Emken *et al.* 1994). Although this does not specifically measure absorption across the gut, it does indicate that the overall bioavailability of αLNA from a meal is similar to that of other unsaturated fatty acids. Direct assessment of absorption of αLNA requires measurement of the output in stool compared with consumption of a known amount in a meal. To date there have been no published data from such an experiment. Measurement of the cumulative concentration of labelled αLNA in stool collected over 5 d following ingestion of 750 mg [U-^{13}C]αLNA by a single individual showed that greater than 96 % of the administered dose was absorbed (GC Burdge, unpublished results). Similar levels of αLNA uptake have also been reported in patients with ileostomies who were fed 100 g linseed oil (Saunders & Sillery, 1988). These findings suggest that absorption of αLNA across the gut and its secretion into the bloodstream is efficient in man.

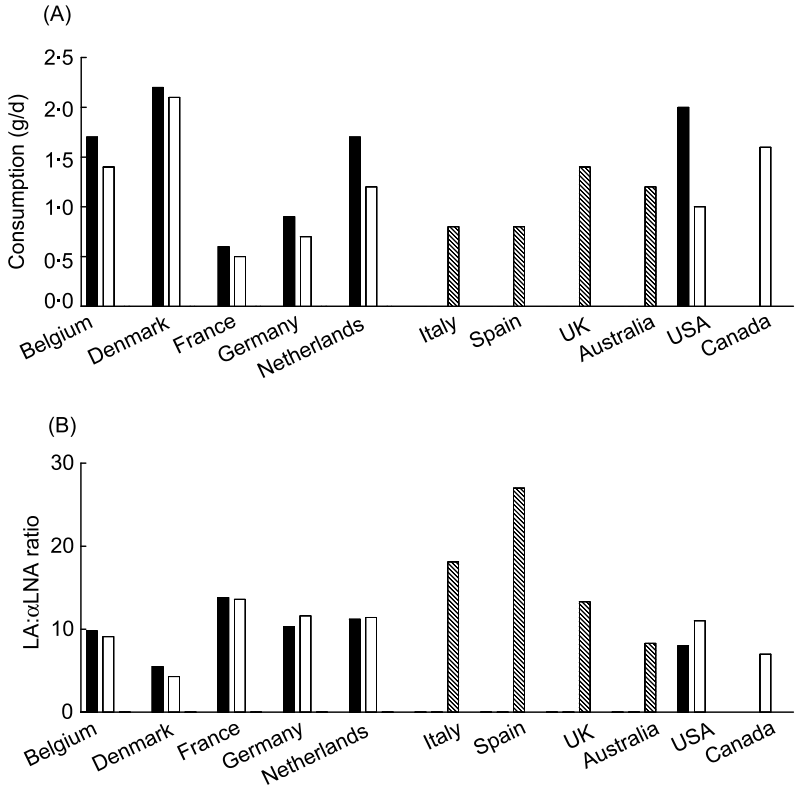

Fig. 1. Daily intakes of α-linolenic acid (αLNA) and linoleic acid (LA) in different nations for men (■), women (□) and men and women (▨). (A) αLNA consumption; (B) LA:αLNA ratio. Values for Canada were from pregnant women. Separate data were not available for men and women from Italy, Spain, UK and Australia. (Data from Ministry of Agriculture, Fisheries & Food, 1997; Hulshof *et al.* 1999; Ollis *et al.* 1999; Kris-Etherton *et al.* 2000; Innis & Elias, 2003).

α-Linolenic acid metabolism in man

αLNA which has been absorbed by the gut passes into the circulation primarily esterified to TAG carried by chylomicron particles (Burdge *et al.* 2002) and so is made available for uptake by tissues and incorporation into pools destined for storage, cell membranes, production of energy, mobilisation and conversion to longer-chain PUFA. The metabolic fates of αLNA in man are summarised in Fig. 2.

Incorporation of α-linolenic acid into adipose tissue

Adipose tissue accounts for approximately 15 % of body mass in men and 23 % of body mass in women. Thus incorporation of αLNA into this storage pool represents a potentially important route of disposal of dietary αLNA and a reserve pool which is available for mobilisation during periods of increased demands. αLNA accounts for about 0·7 % of total fatty acids in neutral lipids in adipose tissue in men and women, while DHA concentration is approximately 0·1 % and EPA is practically undetectable (Tang *et al.* 1993; Kaminskas *et al.* 1999). Thus, it can be calculated that, in a 75 kg man with a fat mass of 15 %, the whole body αLNA reserve in adipose tissue would be approximately 79 g (roughly equivalent to typical intake over 53 d). Likewise, in a 65 kg woman with a fat mass of 23 %, the whole body αLNA reserve in adipose tissue would be approximately 105 g (roughly equivalent to typical intake over 70 d). It is conceivable that such reserves may buffer variations in dietary intake and so prevent development of deficiency of αLNA or of longer-chain metabolites.

During the postprandial period there is a metabolic drive to store fatty acids which is facilitated by the insulin-dependent increase in lipoprotein lipase activity and of fatty acid uptake in adipose tissue. In the fasting state, plasma NEFA are derived primarily from the release of adipose tissue TAG stores by the action of hormone-sensitive lipase. The exchange of αLNA between the blood and adipose

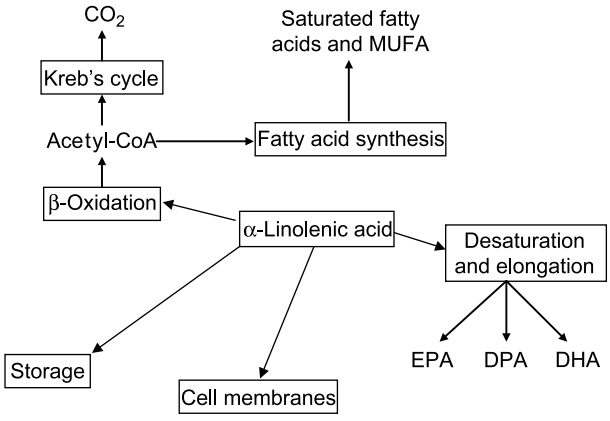

Fig. 2. Summary of the main metabolic fates of α-linolenic acid in man. DPA, docosapentaenoic acid.

tissue compartments has not been characterised in detail in human subjects *in vivo*. However, when men consumed [U-^{13}C]αLNA, labelled αLNA was detected in the plasma NEFA pool within 2 h and reached a peak at 6 h (Burdge *et al.* 2002). One interpretation is that during the early postprandial period labelled αLNA detected in the NEFA pool probably reflects incomplete entrapment of fatty acids released by the hydrolysis of chylomicron TAG (Evans *et al.* 2002). At later time points (for example, about 6 h) the presence of labelled αLNA in plasma NEFA may reflect recently assimilated fatty acids. Overall, the rapid release of αLNA into the NEFA pool, together with the αLNA pool associated with chylomicron remnant particles, would tend to facilitate supply of αLNA to the liver. The concentration of [^{13}C]αLNA in plasma NEFA was 2-fold greater in women than in men over 21 d (Burdge & Wootton, 2002). This suggests sex differences in the metabolism of αLNA in storage pools and potentially greater short-term availability of αLNA for supply to the liver in women.

Comparison of the metabolic fates of EPA and DHA during the postprandial period showed enrichment of DHA in the plasma NEFA pool, presumably as a result of incomplete entrapment of fatty acids released by hydrolysis of chylomicron TAG, compared with EPA (Heath *et al.* 2003). Furthermore, there was greater incorporation of DHA than of EPA into VLDL TAG. This suggests differential uptake of PUFA released by lipoprotein lipase activity which appears to facilitate supply of DHA to the liver and remobilisation on VLDL.

β-Oxidation of α-linolenic acid

Since αLNA is essential in the human diet and is consumed in relatively modest amounts, it is somewhat surprising that it is a substrate for β-oxidation and energy production. However, the appearance of ^{13}C as $^{13}CO_2$ on breath following the ingestion of ^{13}C-labelled αLNA has been clearly demonstrated in a number of studies and the area under the time × enrichment curve has been used to estimate the proportion of ingested αLNA used for energy production. The values produced by such calculations probably represent an approximately 30 % underestimate of the actual proportion of ingested αLNA used in energy production due to trapping of $^{13}CO_2$ in bicarbonate pools (Irving *et al.* 1983). Since the period of collection of CO_2 differs between reports from 9 to 48 h, the estimates of partitioning towards β-oxidation differ from 15 to 33 % (DeLany *et al.* 2000; Vermunt *et al.* 2000; Bretillon *et al.* 2001; Burdge *et al.* 2002; McCloy *et al.* 2004). When subjects were studied over 24 h under comparable conditions, the fractional β-oxidation of αLNA in women was estimated as approximately 22 % of administered dose (Burdge & Wootton, 2002) compared with 33 % in men (Burdge *et al.* 2002). This may reflect lower muscle mass in women, and the potential overall effect would be to increase the proportion of ingested αLNA available for conversion to longer-chain PUFA in women compared with men. The extent of partitioning of αLNA towards β-oxidation, when assessed under identical conditions, was almost twice that of palmitic, stearic and oleic acids, and greater than LA (DeLany *et al.* 2000) (Fig. 3 (A)). This may reflect the higher affinity of carnitine palmitoyl transferase-1 for αLNA (Clouet *et al.* 1989).

One study has reported the effect of altering the *n*-3 PUFA content of the background diet on the proportion of ingested [^{13}C]αLNA recovered as $^{13}CO_2$ on breath (Burdge *et al.* 2003). Three groups of men matched for BMI, age and fasting plasma TAG concentrations consumed a standard meal containing 700 mg [U-^{13}C]αLNA and excretion of $^{13}CO_2$ on breath was measured over 24 h. Subjects then consumed either a control diet (αLNA 1·7 g/d, EPA + DHA 0·4 g/d), a diet containing an increased amount of αLNA (αLNA 9·6 g/d, EPA + DHA 0·4 g/d) or a diet containing an increased amount of EPA + DHA (αLNA 1·7 g/d, EPA + DHA 1·6 g/d) for 8 weeks. There was no difference in energy intake between the groups. The proportion of ingested labelled αLNA recovered as $^{13}CO_2$ on breath was then measured again. There was no significant difference between baseline and the end of the 8-week intervention period in the proportion of labelled αLNA partitioned towards β-oxidation in the group consuming the control, increased αLNA or increased EPA + DHA diets (Fig. 3 (B)). This suggests that the extent of partitioning of αLNA towards β-oxidation is relatively stable over short periods of time and that altering the amount of either αLNA or long-chain *n*-3 PUFA in the diet does not significantly alter this process.

Recycling of carbon from α-linolenic acid into saturated and monounsaturated fatty acids

In addition to conversion to CO_2 by the activity of Kreb's cycle, carbon in acetyl-CoA generated by fatty acid β-oxidation may be recycled and used in fatty acid synthesis *de novo*. This process has been suggested to be important as a source of fatty acids in pregnant and fetal monkeys (Sheaff-Greiner *et al.* 1996) and rats (Cunnane *et al.* 1994, 2003). There is one report which describes recycling of carbon released by β-oxidation of αLNA in human subjects (Burdge & Wootton, 2003). Men (35 years of age) and women (28 years of age) consumed 700 mg [U-^{13}C]αLNA and the concentrations of labelled saturated fatty acids (SFA) and MUFA in plasma were measured over 21 d. Labelled palmitic, stearic, palmitoleic and oleic acids were detected in plasma phosphatidylcholine (PC) and TAG, but not other plasma lipid pools in both men and women. The proportion of label was 6-fold greater in plasma PC compared with TAG in men and 25-fold greater in plasma PC than TAG in women. These data suggest preferential channelling of SFA and MUFA synthesised by the recycling pathway into phospholipids by the liver, which is in contrast to the molecular partitioning of the bulk of the hepatic SFA and MUFA pools towards TAG. Since typical daily consumption of αLNA in the UK is 25-fold and 15-fold greater than EPA and DHA, respectively (Ministry of Agriculture, Fisheries & Food, 1997), it seems unlikely that recycling of carbon from αLNA simply represents a means of disposal of excess αLNA not required for EPA and DHA synthesis.

The total concentration of labelled SFA and MUFA in plasma lipids was 20 % greater in men compared with women (Burdge & Wootton, 2003) (Table 2). This is in agreement with greater partitioning of αLNA towards

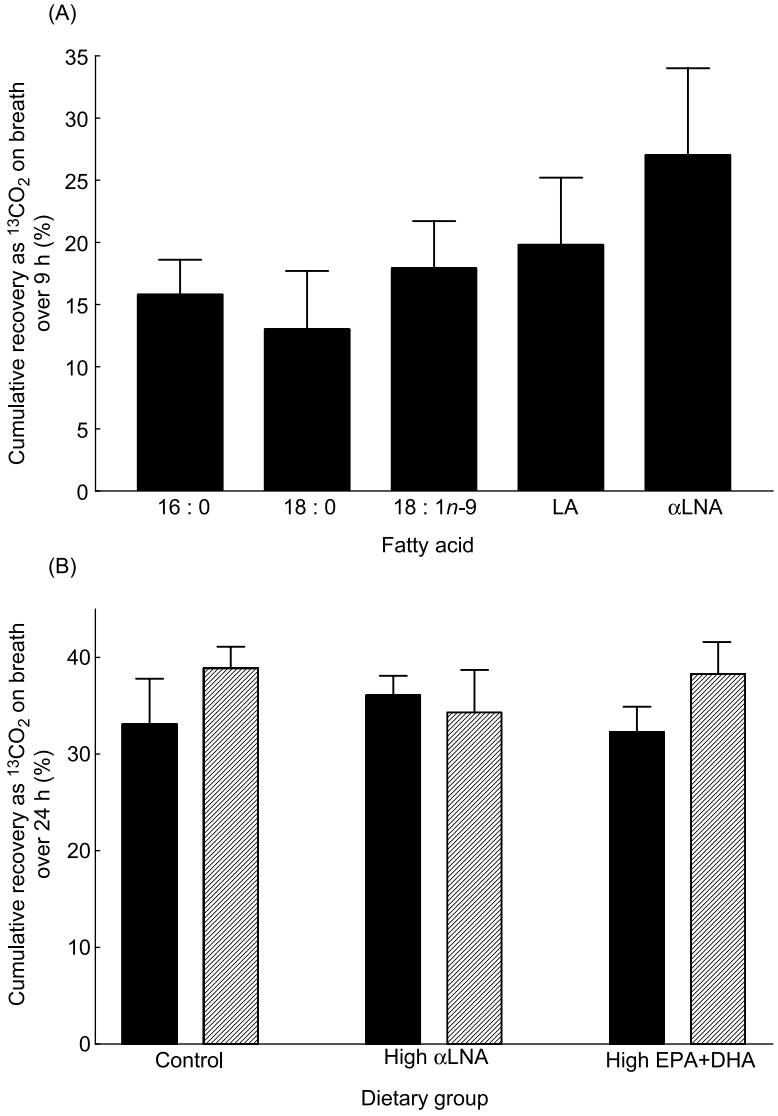

Fig. 3. Recovery of ingested [^{13}C]fatty acids as $^{13}CO_2$ on breath. (A) Comparison of the proportion of different fatty acids recovered as $^{13}CO_2$ on breath under identical conditions (Delany *et al.* 2000). (B) The effect of consuming experimental diets with fatty acid compositions based on the typical pattern of fatty acid consumption in the UK (control); providing 9·6 g α-linolenic acid (αLNA)/d (high αLNA); providing 1·7 g EPA + DHA/d (high EPA + DHA). The proportion of [^{13}C]αLNA recovered as $^{13}CO_2$ on breath is shown at the start (■) and the end (▨) of the dietary intervention (Burdge *et al.* 2003). Values are means, with standard errors represented by vertical bars. 16 : 0, Palmitic acid; 18 : 0, stearic acid; 18 : 1*n*-9, oleic acid; LA, linoleic acid.

β-oxidation in men compared with women (Burdge *et al.* 2002; Burdge & Wootton, 2002). Comparison of the amount of ^{13}C recycled into SFA and MUFA with that incorporated by desaturation and elongation of αLNA into longer-chain PUFA in plasma over 21 d showed marked differences between men and women (Table 2). In men, the carbon recycling greatly exceeded conversion to longer-chain PUFA. In contrast, the amount of carbon recycled into SFA and MUFA in women was about 20 % of that recovered in *n*-3 PUFA (Table 2). One overall implication of these findings is that the extent of partitioning of αLNA towards β-oxidation and carbon recycling may be an important control point in the regulation of the availability of αLNA for conversion to longer-chain PUFA and that the relative partitioning between these metabolic fates differs markedly between men and women. This suggests that the extent of partitioning of αLNA towards β-oxidation may not simply reflect sex differences in muscle mass.

The general pathway for conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids

A pathway for the conversion of the essential fatty acids LA and αLNA to longer-chain PUFA has been described in rat liver (for a review, see Sprecher, 2002) and is summarised in Fig. 4. All reactions occur in the endoplasmic reticulum with the exception of the final reaction which results in the formation of DHA. Since both *n*-6 and *n*-3 PUFA are metabolised by the same desaturation–elongation pathway, there exists potential for competition between these two

Table 2. Recycling of carbon from [U-^{13}C]α-linolenic acid and conversion to longer-chain polyunsaturated fatty acids in men and women (Data from Burdge & Wootton, 2002, 2003)

Fatty acid	AUC over 21 d (μmol/l) Men	Women
Palmitic acid (16 : 0)	22	15
Palmitoleic acid (16 : 1*n*-7)	3	3
Stearic acid (18 : 0)	6	5
Oleic acid (18 : 1*n*-9)	7	4
Total SFA + MUFA	38	27
EPA	0·2	87
DPA	0·2	21
DHA	ND	34
Total *n*-3 PUFA	0·4	142
SFA + MUFA:*n*-3 PUFA ratio	95	0·2

AUC, area under the curve; SFA, saturated fatty acids; DPA, docosapentaenoic acid; ND, not detected.

families of fatty acids. The initial conversion of αLNA to 18 : 4*n*-3 by the action of Δ6-desaturase is the rate-limiting reaction of the pathway. The affinity of Δ6-desaturase for αLNA is greater than for LA (Sprecher, 2002). However, the typically higher concentration of LA than αLNA in cellular pools results in greater conversion of *n*-6 PUFA. The introduction of a double bond at the Δ6 position is followed by the addition of 2C by elongase activity and then by desaturation at the Δ5 position by Δ5-desaturase to form EPA. DPA is synthesised from EPA by the addition of 2C. The conversion of DPA to DHA has been a matter of controversy and two pathways have been suggested for this reaction. First, Δ4-desaturase activity has been suggested to be the primary mechanism for DHA synthesis (Infante & Huszagh, 1998). However, others have demonstrated that the synthesis of DHA involves the formation of 24-carbon

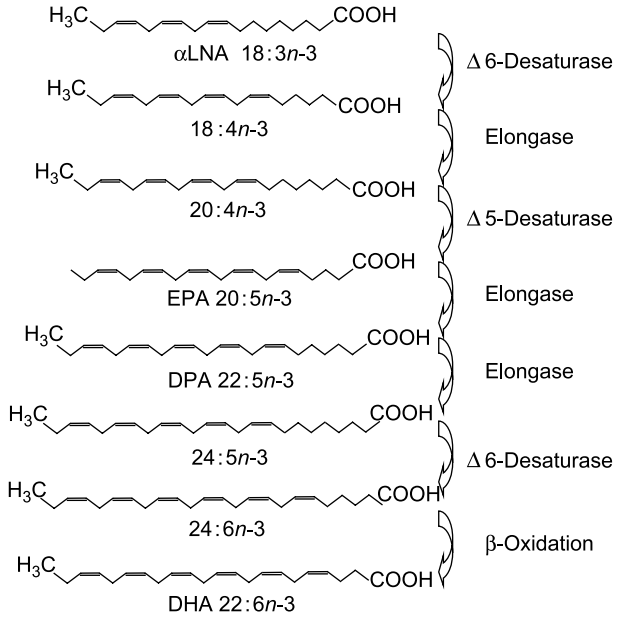

Fig. 4. The general pathway for conversion of α-linolenic acid (αLNA) to longer-chain PUFA (Sprecher, 2002). DPA, docosapentaenoic acid.

intermediates, a second desaturation at the Δ6 position and limited peroxisomal β-oxidation (Sprecher, 2002). Studies in which subcellular organelles were isolated and then recombined (Li *et al.* 2000) and reports of the action of the specific Δ6-desaturase inhibitor SC-26 196 (Harmon *et al.* 2003) strongly support the second pathway. Furthermore, patients with peroxisome disorders such as Zellweger's syndrome have lower plasma DHA concentration, which is the result of a failure to convert DPA to DHA in the absence of an active peroxisomal β-oxidation pathway (Martinez, 2000). Thus the consensus pathway for the conversion of DPA to DHA is as follows: DPA is elongated to 24 : 5*n*-3 which is desaturated at the Δ6-position by the action of Δ6-desaturase activity to form 24 : 6*n*-3. It is unclear whether the same enzyme is responsible for desaturation of αLNA and 24 : 5*n*-3 (Marzo *et al.* 1996; de Antueno *et al.* 2001; D'andrea *et al.* 2002). Then 24 : 6*n*-3 is translocated from the endoplasmic reticulum to the peroxisome where the acyl chain is shorted by 2C by one cycle of the β-oxidation pathway to form DHA. DHA is then translocated back to the endoplasmic reticulum. Although the precise regulation of the translocation steps and limited β-oxidation is not known, it is possible that this represents a locus for metabolic regulation that facilitates the control of DHA synthesis independently from the preceding steps of the pathway.

The activity of the desaturation–elongation pathway in the liver is the most important in terms of supply of αLNA metabolites to other tissues. However, expression of Δ6 and Δ5 desaturases has also been reported in human heart, brain, placenta, skeletal muscle, kidney, and pancreas (Cho *et al.* 1999). An active desaturation–elongation pathway has also been reported in immortalised human T cells (Jurkat cells), but not B cells (Marzo *et al.* 1995), and in sebaceous glands (Ge *et al.* 2003). Human epidermis appears to possess Δ6-desaturase, but not Δ5-desaturase activity (Chapkin *et al.* 1986). Whether the activity of the desaturation–elongation pathway contributes quantitatively to meeting the demands of these tissues for long-chain PUFA remains to be determined.

α-Linolenic acid conversion to longer-chain *n*-3 polyunsaturated fatty acids in human adults

Current understanding of the extent to which man can convert αLNA to longer-chain PUFA is based on the findings of studies reporting the outcomes of chronic increases in intake of αLNA on concentrations of *n*-3 PUFA in plasma, cell and tissue lipid pools, and of shorter-term studies in which subjects consume a bolus of αLNA labelled with a stable isotope.

Effects of chronically increased α-linolenic acid consumption

A number of studies have reported the effects of consuming increased amounts of αLNA, usually via consumption of oils with a high αLNA content or of products made with those oils (for example, spreads), on the fatty acid composition of plasma or cell lipids (Tables 3 and 4). These studies were conducted either in men or in mixed

Table 3. Studies investigating the effect of increased α-linolenic acid (αLNA) consumption on the fatty acid composition of blood lipids in human subjects

Subjects	αLNA intake (g/d)†	How αLNA provided	Duration (weeks)	Blood lipid fraction	Change in proportion of total fatty acids from baseline (%) EPA	DPA	DHA	Reference
M + F	7·8	Flaxseed oil capsules	2	Plasma PC	108*	NA	−14	Sanders & Younger (1981)
M	13·7	Flaxseed oil + spread	4	Plasma PL	143*	NA	14	Mantzioris *et al.* (1994)
				Plasma CE	150*	NA	20	
				Plasma TAG	200*	NA	20	
M	20‡	Flaxseed oil	8	Total serum	0	20	38	Kelley *et al.* (1993)
M + F	9§	Muffins made with flaxseeds	4	Plasma PL	33*	36*	−3	Cunnane *et al.* (1995)
M	15·4	Flaxseed oil + spread	4	Plasma TAG	300*	250*	−33	Li *et al.* (1999)
				Plasma PL	367*	50*	−5	
				Plasma TAG	300*	50	0	
M + F	6·3	Spread	52	Serum CE	40*	NA	50	Bemelmans *et al.* (2002)
M	4·7	Flaxseed oil capsules	12	Plasma PL	60*	NA	3	Wallace *et al.* (2003)
M + F	4·5	Spread	24	Plasma PL	82*	5	−2	Finnegan *et al.* (2003*b*)
	9·5				133*	39*	6	
M + F	0·75 then 1·5§	αLNA ethyl ester in capsules	4 then 4	Plasma PL	8 then 15	0 then 7	−3 then −11	James *et al.* (2003)

DPA, docosapentaenoic acid; M, male; F, female; PC, phosphatidylcholine; NA, data not available; PL, phospholipid; CE, cholesteryl ester; TAG, triacylglycerol.
*Mean values were significantly different from baseline ($P < 0·05$).
†αLNA intake refers to total intake unless otherwise specified.
‡Approximate intake.
§Excluding background diet contribution of approximately 1 g/d.

Table 4. Studies investigating the effect of increased α-linolenic acid (αLNA) consumption on the fatty acid composition of circulating cells in human subjects

Subjects	αLNA intake (g/d)†	How αLNA provided	Duration (weeks)	Cell lipid fraction	Change in proportion of total fatty acids from baseline (%) EPA	DPA	DHA	Reference
M + F	7·8	Flaxseed oil capsules	2	Platelet PL	100*	NA	−3	Sanders & Younger (1981); Sanders & Roshanai (1983)
M	8·5	Rapeseed oil-based foods	2·5	Platelet PC	100	0	−27*	Weaver *et al.* (1990)
				Platelet PE	133*	51*	−5	
M	8 % of fatty acids	Rapeseed oil-based foods	8	Platelet PL	14*	13	20	Kwon *et al.* (1991)
M	2·1 % energy	Rapeseed oil-based foods	3	Total platelet lipid	−8	NA	20	Mutanen *et al.* (1992)
M	20‡	Flaxseed oil	8	Total mononuclear cell§ lipid	0	45	−36	Kelley *et al.* (1993)
M	13·7	Flaxseed oil + spread	4	Neutrophil PL	67*	NA	0	Mantzioris *et al.* (1994)
M + F	18‡ (8·5 % energy)	Flaxseed oil	3	Platelet PL	140*	45*	−11	Allman *et al.* (1995)
M	13·7	Flaxseed oil + spread	4	Mononuclear cell§ PL	133*	NA	−9	Caughey *et al.* (1996)
M	15·4	Flaxseed oil + spread	4	Platelet PL	150*	56*	−10	Li *et al.* (1999)
M	3·5	Flaxseed oil + foods	6	Total platelet lipid	200*	45*	−50	Allman-Farinelli *et al.* (1999)
M	4·7	Flaxseed oil capsules	12	Neutrophil PL	30	NA	15	Healy *et al.* (2000)
M + F	0·75 then 1·5‖	αLNA ethyl ester in capsules	4 then 4	Erythrocyte PL	0 then 11	0 then 3	−4 then −11	James *et al.* (2003)
M + F	4·5	Spread	24	Mononuclear cell§ PL	0	6	−28*	Kew *et al.* (2003)
	9·5				33*	0	−20*	

DPA, docosapentaenoic acid; M, male; F, female; PL, phospholipid; NA, data not available; PC, phosphatidylcholine; PE, phosphatidylethanolamine.
*Mean values were significantly different from baseline ($P < 0·05$).
†αLNA intake refers to total intake unless otherwise specified.
‡Approximate intake.
§Mononuclear cells are a mix of lymphocytes and monocytes (ratio approximately 85:15).
‖Excluding background diet contribution of approximately 1 g/d.

groups of men and women, used intakes of αLNA ranging from less than 1 to 20 g/d, and were of a few weeks' to many months' duration (Tables 3 and 4). Despite differences in the study design, physical form in which the αLNA was presented and the duration of the studies, overall they consistently demonstrate increased proportions of EPA in both plasma and cell lipids when αLNA intake is increased (Tables 3 and 4). The relationship between increased αLNA intake and increased EPA concentration in plasma phospholipids is a significant linear one (r 0·846; $P = 0{\cdot}004$; Fig. 5). The relationship for cell phospholipids is also likely to be linear, but there are insufficient data for a single cell type to allow this to be clearly identified at this stage. There is also some variation in the response between studies which might reflect differences in age and sex mix of the subjects studied, differences in the fatty acid content of the background diet, differences in the way in which αLNA was provided and differences in analytical procedures used.

Because of competition for metabolism between LA and αLNA, the LA content of the diet may influence conversion of αLNA to longer-chain derivatives. If this is so, then the EPA content of blood and cell lipids should be greater at a given intake of αLNA if LA intake is decreased. A study by Chan *et al.* (1993) demonstrated that this is indeed the case. Subjects consumed diets providing 7 g αLNA/d for 18 d against a background of either 21 or 50 g LA/d. The proportion of EPA was greater after the low- compared with the high-LA background in plasma PC (0·8 *v.* 0·3 % of fatty acids), plasma phosphatidylethanolamine (0·9 *v.* 0·3 % of fatty acids) and platelet PC (0·25 *v.* 0·1 % of fatty acids).

Several studies also demonstrate increased proportions of DPA in plasma and cell lipids when αLNA consumption is increased (Tables 3 and 4). The studies also consistently demonstrate that increased consumption of αLNA does not result in increased proportions of DHA in plasma or cell lipids (Tables 3 and 4). Indeed many studies report a tendency for DHA to decline when αLNA consumption is markedly increased, although few studies have identified this as a statistically significant effect (Tables 3 and 4).

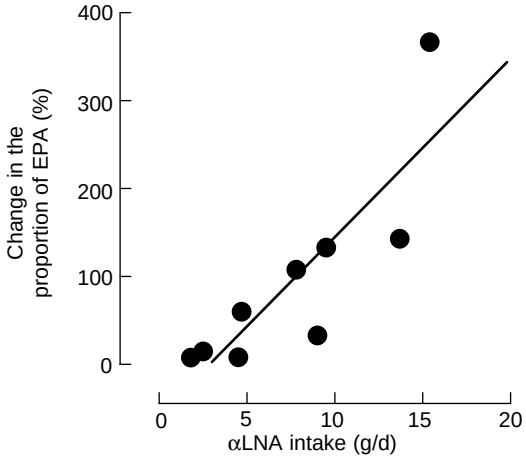

Fig. 5. The relationship between consumption of α-linolenic acid (αLNA) and the change in the concentration of EPA in plasma phospholipids. Data are taken from references cited in Table 3.

Overall, these studies demonstrate that chronically increased consumption of αLNA results in conversion to EPA resulting in increases in EPA concentration in plasma and cell pools, while the extent of conversion to DHA is insufficient to increase the concentration of this fatty acid.

Estimates of α-linolenic acid conversion from stable-isotope tracer studies

A number of studies using αLNA labelled with either ^{13}C or ^{2}H have provided estimates of the extent of conversion to longer-chain PUFA in man. The advantages and limitations of these techniques have been reviewed (Emken, 2001). There remain unresolved issues regarding standardisation of quantification of data (particularly how conversion between fatty acids should be estimated), kinetic modelling, variation between subjects including age and sex, the method of administration of the labelled fatty acid, the duration of the study, the extent to which the background diet is controlled and the use of measurements of labelled fatty acids in blood (including which lipid pool should be measured) as a marker of fatty acid metabolism within tissues. Together these factors have resulted in considerable heterogeneity in the findings of such studies (Emken *et al.* 1994, 1999; Salem *et al.* 1999; Vermunt *et al.* 2000; Pawlosky *et al.* 2001, 2003*a*,*b*; Burdge *et al.* 2002, 2003; Burdge & Wootton, 2002; Goyens *et al.* 2005; Hussein *et al.* 2005) (Table 5). This presents a considerable challenge to any attempt to reach a consensus view on αLNA metabolism in man. Nevertheless, at present there are no practical alternatives to these stable-isotope tracer techniques to study human αLNA metabolism *in vivo.*

The outcomes of stable-isotope tracer studies designed to investigate conversion of αLNA to longer-chain PUFA in man are summarised in Table 5. The general consensus of these studies is that the proportion of αLNA entering the desaturation–elongation pathway and converted to EPA and DPA is low. The extent of conversion of αLNA to DHA is even less clear (Table 5). The highest estimated fractional conversion is 4 % (Emken *et al.* 1994), while most other studies have reported lower estimates of conversion (0·05 % or less) (Emken *et al.* 1999; Salem *et al.* 1999; Vermunt *et al.* 2000; Pawlosky *et al.* 2001, 2003*a*,*b*; Burdge *et al.* 2003; Goyens *et al.* 2005; Hussein *et al.* 2005) and one study failed to detect significant incorporation of stable isotope into DHA above background ^{13}C enrichment (Burdge *et al.* 2002).

Pawlosky *et al.* (2001) have suggested estimates for the efficiency of conversion of individual steps in the desaturation–elongation pathway from kinetic analysis based on the concentrations of individual ^{2}H-labelled fatty acids in plasma from a mixed group of men and women consuming a beef-based diet. The findings of the study were that the efficiency of conversion of αLNA to EPA was 0·2 %, of EPA to DPA 65 % and of DPA to DHA 37 %. Thus, the overall efficiency of conversion from αLNA is 0·2 % to EPA, 0·13 % to DPA and 0·05 % to DHA. This is in general agreement with the other studies summarised in Table 5 and with the assumption that the first reaction catalysed by Δ6-desaturase is the rate-limiting step of the pathway.

Table 5. Estimated conversion of α-linolenic acid (α-LNA) to longer-chain polyunsaturated fatty acids

Reference	Subjects	Isotope and dose	Outcome measures			
				EPA	DPA	DHA
Emken *et al.* (1999)	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)	M	^{2}H-labelled mixed TAG, 3·1 g. Subjects consumed 6·5 or < 0·1 g DHA/d for 90 d before experiment.	AUC concentrations in total plasma lipids. Higher DHA consumption decreased EPA and DHA synthesis by 76 and 88 %, respectively			
Salem *et al.* (1999)	Adults*	^{2}H-labelled ethyl ester, 1 g	Concentrations in total plasma lipids.	57 ng/ml†	ND	< 2 ng/ml†
Vermunt *et al.* (2000)	M + F	[U-^{13}C]methyl ester, 45 mg	Peak concentrations adjusted for estimated total blood volume	120 μg	50 μg	About 10 μg
Pawlosky *et al.* (2001)	M + F	^{2}H-labelled ethyl ester, 1 g	Mathematical modelling of kinetic parameters following consumption of a beef-based diet. Data expressed here as conversion efficiency from αLNA	0·2 %	0·13 %	0·05 %
Burdge *et al.* (2002)	M	[U-^{13}C]α-LNA, free fatty acid, 0·7 g	Concentrations in plasma TAG, NEFA and PC over 21 d. Fractional conversion estimated from time × concentration AUC	8 %	8 %	ND
Burdge & Wootton (2002)	F	[U-^{13}C]αLNA, free fatty acid, 0·7 g	Concentrations in plasma TAG, NEFA, CE and PC over 21d. Fractional conversion estimated from time ×concentration AUC	21 %	6 %	9 %
Pawlosky *et al.* (2003*b*)	M + F	^{2}H-labelled ethyl ester, 1 g	Mathematical modelling of kinetic parameters. Increased fish consumption decreased the rate constant for conversion of DPA to DHA by 70 % compared with a beef-based diet			
Burdge *et al.* (2003)	M	[U-^{13}C]free fatty acid, 0·7 g	Repeated analysis of subjects at baseline and after consuming control, αLNA- or EPA + DHA-enriched diets for 8 weeks. EPA + DPA, but not DHA, synthesis reduced by the EPA + DHA-enriched diet, but no effect of increased αLNA consumption			
Pawlosky *et al.* (2003*a*)	M *v.* F	^{2}H-labelled ethyl ester	Fractional conversion DPA to DHA. Conversion about 2-fold greater in women than men on *ad libitum* and beef-based diet. Reduced to the same level as men by consuming a fish-based diet			
Hussein *et al.* (2005)	M	[U-^{13}C]free fatty acid	Consumption of 17 g αLNA/d or 17 g LA/d for 12 weeks followed by tracer study			
			High-αLNA diet	0·29 %	0·05 %	< 0·01 %
			High-LA diet	0·19 %	0·02 %	< 0·01 %
			Both diets	0·26 %	0·04 %	< 0·01 %
Goyens *et al.* (2005)	Adults*	[U-^{13}C]free fatty acid. 30 mg bolus plus 8 × 20 mg daily doses	Kinetic model. 7 % of ingested αLNA was incorporated into plasma PL. 99·8 % of this was converted to EPA (6·98 % of ingested), and 1 % each to DPA and DHA (0·07 % of ingested in each fatty acid)			

DPA, docosapentaenoic acid; M, male; F, female; TAG, triacylglycerol; AUC, area under the curve; PC, phosphatidylcholine; ND, not detected; CE cholesteryl ester; PL, phospholipid.
* Distribution by sex not disclosed.
† Peak concentrations.

Several studies have reported the effects of modifications to the background diet on the extent of αLNA conversion to long-chain *n*-3 PUFA determined using stable-isotope tracers. The effect of increased consumption of EPA + DHA or DHA alone on this process is of interest because of the potential for feedback inhibition of this pathway. Emken *et al.* (1999) compared the effect of consuming diets either containing $<0{\cdot}1$ g DHA/d or supplemented with 6·5 g purified DHA/d. There was a 76 % reduction in EPA synthesis and an 88 % reduction in DHA synthesis in the group receiving the DHA supplement. Others have reported a decrease in the conversion efficiency of DPA to DHA following the consumption of a fish-based diet (containing EPA + DHA) compared with a beef-based diet in a mixed group of men and women (Pawlosky *et al.* 2003*b*). However, when the fractional conversion of DPA to DHA was calculated separately for men and women, the decrease in DHA synthesis as a result of consuming a fish-based diet was only found in the female subjects (Pawlosky *et al.* 2003*a*). Consumption of 1·6 g EPA + DHA/d for 8 weeks decreased EPA and DPA, but not DHA, synthesis when αLNA conversion was compared before and after the intervention in the same individuals (Burdge *et al.* 2003). These studies indicate that increased consumption of long-chain *n*-3 PUFA acts to down regulate their synthesis from αLNA, possibly by activation of peroxisomal proliferator-activated receptor-α leading to inhibition of Δ6-desaturase transcription (Tang *et al.* 2003). One recent study showed that consuming either 17 g LA/d or 17 g αLNA/d inhibited the conversion of the alternate series of fatty acids (Hussein *et al.* 2005). In contrast, Vermunt *et al.* (2000) found that increased αLNA intake (8 g/d) decreased EPA, DPA and DHA synthesis, although others have not found this (Burdge *et al.* 2003).

Overall, substantial increases in the intakes of individual fatty acids are able to modify the conversion of αLNA, although there are inconsistencies in the magnitude of these effects between reports.

Since there is a positive linear relationship between the amount of αLNA consumed and the change in plasma phospholipid EPA concentration (Fig. 5), it is possible to evaluate the accuracy of the estimates of αLNA conversion provided by studies using stable-isotope tracers. It is assumed that doubling the provision of EPA will increase its concentration in plasma phospholipid by 100 %. The daily intake of EPA in the UK is about 0·06 g (Ministry of Agriculture, Fisheries & Food, 1997). Thus a further 0·06 g would be required to double the concentration of EPA in the circulation. In order to make a direct comparison between pools, estimated fractional conversion of αLNA based upon the measurement of ^{13}C incorporation into plasma phospholipid will be used, which is about 5 % in men (Burdge *et al.* 2002). In the UK, where this study was conducted, men consume about 1·5 g αLNA/d. Partitioning towards β-oxidation removes about 33 % of αLNA from the pool derived from the diet which is available for conversion, leaving 1·0 g. As there are no estimates for the fractional partitioning of αLNA into storage or membrane pools it will be assumed that all of the remaining αLNA is available for conversion, which obviously results in an overestimation of the synthesis of EPA. If it is assumed that about 5 % is converted to EPA, then 1 g αLNA will provide 0·05 g EPA, which would almost double the provision of EPA. Thus, the consumption of 1 g of αLNA above the background diet would be expected to double the concentration of EPA in the circulation. Others have suggested that, based on kinetic models, 0·2 % of αLNA is converted to EPA (Pawlosky *et al.* 2001), which would provide 0·002 g EPA, which would mean that 300 g αLNA would need to be consumed in order to provide an additional 0·06 g above the intake in the background diet. The data in Fig. 5 show that 5 g is required to double the provision of EPA, and so neither of these estimates of αLNA conversion based upon stable-isotope tracer studies is entirely correct. There are two possible explanations. First, the true level of conversion lies between the two experimental values and is in the order of 2 % if it is assumed that 1·7 g (i.e. 33 % of 5 g) is lost in β-oxidation and all of the remaining αLNA is available for desaturation and elongation. This is unlikely to be true. Second, if the estimate of 5 % conversion was correct, then the pool available for conversion must be less than 1 g by a factor of 5. One possible explanation is that 80 % of αLNA which is ingested and is not used to produce energy enters pools which are not available for conversion to longer-chain PUFA, such as storage pools in adipose tissue or structural pools in cell membranes. If so, the pool of αLNA available for conversion would be 0·2 g for each 1 g consumed. For example, if 5 g αLNA are consumed, β-oxidation would account for 1·7 g, and 2·3 g would enter storage or membrane pools, leaving 1·0 g which would be available for conversion. Of this, 5 % would be used to provide 0·05 g EPA. The proportion of ingested αLNA incorporated into pools not used for conversion or β-oxidation, presumably storage or cell membranes, is comparable with the estimated upper range (57 %) of incorporation of [^{13}C]αLNA into total adipose tissue (McCloy *et al.* 2004). The calculations presented here are obviously an oversimplification of the processes which determine the extent to which dietary αLNA is converted to EPA. However, these estimates do suggest a level of fractional conversion of αLNA to EPA, which can explain the data from dietary intervention studies, and are consistent with the findings of at least some of the stable-isotope tracer studies.

α-Linolenic acid metabolism: sex differences

The majority of investigations of αLNA metabolism in human subjects have focused on groups of relatively young healthy individuals, either men or mixed groups of men and women. There is relatively little information regarding the effects of sex on this process. Two reports have specifically studied αLNA conversion in women of reproductive age. Burdge & Wootton (2002) showed that conversion of αLNA to EPA and DHA in women aged about 28 years was substantially greater (2·5-fold and >200-fold, respectively) than in a comparable study of men of similar age (Burdge *et al.* 2002) (Table 5). This finding is strongly supported by kinetic analysis, which showed that the rate-constant coefficient for the conversion of DPA to DHA was approximately 4-fold greater in women compared with men (Pawlosky *et al.* 2003*a*) (Table 5). This may reflect a greater availability of αLNA for conversion in women than

in men that may perhaps, in part, be due to lower partitioning towards β-oxidation. However, since the rate-constant coefficient for the conversion of DPA to DHA was greater in women than men (Pawlosky *et al.* 2003*a*), it is likely that there is a sex-related difference in the activity of the desaturation–elongation pathway.

One possible explanation for the greater synthesis of EPA and DHA from αLNA in women compared with men is the action of oestrogen. DHA synthesis was almost 3-fold greater in women using an oral contraceptive pill containing 17α-ethylnyloestradiol than in those who did not (Burdge & Wootton, 2002). The suggestion that oestrogen may increase the activity of the desaturation–elongation pathway is consistent with the finding that oestrogen-based hormone replacement therapy in postmenopausal women resulted in greater plasma dihomo-γ-linolenic and arachidonic acid concentrations than before treatment (Ottosson *et al.* 1984). Furthermore, DHA concentration in the plasma cholesteryl ester fraction has recently been shown to be greater in women (0·53 % total fatty acids) compared with men (0·48 % total fatty acids) consuming diets controlled for energy and αLNA content, although DHA is a minor component of this plasma lipid pool (Giltay *et al.* 2004*b*). Interestingly, administration of 17α-ethylnyloestradiol to male to female transsexuals increased the concentration of DHA in plasma cholesteryl esters by 42 % which is in agreement with the effects of oral contraceptive pill use on αLNA conversion (Burdge & Wootton, 2002). Conversely, testosterone decreased DHA concentration by 22 % in female to male transsexuals (Giltay *et al.* 2004*b*). Hormone replacement therapy in postmenopausal women also increased plasma cholesteryl ester arachidonic acid and DHA concentrations (Giltay *et al.* 2004*a*). Our own data (L Bakewell, PC Calder and GC Burdge, unpublished results) show that women (aged 18 to 35 years) have a 50 % higher concentration of DHA in plasma PC compared with men of similar age and BMI (>20 and $<30\,kg/m^2$) (Fig. 6). Together these data strongly support the suggestion that sex hormones, in particular oestrogen, regulate the activity of the desaturation–elongation pathway in man.

One possible biological role for greater capacity for αLNA conversion in women may be in meeting the demands of the fetus and neonate for DHA. The developing human fetus assimilates at least 400 mg DHA per week during the third trimester (Lauritzen *et al.* 2001). Since this estimate reflects only brain, adipose tissue and liver requirements, the overall demands for DHA are likely to be substantially greater. Desaturase activities in the developing human liver appear to be lower than in adults (de Gomez Dumm & Brenner, 1975; Sauerwald *et al.* 1977; Poisson *et al.* 1993; Salem *et al.* 1996; Carnielli *et al.* 1996), and so the capacity of the fetus and neonate to satisfy their demands for DHA may be limited. Thus, assimilation of DHA by the fetus has to be met primarily by supply of DHA by the mother. In pregnant women, plasma PC DHA concentration increases by approximately 33 % between 16 weeks (170 μmol/l) and 40 weeks (230 μmol/l) gestation (Postle *et al.* 1995). When the increase in maternal blood volume during pregnancy is taken into account (Gregersen & Rawson, 1959), in women there is an overall doubling of DHA in the circulation. Since circulating oestrogen concentration rises during pregnancy due to synthesis and secretion by the placenta, one possibility is that αLNA conversion may increase during gestation. This is supported by studies in rats that indicate that the increased plasma PC DHA concentration during pregnancy is the result of physiological adaptations to hepatic phospholipid (Burdge *et al.* 1994) and αLNA (Larque *et al.* 2003) metabolism. If true in women, one implication would be that the 50 % variation between pregnant women in plasma PC DHA concentration at term (Postle *et al.* 1995) may reflect differences in αLNA metabolism in addition to any dietary effects. This may influence the supply of DHA to the fetus and, in turn, the development and function of neural tissues.

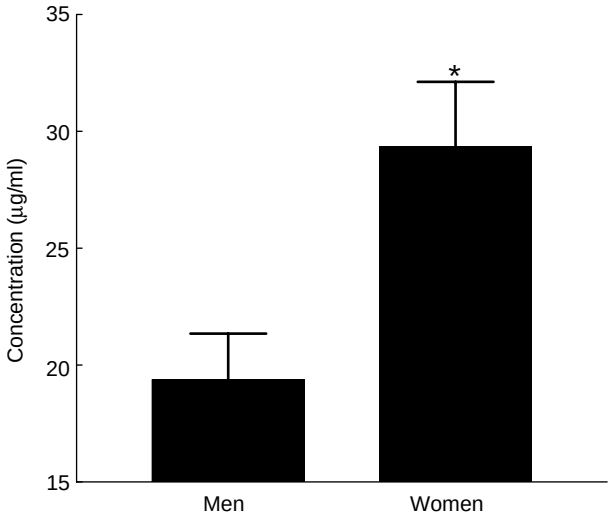

Fig. 6. The concentration of DHA in plasma phosphatidylcholine in men and women consuming their habitual diets. Values are means, with standard errors represented by vertical bars. * Mean value was significantly different from that for men ($P = 0{\cdot}02$) (L Bakewell, PC Calder and GC Burdge, unpublished results).

Consumption of 10·7 g αLNA/d by lactating women increased maternal plasma, erythrocyte and breast-milk αLNA concentration (Francois *et al.* 2003). The effect on breast-milk EPA and DPA concentrations is less clear, as the difference between baseline DPA concentration (0·19 ± 0·05 %) and that after 4 weeks of supplementation (0·17 ± 0·02 %) does not support the claim that the DPA content of milk increased over time (Francois *et al.* 2003). Increased consumption of αLNA did not alter breast-milk DHA concentration (Francois *et al.* 2003). This is consistent with the finding that newly synthesised arachidonic acid is a minor component of the arachidonic acid content of breast milk (Del Prado *et al.* 2001). One interpretation is that the incorporation of PUFA into milk may be dependent upon mobilisation of stores accumulated in adipose tissue before conception and during pregnancy. If so, this emphasises the importance of adequate nutrition of women both before and during pregnancy. Furthermore, since prolactin suppresses oestrogen activity, the activity of the desaturation–elongation pathway may be down regulated in lactating compared with non-pregnant and pregnant women, and so these results do not exclude the possibility of increased DHA synthesis during pregnancy.

Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids: the legacy of our ancestors?

The function of the brain is arguably the major distinguishing characteristic of *Homo sapiens*. The human brain weighs about 1300 g, of which 60 % is lipid. Long-chain PUFA are critically important for neurological development and function, in particular DHA, and deficit in the DHA content of neural membranes is associated with impaired function (Fernstrom, 1999). Thus the interaction between the availability of long-chain PUFA, in particular DHA, and the development of the brain is likely to have been a critical process in the evolution of *H. sapiens*. The growth in both size and functional capacity which accompanied the evolution of *H. sapiens* from our common ancestor with the chimpanzees 5·8–7·1 million years ago (Hacia, 2001) would have required an increase in the availability of DHA to support the increased synthesis of brain tissues during development and to replenish the turnover of neural membrane phospholipids. The level of DHA in the meat of species living on the African savanna, although greater than domesticated species, is relatively low (Broadhurst *et al.* 2002) and the hunting skills required too great for this to be a major source of DHA to support the early evolution of the hominid brain (Muskiet *et al.* 2004). An alternative theory of the ancestral hunter–gatherer which emphasises the role of long-chain PUFA in the evolution of the brain suggests that hominids evolved along the margins of lakes and rivers, and along the seashore where fish provided a richer source of DHA than would have been obtained by a hunter–gatherer lifestyle (Broadhurst *et al.* 2002; Gibbons, 2002*a*; Muskiet *et al.* 2004). This is supported by the relatively large numbers of remains of early hominids and of their tools at Lake Turkana in the East African Rift Valley (now Kenya), in the Southern African Cape and in the Central African Chad Basin (Gibbons, 2002*b*). Furthermore, when early humans left Africa, they did so along a coastal route (Macaulay *et al.* 2005). One interpretation is that evolution of the human brain may have occurred in the presence of an adequate dietary supply of preformed DHA, and that there was little need for synthesis of DHA from αLNA. Furthermore, experiments in animal models suggest that recycling of DHA in cell membranes of the brain and the retina is highly efficient (Wiegand *et al.* 1991; Chang *et al.* 1999). In rats, 97 % of DHA in the brain is derived from the recycling of DHA from membrane sources (Chang *et al.* 1999). If this is also found in human adults, this suggests that the actual daily requirements for DHA to replenish neural membranes may be very small, and so could be provided by relatively low dietary intakes. In the context of the evolution of the brain, such adaptation would tend to buffer its function against variations in the intake of DHA from the diet. The development of the brain represents the period of greatest sensitivity to deficits in supply of DHA. Since desaturase activities in fetal liver are unlikely to satisfy demands for DHA (Salem *et al.* 1996), the developing fetal brain is highly dependent upon a supply of preformed DHA from the mother. In this context, the capacity to increase maternal DHA synthesis by the action of oestrogen may be of particular importance in ensuring a supply of DHA to the fetus. Speculatively, this ability may have evolved to protect the developing fetal brain from a deficit in DHA accumulation, a risk which would be substantially greater if supply of DHA from the maternal diet was the sole source of DHA for supply to the fetus. Furthermore, accumulation of DHA into adipose tissue during the third trimester is about 10-fold greater (380 mg/week) than into the fetal brain (35 mg/week) (Clandinin *et al.* 1981). This suggests that one of the reasons why man (Poissonnet *et al.* 1984), unlike other primates (Adolph & Heggeness, 1971; Lewis *et al.* 1983), has evolved the capacity to accumulate adipose stores in late gestation may be to provide a nutritional buffer during the transition from placental to oral supply (Kuzawa, 1998; Correia *et al.* 2004), thus preventing a deficit in DHA. This store is likely to depend upon supply of preformed DHA from the mother.

Overall, the capacity of modern man to convert αLNA to longer-chain PUFA, in particular DHA, appears to be consistent with our evolutionary past and the fatty acid content of our ancestral diet. This is reflected in differences in the efficiency of conversion of αLNA to DHA between men and women. In women, capacity to up regulate may be important for protecting the development of the fetal brain against deficit in DHA accumulation during pregnancy. The contrastingly low level of DHA synthesis in men may reflect the activity of recycling mechanisms that may limit the demands of neural tissue for DHA.

α-Linolenic acid and human health-related outcomes

Long-chain n-*3 polyunsaturated fatty acids and human health*

Studies of disease incidence in native Inuit populations in Greenland, Canada and Alaska identified less cardiovascular and inflammatory disease than expected (Dyerberg *et al.* 1978; Kromann & Green, 1980; Bjerregaard & Dyerberg, 1988; Newman *et al.* 1993). This has been attributed to the very high dietary intake of long-chain *n*-3 PUFA among these populations (Bang *et al.* 1976). Epidemiological studies in Western Europe, the USA and Japan indicate decreased risk of CVD (for references, see Bucher *et al.* 2002; Kris-Etherton *et al.* 2002; Calder, 2004*a*; He *et al.* 2004) and inflammation (Shoda *et al.* 1996; Pischon *et al.* 2003; Lopez-Garcia *et al.* 2004) with increasing habitual consumption of fish, oily fish, or long-chain *n*-3 PUFA. Supplementation studies with long-chain *n*-3 PUFA, usually as fish oil, have demonstrated beneficial impacts on a multitude of cardiovascular risk factors including fasting TAG concentrations (for reviews, see Harris, 1996; Roche & Gibney, 1999), postprandial lipaemia (for a review, see Williams, 1997), blood pressure (for a meta-analysis, see Geleijnse *et al.* 2002), platelet aggregation (Sanders *et al.* 1981; Goodnight *et al.* 1981; von Schacky *et al.* 1985), blood coagulation and fibrinolysis (see British Nutrition Foundation, 1992), heart rate variability (Christensen *et al.* 1996, 2001), cardiac arrhythmias (Schrepf *et al.* 2004), vascular reactivity (Chin *et al.* 1993; McVeigh *et al.* 1994; Goode *et al.* 1997; Tagawa *et al.* 1999), and inflammation (for reviews, see Calder, 2001, 2002, 2003, 2005). Paradoxically there are reports that long-chain *n*-3 PUFA increase the susceptibility of LDL to

oxidation *ex vivo* (Suzukawa *et al.* 1995; Sorensen *et al.* 1998; Finnegan *et al.* 2003*b*), which, if it occurred *in vivo*, would be a deleterious, pro-atherogenic effect. Long-chain *n*-3 PUFA also affect immune function (for reviews, see Calder, 2001; Calder *et al.* 2002). If the effects of long-chain *n*-3 PUFA are representative of general effects of this class of fatty acids, then αLNA too could beneficially affect a range of cardiovascular risk factors and so could lower the risk of CVD in particular, but also inflammatory, and perhaps other, conditions. Alternatively through conversion to its longer-chain, more unsaturated derivatives which are found in fish oil, αLNA could exert the same health benefits ascribed to EPA and DHA. This has become an attractive notion because there are more sources of αLNA (nuts, seeds, vegetable oils) than of long-chain *n*-3 PUFA; αLNA-rich oils are readily available, inexpensive and sustainable; αLNA-rich oils are more easily incorporated into existing foodstuffs (spreads, cooking oils, cakes, biscuits, snacks etc) than are fish oils, thus representing less of a challenge to food technologists; and high amounts of αLNA do not affect the taste, smell and shelf life of foodstuffs in the same way that inclusion of high amounts of long-chain *n*-3 PUFA can and so are more attractive to food producers, food retailers and consumers. In addition, the lower propensity of αLNA to free radical attack, compared with EPA and DHA, means that the risk of increased oxidative damage to lipoproteins such as LDL and to cell membranes is much lower. Finally, the view that the current intake of the *n*-6 PUFA LA is too high (Simopoulos, 2001; Lands, 2003) and increases the risk of thrombotic, inflammatory and allergic sequelae, provides an opportunity for αLNA to decrease disease risk as a result of being a replacement in the diet for LA. For these reasons there has been significant interest in the effects of dietary αLNA intake on CVD and on its risk factors.

α-Linolenic acid and cardiovascular disease

Epidemiological associations

Several studies report a protective effect of αLNA towards CVD. A population-based case–control study in Costa Rica reported that high adipose tissue αLNA content, a marker for intake, was associated with lower risk of myocardial infarction (Baylin *et al.* 2003). Dietary αLNA intake was significantly inversely associated with mortality from CHD in the Multiple Risk Factor Intervention Trial (Dolecek, 1992). The highest quintile of αLNA intake (1·36 g/d) was associated with 45 % fewer coronary deaths in women compared with the lowest quintile (0·71 g/d) (Hu *et al.* 1999). Dietary αLNA intake was inversely associated with risk of myocardial infarction among men (Ascherio *et al.* 1996); there was a relative risk of 0·41 for each 1 % increase in energy intake as αLNA. In a study of over 4400 men and women the highest quintile of αLNA intake (1·1 g/d) was associated with 40 % lower mortality from coronary artery disease compared with the lowest quintile of intake (0·5 g/d) (Djousse *et al.* 2001). In addition, dietary αLNA intake was inversely related to carotid atherosclerosis (Djousse *et al.* 2003) and to calcified atherosclerotic plaque in the coronary arteries (Djousse *et al.* 2005). In contrast to these findings, other epidemiological studies found no association between αLNA intake and CHD risk (Simon *et al.* 1995; Oomen *et al.* 2001) or mortality (Pietinen *et al.* 1997; Oomen *et al.* 2001). A recent meta-analysis of five prospective cohort studies (Dolecek, 1992; Ascherio *et al.* 1996; Pietinen *et al.* 1997; Hu *et al.* 1999; Oomen *et al.* 2001) concluded that high αLNA intake is associated with decreased risk of fatal CHD (Brouwer *et al.* 2004).

Intervention studies

The well-known Lyon Heart Study reported a substantial reduction in coronary events and deaths among myocardial infarction survivors following a Mediterranean-style diet which included an αLNA-rich margarine providing 1·5 g αLNA/d (de Lorgeril *et al.* 1994). However, this intervention also involved several other dietary changes and so the contribution, if any, of increased αLNA intake to the positive outcomes cannot be identified.

α-Linolenic acid and cardiovascular risk factors

Blood lipids

A number of studies have investigated the effect of increased αLNA consumption on blood cholesterol, LDL and HDL concentrations (Table 6). While some of these studies report little effect of αLNA intervention, several indicate that αLNA is similar to LA with respect to blood cholesterol concentration (Kestin *et al.* 1990; Chan *et al.* 1991; Mantzioris *et al.* 1994; Arjmandi *et al.* 1998; Pang *et al.* 1998; Sodergren *et al.* 2001; Bemelmans *et al.* 2002; Finnegan *et al.* 2003*b*; Zhao *et al.* 2004). Likewise some of these studies indicate similar effects of αLNA and LA on LDL- and HDL-cholesterol concentrations (Kestin *et al.* 1990; Chan *et al.* 1991; Arjmandi *et al.* 1998; Sodergren *et al.* 2001; Bemelmans *et al.* 2002; Finnegan *et al.* 2003*b*; Zhao *et al.* 2004). However, two studies (Mantzioris *et al.* 1994; Pang *et al.* 1998) suggest that αLNA is not as effective as LA in lowering LDL-cholesterol concentrations. Furthermore, studies reporting that αLNA decreased HDL concentration (Nestel *et al.* 1997; Rallidis *et al.* 2003) did not see this effect with LA, while Bemelmans *et al.* (2002) found that HDL concentration was significantly lower (by 4 % or 0·05 mM) after αLNA than after LA. Taken together these data would suggest that, while αLNA is cholesterol- and LDL-lowering, it is less effective than LA and that αLNA, unlike LA, may decrease HDL concentration.

Studies investigating the effect of αLNA on fasting plasma TAG concentrations are contradictory, with most reporting no change from baseline, several reporting an increase or a tendency to an increase, and two reporting a decrease (for references, see Table 6). These differences in outcome may relate to the fat content of the background diet, to habitual dietary fat composition, to whether subjects were placed on a standardised diet before the αLNA intervention, to the amount of αLNA and of other fatty acids supplied, and to the degree of triacylglycerolaemia at baseline. It is interesting that the two studies reporting decreased plasma TAG concentrations used very high αLNA intakes (Singer *et al.* 1986; Zhao *et al.* 2004). Some studies permit the effect of αLNA on fasting TAG

Table 6. Controlled intervention studies in human subjects investigating the effect of α-linolenic acid (αLNA) on blood lipid concentrations

Subjects	αLNA intake (g/d)*	Duration (weeks)	How αLNA provided	Outcomes investigated	Effect of αLNA (compared with baseline)	Reference
M + F; age 23–30 years	9·4	2	Flaxseed oil capsules	Total cholesterol	None	Sanders & Roshani (1983)
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F; mean age 33 years	38	2	Flaxseed oil	Total cholesterol	None	Singer *et al.* (1986)
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	Decrease (25 %)	
M; mean age 47 years	9·2	6	Milkshake containing flaxseed oil	Total cholesterol	None	Kestin *et al.* (1990)
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				VLDL-cholesterol	None	
				Triacylglycerol	None	
M; age 20–34 years	Approximately 7	2·5	Rapeseed oil or soyabean oil	Total cholesterol	Decrease (18 %)	Chan *et al.* (1991)
				LDL-cholesterol	Decrease (22 %)	
				VLDL-cholesterol	Decrease (41 %)	
				Apo B	Decrease (19 %)	
				Apo A1	Decrease (9 %)	
M; age 21–37 years	20†	8	Flaxseed oil	Total cholesterol	None	Kelley *et al.* (1993)
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
				Apo B	None	
				Apo A1	None	
M; age 25–44 years	13·7	4	Flaxseed oil + spread	Total cholesterol	None	Mantzioris *et al.* (1994)
				LDL-cholesterol	None	
				HDL-cholesterol	Increase (20 %)	
				Triacylglycerol	None	
M + F; mean age 25 years	9‡	4	Muffins made with flaxseeds	Total cholesterol	Decrease (6 %)	Cunnane *et al.* (1995)
				LDL-cholesterol	Decrease (9 %)	
				HDL-cholesterol	None	
				Triacylglycerol	None (tendency to increase; 8 %)	
M + F; mean age 30 years	2·5	12	Flaxseed oil capsules	Total cholesterol	None	Layne *et al.* (1996)
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F; age 20–44 years	5·9	4	Flaxseed oil capsules	Total cholesterol	None	Freese & Mutanen (1997)
				HDL-cholesterol	None	
				Triacylglycerol	None	
Overweight M + F; age 45–63 years	20·0	4	Spread + foods made with flaxseed oil	Total cholesterol	None	Nestel *et al.* (1997)
				LDL-cholesterol	None	
				HDL-cholesterol	Decrease (8 %)	
				Triacylglycerol	None	
M; age 18–35 years	10·1	3 and 6	αLNA-rich foods	Total cholesterol	None	Pang *et al.* (1998)
				LDL-cholesterol	None	
				HDL-cholesterol	None	

Table 6. *Continued*

Subjects	αLNA intake (g/d)*	Duration (weeks)	How αLNA provided	Outcomes investigated	Effect of αLNA (compared with baseline)	Reference
				Triacylglycerol	None	
F; mean age 56 years	8·5	6	Muffins and bread made with flaxseeds and flaxseed oil	Total cholesterol	Decrease (7 %)	Arjmandi *et al.* (1998)
				LDL-cholesterol	Decrease (16 %)	
				HDL-cholesterol	None	
				Triacylglycerol	None (tendency to increase; 11 %)	
				Lipoprotein (a)	Decrease (6 %)	
M; mean age 34 years	3·7, 15·4	4	Flaxseed oil + spread	Total cholesterol	None	Li *et al.* (1999)
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F; age 67–91 years	4·2	40	Perilla oil	Total cholesterol	None	Ezaki *et al.* (1999)
				LDL-cholesterol	None	
				HDL-cholesterol	None	
				VLDL-cholesterol	None	
				Triacylglycerol	None	
				VLDL-triacylglycerol	None	
M + F; mean age 27 years	10	4	Spread + foods made with rapeseed oil	Total cholesterol	Decrease (15 %)	Junker *et al.* (2001)
				LDL-cholesterol	Decrease (18 %)	
				HDL-cholesterol	Decrease (12 %)	
				Triacylglycerol	None	
M + F; age 30–65 years	9·2	4	Spread + rapeseed oil + foods made with rapeseed oil	Total cholesterol	Decrease	Sodergren *et al.* (2001)
				LDL-cholesterol	Decrease (11 %)	
				HDL-cholesterol	Decrease	
				VLDL-cholesterol	None	
				Triacylglycerol	None	
				Apo A1	None	
				Apo B	None	
				Lipoprotein (a)	None	
M + F; age 28–65 years	3	6	Rapeseed oil	Total cholesterol	Decrease (6 %)	Karvonen *et al.* (2002)
				LDL-cholesterol	Decrease (12 %)	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F; age 28–65 years	11·4	6	Camelina oil	Total cholesterol	Decrease (8 %)	Karvonen *et al.* (2002)
				LDL-cholesterol	Decrease (12 %)	
				HDL-cholesterol	None	
				Triacylglycerol	None	
M + F at risk of CVD; age 30–70 years	6·3	104	Spread	Total cholesterol	None	Bemelmans *et al.* (2002)
				LDL-cholesterol	Decrease (12 %)	
				HDL-cholesterol	Increase (7 %)	
				Triacylglycerol	Increase (10 %)	
M + F; age 25–72 years	4·5 and 9	24	Spread	Total cholesterol	None	Finnegan *et al.* (2003*b*)
				LDL-cholesterol	None	

Table 6. *Continued*

Subjects	αLNA intake (g/d)*	Duration (weeks)	How αLNA provided	Outcomes investigated	Effect of αLNA (compared with baseline)	Reference
				HDL-cholesterol	None	
				Triacylglycerol	None (tendency to increase; 10 %)	
				Apo B	None	
M; mean age 51 years	8‡	12	Flaxseed oil	Total cholesterol	None	Rallidis *et al.* (2003)
				LDL-cholesterol	None	
				HDL-cholesterol	Decrease (4 %)	
				Triacylglycerol	None	
M + F; age 36–65 years	About 17·5 (6·5 % energy)	6	Walnuts, walnut oil + flaxseed oil	Total cholesterol	Decrease (11 %)	Zhao *et al.* (2004)
				LDL-cholesterol	Decrease (11 %)	
				Triacylglycerol	Decrease (18 %)	
				Apo B	Decrease (10 %)	

M, male; F, female.
* αLNA intake refers to total intake unless otherwise specified.
† Approximate intake.
‡ Excluding background diet contribution of approximately 1 g/d.

concentrations to be directly compared with that of LA. Kestin *et al.* (1990), Nestel *et al.* (1997), Pang *et al.* (1998), Rallidis *et al.* (2003) and Zhao *et al.* (2004) found that the effects of a high-αLNA diet were not different from those of an LA-rich diet. However, Arjmandi *et al.* (1998) and Finnegan *et al.* (2003*b*), who found that αLNA tended to increase TAG concentration, did not see this effect with a high-LA diet, while Bemelmans *et al.* (2002) reported that plasma TAG concentration was significantly higher (12 % or 0·24 mM) after αLNA than after LA. These studies are suggestive that αLNA has a TAG-raising effect compared with LA.

Postprandial lipaemia

Lichtenstein *et al.* (1993) reported no differences in the postprandial TAG response to a test meal in subjects who consumed diets rich in rapeseed, maize or olive oils. More recently, Finnegan *et al.* (2003*b*) found no effect of diets containing 4·5 or 9 g αLNA/d on the postprandial TAG response to a test meal, compared with study entry or with an LA-rich diet.

Low-density lipoprotein oxidation

Nestel *et al.* (1997) found that the lag time of Cu-induced oxidation of LDL was significantly lower (by 14 min; about 22 %) after consumption of an αLNA-rich (20 g/d) low-fat (26 % energy from fat) diet compared with a monounsaturated fat-rich low-fat-diet. In addition, the content of thiobarbituric acid reactive substances in the oxidised LDL was higher if the LDL came from subjects on the αLNA-rich diet (Nestel *et al.* 1997). However, Finnegan *et al.* (2003*b*) reported no effect of diets containing 4·5 or 9 g αLNA/d on lag time of LDL oxidation, compared with study entry or with an LA-rich diet. Ezaki *et al.* (1999) found no effect of 4·2 g αLNA/d on plasma lipid peroxide or oxidised LDL concentrations in elderly Japanese subjects.

Blood pressure

Berry & Hirsch (1986) noted that a 1 % increase in adipose tissue αLNA content was associated with a 5 mmHg decrease in systolic and diastolic blood pressure. Salonen *et al.* (1988) reported an inverse association between αLNA consumption and blood pressure in a large prospective study. However, 38 g αLNA/d for 2 weeks did not affect systolic or diastolic blood pressure in normotensive or hypertensive subjects (Singer *et al.* 1986). Likewise, 9·2 g αLNA/d did not affect systolic or diastolic blood pressure in a 6-week intervention study in normotensive hypercholesterolaemic subjects (Kestin *et al.* 1990). Furthermore, 6·3 g αLNA/d for 2 years did not affect systolic or diastolic blood pressure in subjects with multiple cardiovascular risk factors (Bemelmans *et al.* 2002); some of these subjects were under antihypertensive therapy.

Vascular reactivity

Nestel *et al.* (1997) found increased arterial compliance (aortic flow velocity and aortic root driving pressure) after 4

weeks of 20 g αLNA/d (9 % energy) (and 2·7 % energy as LA) in exchange for oleic acid in obese subjects.

Haemostasis and platelet aggregation

Increased consumption of αLNA results in an increased content of EPA and DPA in platelets (see Table 4) in parallel with a decreased content of arachidonic acid. This might be expected to affect platelet aggregation, since this is regulated in part by arachidonic acid- and EPA-derived eicosanoids (Needleman *et al.* 1979; Knapp *et al.* 1986). Indeed, healthy men consuming a high-αLNA diet (8·5 % energy from αLNA; approximately 18 g/d) had decreased collagen-induced platelet aggregation compared with those on an LA-rich diet (12 % energy from LA) (Allman *et al*. 1995). Freese *et al.* (1994) reported a decrease in ADP-induced platelet aggregation in hyperlipaemic subjects consuming 2·1 % energy as αLNA (and 6·5 % energy as LA) compared with a low-αLNA diet (0·3 % energy from αLNA and 8 % energy from LA). However, there are reports that substantially increased consumption of αLNA (3·7, 5·9 or 15·4 g/d) does not affect platelet aggregation induced by collagen (Freese & Mutanen, 1997, Li *et al.* 1999). Furthermore, there was no effect of 6·8 or 10 g αLNA/d on ADP-induced platelet aggregation (Wensing *et al.* 1999; Junker *et al.* 2001).

A number of intervention studies report little effect of αLNA on coagulation and fibrinolytic factors, including factor VII, factor XII, fibrinogen, plasminogen activator inhibitor-1 or tissue plasminogen activator concentration or factor VII, plasminogen activator inhibitor-1 or tissue plasminogen activator activity (Table 7). Intakes of αLNA studied have been in the range of 3·7 to 15·4 g/d (see Table 7).

Inflammation

Increased consumption of αLNA results in an increased content of EPA and DPA in the membranes of inflammatory cells such as neutrophils and monocytes (see Table 4) in parallel with a decreased content of arachidonic acid. This might be expected to affect inflammation, since this is regulated in part by arachidonic acid- and EPA-derived eicosanoids (see Lewis *et al.* 1990; Tilley *et al.* 2001; Calder 2003, 2004*b*, 2005). A recent study reported an inverse association between αLNA intake and plasma concentrations of IL-6 and soluble vascular cell adhesion molecule-1 but not of C-reactive protein (CRP), soluble TNF receptor-2, soluble E-selectin or soluble intercellular adhesion molecule-1 in samples taken from the Nurses' Health Study 1 cohort (Lopez-Garcia *et al.* 2004). After adjustment for age, BMI, physical activity, smoking status, alcohol consumption and intakes of LA and SFA, there were significant negative associations between αLNA intake and plasma CRP, IL-6, and soluble E-selectin concentrations (Lopez-Garcia *et al.* 2004). These observations would suggest that increasing αLNA intake would result in decreased inflammation. Table 8 summarises intervention studies that have investigated the effect of αLNA on inflammatory outcomes in human subjects; some studies have examined the circulating concentrations of inflammatory markers such as CRP, soluble adhesion molecules or cytokines (Junker *et al.* 2001; Thies *et al.* 2001*a*; Rallidis *et al.* 2003, 2004; Bemelmans *et al.* 2004; Zhao *et al.* 2004), while other studies have examined inflammatory cell responses *ex vivo* (Caughey *et al.* 1996; Healy *et al.* 2000; Thies *et al.* 2001*a*; Kew *et al.* 2003; Wallace *et al.* 2003). Caughey *et al.* (1996) reported that 13·7 g αLNA/d for 4 weeks resulted in a decrease in *ex vivo* production of prostaglandin E_2, TNF-α and IL-1β by endotoxin-stimulated mononuclear cells by 33, 27 and 30 %, respectively. By comparison, fish oil providing 2·7 g EPA + DHA/d decreased production of these inflammatory mediators by 55, 70 and 78 % respectively (Caughey *et al.* 1996). Thus on a g/d basis, long-chain *n*-3 PUFA are about eight to fourteen times more potent than αLNA with respect to this outcome in healthy subjects. In contrast to the observations of Caughey *et al.* (1996), several studies using lower intakes of αLNA (2 to 9·5 g/d) did not find effects on neutrophil chemotaxis, neutrophil respiratory burst, monocyte respiratory burst, TNF-α, IL-1β and IL-6 production by endotoxin-stimulated mononuclear cells, all studied *ex vivo*, or on soluble adhesion molecule concentrations (for details, see Table 8). Furthermore, a study by Rallidis *et al.* (2004) reported no effect of 8 g αLNA/d on soluble intercellular adhesion molecule-1 or soluble E-selectin concentrations and a similar decrease in soluble vascular cell adhesion molecule-1 concentration in both αLNA and control groups. Likewise, Bemelmans *et al.* (2004) found no effect of 6·3 g αLNA/d on soluble intercellular adhesion molecule-1 concentration at 1 and 2 years of intervention. Taken together, these data suggest that increasing αLNA intake to >9 g/d is required in order for marked anti-inflammatory effects to be seen. Even then, the effects will be much more modest than those exerted by long-chain *n*-3 PUFA (Caughey *et al.* 1996). However, both Rallidis *et al.* (2003) and Bemelmans *et al.* (2004) did find a significant decrease in CRP concentration, suggesting that this may be a marker that is more sensitive to intakes of αLNA that do not affect soluble adhesion molecule or cytokine concentrations or *ex vivo* inflammatory cell responses. One study using a very high intake of αLNA (approximately 17·5 g/d) reported significant decreases in both CRP and soluble adhesion molecule concentrations (Zhao *et al.* 2004). Interestingly, the authors found that the changes in these inflammatory markers were significantly related to changes in serum concentrations of EPA or EPA + DPA, but not of αLNA (Zhao *et al.* 2004). This suggests that the observed effects are due to the long-chain *n*-3 PUFA rather than to αLNA *per se*. Thus the probable explanation for the lack of anti-inflammatory effect of αLNA at modest (Healy *et al.* 2000; Thies *et al.* 2001*a*; Kew *et al.* 2003; Wallace *et al.* 2003), and even at rather high (Kew *et al.* 2003), intakes is that there has been insufficient conversion of αLNA to the more active EPA.

α-Linolenic acid and immune function

A limited number of studies have investigated the effect of αLNA on immune functions in human subjects, apart from those with an inflammatory component. Table 9 summarises the studies that exist in this area. These studies have

Table 7. Controlled intervention studies in human subjects investigating the effect of α-linolenic acid (αLNA) on haemostatic factors

Subjects	αLNA intake (g/d)*	Duration (weeks)	How αLNA provided	Outcomes investigated	Effect of αLNA (compared with baseline)	Reference
M; age 21–37 years	20†	8	Flaxseed oil	Prothrombin time	None	Kelley *et al.* (1993)
				Partial prothrombin time	None	
M + F; age 20–44 years	5·9	4	Flaxseed oil	Factor VII activity	None	Freese & Mutanen (1997)
				PAI-I activity	None	
				Fibrinogen concentration	None	
				Anti-thrombin III activity	None	
M; age 22–44 years	3·7, 15·4	4	Flaxseed oil + spread	Factor VII concentration	None	Li *et al.* (1999)
				Fibrinogen concentration	None	
				Prothrombin time	None	
				Anti-thrombin III activity	None	
				Plasminogen concentration	None	
M; age 18–25 years	3·5	6	Flaxseed oil + flaxseed oil in foods	Factor VII concentration and activity	None	Allman-Farinelli *et al.* (1999)
				Fibrinogen concentration	None	
				t-PA concentration and activity	None	
				PAI-1 concentration and activity	None	
M + F; age 67–91 years	4·2	40	Perilla oil	Fibrinogen concentration	None	Ezaki *et al.* (1999)
				PAI-1 concentration	None	
				Prothrombin time	None	
M + F; mean age 27 years	10	4	Spread + foods made with rapeseed oil	Factor II concentration	None	Junker *et al.* (2001)
				Factor VII concentration and activity	None	
				Factor X concentration	None	
				Factor XI concentration	None	
				Factor XII concentration and activity	None	
				t-PA activity	None	
				PAI-I activity	None	
				D-dimer concentration	None	
				Fibrinogen concentration	None	
				t-PA activity	None	
				PAI-1 activity	None	
M + F risk of CVD; age 30–70 years	6·3	104	Spread	Fibrinogen concentration	Decrease (by 0·18 g/l)	Bemelmans *et al.* (2002)
M + F; age 25–72 years	4·5 and 9	24	Spread	Factor VII concentration	None	Finnegan *et al.* (2003*a*)
				Factor XII concentration	None	
				Fibrinogen concentration	None	
				t-PA concentration and activity	None	
				PAI-1 concentration and activity	None	

M, male; F, female; PAI, plasminogen activator inhibitor; t-PA, tissue plasminogen activator.
* αLNA intake refers to total intake.
† Approximate intake.

Table 8. Controlled intervention studies in human subjects investigating the effect of α-linolenic acid (αLNA) on inflammatory markers

Subjects	αLNA intake (g/d)*	Duration (weeks)	How αLNA provided	Outcomes investigated	Effect of αLNA (compared with baseline)	Reference
M; age 22–44 years	13·7	4	Flaxseed oil + spread	Production of prostaglandin E_2 by PBMC	Decrease (33 %)	Caughey *et al.* (1996)
				Production of TNF-α and IL-1β by PBMC	Decrease (27 and 30 %, respectively)	
M; age 18–40 years	4·7	12	Flaxseed oil capsules	Neutrophil chemotaxis	None	Healy *et al.* (2000)
				Neutrophil respiratory burst	None	
M + F; mean age 27 years	10	4	Spread + foods made with rapeseed oil	Serum concentration of CRP	None	Junker *et al.* (2001)
				Serum concentration of sL-selectin	None	
M + F; age 60–74 years	2†	12	Flaxseed oil capsules	Neutrophil respiratory burst	None	Thies *et al.* (2001*a*)
				Monocyte respiratory burst	None	
				Production of TNF-α, IL-1β and IL-6 by PBMC	None	
				Plasma concentrations of sVCAM-1, sICAM-1 and sE-selectin	None	
M + F; age 25–72 years	4·5 and 9	24	Spread	Neutrophil respiratory burst	None	Kew *et al.* (2003)
				Monocyte respiratory burst	None	
				Production of TNF-α, IL-1β and IL-6 by PBMC	None	
M; age 18–40 years	4·7	12	Flaxseed oil capsules	Production of TNF-α, IL-1β and IL-6 by PBMC	None	Wallace *et al.* (2003)
M; mean age 51 years	8†	12	Flaxseed oil	Serum concentrations of CRP, SAA and IL-6	Decrease (all approximately 25 %)	Rallidis *et al.* (2003, 2004)
				Serum concentrations of sVCAM-1, sICAM-1 and st-selectin	Decrease in sVCAM-1 (15 %) but not the others (sVCAM-1 concentration also decreased (11 %) in the control group)	
M + F; age 36–65 years	About 17·5 (6·5 % energy)	6	Walnuts, walnut oil + flaxseed oil	Serum concentration of CRP	Decrease (75 %)	Zhao *et al.* (2004)
				Serum concentrations of sVCAM-1, sICAM-1 and sE-selectin	Decrease (20, 16 and 15 %, respectively)	
M + F at risk of CVD; age 30–70 years	6·3	52 and 104	Spread	Serum concentrations of CRP, sICAM-1, IL-6 and IL-10	Decrease in CRP (12·5 %) but not the others	Bemelmans *et al.* (2004)

M, male; PBMC, peripheral blood mononuclear cells; F, female; CRP, C-reactive protein; sVCAM, soluble vascular cell adhesion molecule; sICAM, soluble intercellular adhesion molecule; sE-selectin, soluble E-selectin; SAA, serum amyloid A.

*αLNA intake refers to total intake unless otherwise specified.

† Excluding background diet contribution of approximately 1 g/d.

Table 9. Controlled intervention studies in human subjects investigating the effect of α-linolenic acid (αLNA) on immune functions, other than inflammation

Subjects	αLNA intake (g/d)*	Duration (weeks)	How αLNA provided	Outcomes investigated	Effect of αLNA (compared with baseline)	Reference
M; age 21–37 years	20†	8	Flaxseed oil	Serum concentrations of IgG, IgA and complement C3 and C4	None	Kelley *et al.* (1991)
				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	None	
				DTH response	Decrease (23 %)	
M + F; age 60–74 years	2‡	12	Flaxseed oil capsules	Neutrophil phagocytosis	None	Thies *et al.* (2001*a*,*b*,*c*)
				Monocyte phagocytosis	None	
				T cell proliferation	None	
				Production of IL-2, and IFN-γ by PBMC	None	
				NK cell activity	None	
M + F; age 25–72 years	4·5 and 9	24	Spread	Neutrophil phagocytosis	None	Kew *et al.* (2003)
				Monocyte phagocytosis	None	
				T cell proliferation	None	
				Production of IL-2, IFN-γ and IL-4 by PBMC	None	
				DTH response	None	
M; age 18–40 years	4·7	12	Flaxseed oil capsules	T cell proliferation	None	Wallace *et al.* (2003)
				Production of IL-2, IFN-γ, IL-4 and IL-10 by PBMC	None	

M, male; SIgA, secretory IgA; PBMC, peripheral blood mononuclear cells; DTH, delayed type hypersensitivity; F, female; IFN, interferon; NK, natural killer.
* αLNA intake refers to total intake unless otherwise specified.
† Approximate intake.
‡ Excluding background diet contribution of approximately 1 g/d.

investigated *ex vivo* immune cell functions such as phagocytosis of bacteria, lymphocyte proliferation and cytokine production in response to mitogenic stimulation and *in vivo* responses to antigen challenge (delayed type hypersensitivity response). It is evident that increasing αLNA intake to as much as 9 g/d has no effect on those human immune functions examined in these studies (Table 9). One study using a very high intake of αLNA (approximately 20 g/d) reported significant decreases in some immune parameters, but not others (Kelley *et al.* 1991). T cell proliferation was decreased in response to one mitogen but not others and the significance of this is not clear. However, the observed decreased delayed type hypersensitivity response, which is the summation of the *in vivo* response to intradermal challenge with seven antigens, is in accordance with decreased T cell function. Thus, very high intakes of αLNA might impair T cell-mediated immune responses.

Conclusions

Epidemiological studies show that αLNA intake is inversely associated with risk of CVD. This inverse relationship may explain the benefit on mortality seen in an intervention study that involved increased αLNA intake (de Lorgeril *et al.* 1994). Intervention studies are indicative that substantially increased αLNA intake can beneficially affect a range of cardiovascular risk factors including blood cholesterol (Chan *et al.* 1991; Cunnane *et al.* 1995; Arjmandi *et al.* 1998; Junker *et al.* 2001; Sodergren *et al.* 2001; Karvonen *et al.* 2002; Zhao *et al.* 2004), LDL-cholesterol (Chan *et al.* 1991; Cunnane *et al.* 1995; Arjmandi *et al.* 1998; Junker *et al.* 2001; Sodergren *et al.* 2001; Bemelmans *et al.* 2002; Karvonen *et al.* 2002; Zhao *et al.* 2004) and TAG (Singer *et al.* 1986; Zhao *et al.* 2004) concentrations, vascular reactivity (Nestel *et al.* 1997), platelet aggregation (Allman *et al.* 1995) and inflammation (Caughey *et al.* 1996; Zhao *et al.* 2004). However, the effect on cholesterol concentration is similar to that of LA, while αLNA is less potent than LA at decreasing LDL concentration (Mantzioris *et al.* 1994; Pang *et al.* 1998). Furthermore αLNA can decrease HDL concentration (Nestel *et al.* 1997; Junker *et al.* 2001; Rallidis *et al.* 2003) which LA does not do and, in contrast to the effects of long-chain *n*-3 PUFA, may increase TAG concentration. More modest increases in αLNA intake do not affect blood lipid concentrations, postprandial lipaemia, blood pressure, platelet aggregation, haemostatic factors, inflammation or immune function. These effects contrast with those of long-chain *n*-3 PUFA and it is apparent from studies where αLNA and long-chain *n*-3 PUFA have been compared that αLNA is substantially less potent (for example, Caughey *et al.* 1996). The reason why very high intakes of αLNA may induce qualitatively similar effects to those of long-chain *n*-3 PUFA (for example, decreasing TAG concentration, platelet aggregation and inflammation), while lower intakes do not, may be that the high intakes allow sufficient synthesis of EPA to occur to induce biologically effective changes in EPA concentration. This is borne out by the observations of Zhao *et al.* (2004) that the αLNA-induced changes in inflammatory markers were significantly related to changes in serum concentrations of EPA or EPA + DPA, but not of αLNA. Similar to this, Caughey *et al.* (1996) related the effects of αLNA on inflammatory cytokine production to the increased content of EPA in mononuclear cells. Thus, the probable explanation for the lack of biological effect of more modest intakes of αLNA is that there has been insufficient conversion of αLNA to the more active EPA. From this point of view increased intake of αLNA offers little real advantage compared with increased intake of long-chain *n*-3 PUFA, and may even be disadvantageous if αLNA does increase blood TAG concentration. One clear advantage of increased αLNA intake is that it seems unlikely to make LDL more sensitive to oxidation, a process which can be promoted by long-chain *n*-3 PUFA. Once again, the fact that one study using a very high intake of αLNA reported increased susceptibility of LDL to oxidation (Nestel *et al.* 1997) may reflect the fact that the LDL may have been significantly richer in EPA and DPA as a result of αLNA conversion.

Overall conclusions

Human conversion of αLNA to its longer-chain derivatives, especially DHA, is limited, although conversion is greater in women than men. The limited extent to which αLNA is converted to its longer-chain metabolites may explain, at least in part, the relative lack of effectiveness of increased consumption of αLNA on risk factors for CVD, inflammatory markers, and immune function. Dietary intervention studies with αLNA have been conducted either in men or in mixed groups of men and women, and the effect of increased αLNA intake on health outcomes in young women has not been investigated. Since young women possess greater capacity for conversion to EPA and DHA, it is possible that they may exhibit a greater response in terms of health outcomes to increased dietary intake of αLNA. For example, increased αLNA intake may lead to greater reserves of EPA and DHA in adipose tissue which could be mobilised and supplied to the fetus during pregnancy. Furthermore, increased dietary intake of αLNA by young women may provide a means of ameliorating inflammatory disease symptoms. Thus there is a need for more research investigating the role of αLNA in promoting health in young women.

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