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## Conversion of $\alpha$ -linolenic acid to longer-chain polyunsaturated fatty acids in human adults

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**Abstract** – The principal biological role of  $\alpha$ -linolenic acid ( $\alpha$ LNA; 18:3n-3) appears to be as a precursor for the synthesis of longer chain n-3 polyunsaturated fatty acids (PUFA). Increasing  $\alpha$ LNA intake for a period of weeks to months results in an increase in the proportion of eicosapentaenoic acid (EPA; 20:5n-3) in plasma lipids, in erythrocytes, leukocytes, platelets and in breast milk but there is no increase in docosahexaenoic acid (DHA; 22:6n-3), which may even decline in some pools at high  $\alpha$ LNA intakes. Stable isotope tracer studies indicate that conversion of  $\alpha$ LNA to EPA occurs but is limited in men and that further transformation to DHA is very low. The fractional conversion of  $\alpha$ LNA to the longer chain n-3 PUFA is greater in women which may be due to a regulatory effect of oestrogen. A lower proportion of  $\alpha$ LNA is used for  $\beta$ -oxidation in women compared with men. Overall,  $\alpha$ LNA appears to be a limited source of longer chain n-3 PUFA in humans. Thus, adequate intakes of preformed long chain n-3 PUFA, in particular DHA, may be important for maintaining optimal tissue function. Capacity to up-regulate  $\alpha$ LNA conversion in women may be important for meeting the demands of the fetus and neonate for DHA.

### n-3 polyunsaturated fatty acids / humans / $\alpha$ -linolenic acid / metabolism

**Abbreviations:**  $\alpha$ LNA:  $\alpha$ -linolenic acid; CE: cholesteryl ester; DHA: docosahexaenoic acid; DPAn-3: docosapentaenoic acid; EE<sub>2</sub>: 17 $\alpha$ -ethinyloestradiol; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acid; NEFA: non-esterified fatty acid; PC: phosphatidylcholine; PL: phospholipid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; TAG: triacylglycerol.

### 1. INTRODUCTION

$\alpha$ -Linolenic acid (18:3n-3,  $\alpha$ LNA) is an essential fatty acid in the diet of humans and is the principal n-3 polyunsaturated fatty acid (PUFA) in the western diet. The major dietary sources of  $\alpha$ LNA are green leaves,

and oils used on cooking such as rapeseed oil and soybean oil where it accounts for up to 10% of total fatty acids. Some seeds (e.g., flaxseed (also known as linseed)) and nuts (e.g., walnut) are particularly rich in  $\alpha$ LNA, as are the oils extracted from those seeds and nuts. Typical consumption of  $\alpha$ LNA in

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**Table I.** Consumption of linoleic and  $\alpha$ -linolenic acids among adults in some European countries, and in Australia and North America.

	Fatty acid intake (g·day <sup>-1</sup> )					
	Linoleic acid		$\alpha$ -Linolenic acid		LA: $\alpha$ LNA ratio	
	Men	Women	Men	Women	Men	Women
Belgium	16.6	12.8	1.7	1.4	9.8	9.1
Denmark	12.0	9.0	2.2	2.1	5.5	4.3
France	8.3	6.8	0.6	0.5	13.8	13.6
France <sup>c</sup>	10.6	8.1	0.9	0.7	11.8	11.6
Germany	9.3	8.0	0.9	0.7	10.3	11.4
Netherlands	19.0	13.2	1.7	1.2	28.8	11.0
Italy <sup>a</sup>		14.5		0.8		18.1
Spain <sup>a</sup>		21.6		0.8		27.0
UK <sup>a</sup>		14.4		1.4		10.3
Australia <sup>a</sup>		9.9		1.2		8.3
USA	16.0	11.0	2.0	1.0	8.0	11.0
Canada		11.2 <sup>b</sup>		1.6 <sup>b</sup>		7.0 <sup>b</sup>

<sup>a</sup> Separate data are not available for men and women.

<sup>b</sup> Pregnant women. Data for UK from [1]; data for Australia from [3]; data for USA from [4]; data for Canada from [5].

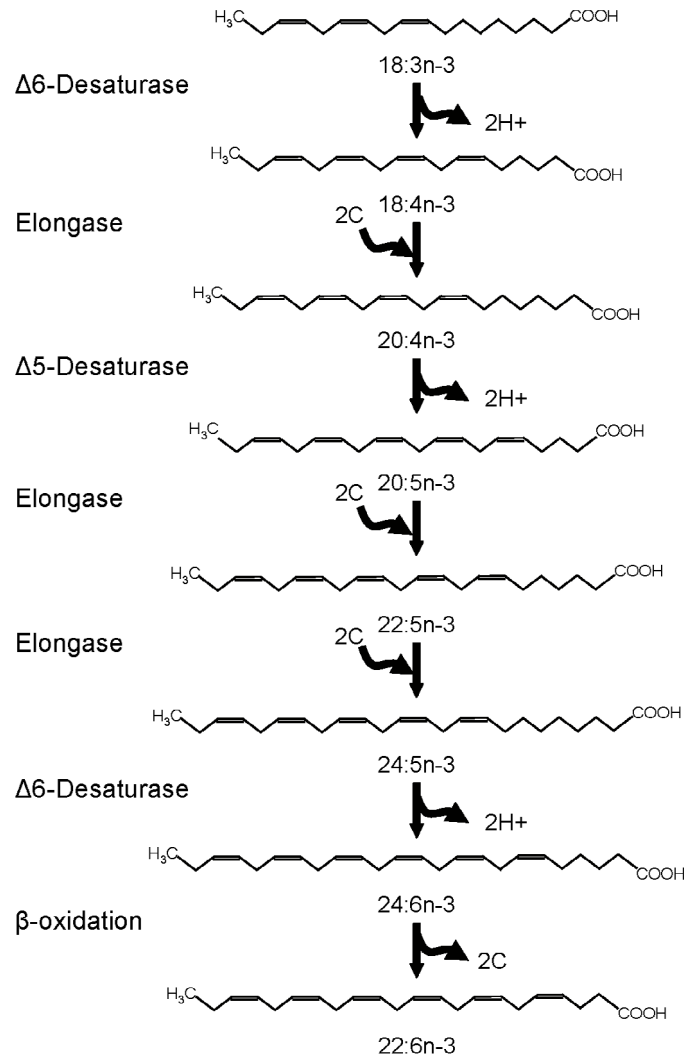
<sup>c</sup> Data for France from [6], other data from [2].

Europe, Australia and North America ranges between 0.6 to 1.7 g per day in men and 0.5 to 1.4 g per day in women [1–5] (Tab. I). This is typically about 10-fold lower than consumption of the n-6 essential fatty acid linoleic acid (18:2n-6) [1–6] (Tab. I). However, even among fairly similar westernised populations, the relative intakes of these fatty acids differ dramatically (Tab. I).

$\alpha$ LNA can be converted to longer-chain n-3 PUFA such as eicosapentaenoic acid (EPA; 20:5n-5) and docosahexaenoic acid (DHA; 22:6n-3) by the pathway shown in Figure 1. Whether the essentiality of  $\alpha$ LNA in the diet primarily reflects the activity of  $\alpha$ LNA itself or of longer-chain PUFA synthesised from  $\alpha$ LNA is a matter for debate [7]. The concentration of  $\alpha$ LNA in phospholipids in plasma, cells and tissues is typically less than 0.5% of total fatty acids. Thus, the  $\alpha$ LNA content of these pools is likely to exert a fairly limited influence on biological function. In the United Kingdom dietary intakes of EPA and DHA are

approximately 25- and 15-fold lower, respectively, than those of  $\alpha$ LNA [1] and similar differences are seen in other countries [3–6]. However, the concentrations of these PUFA in plasma, cell and tissue phospholipids are greater than those of  $\alpha$ LNA. This apparent mismatch between dietary intakes and concentrations in plasma, cell and tissue lipids further suggests that the primary biological role of  $\alpha$ LNA is as a substrate for EPA and DHA synthesis. However, it is possible that the low concentration of  $\alpha$ LNA in plasma, cell and tissues may also reflect negative selection in the incorporation of  $\alpha$ LNA into plasma and membrane lipid pools.

The effect of  $\alpha$ LNA deficiency on neurological function supports the role of  $\alpha$ LNA as a precursor to longer chain n-3 PUFA which are critical in the function of the central nervous system [8]. The DHA content of neural membrane phospholipids modulates the activities of several signalling pathways in the brain [6] and is critical for optimal retinal function [9, 10]. Fifty



**Figure 1.** A general pathway for the conversion of  $\alpha$ -linolenic acid into longer-chain n-3 polyunsaturated fatty acids (based on reference [35]).

percent of children and 30% of adults receiving long-term total parenteral nutrition lacking  $\alpha$ LNA exhibited visual dysfunction, which suggests decreased availability of DHA for incorporation into neural membranes [11]. The offspring of monkeys fed an n-3 PUFA deficient diet during preg-

nancy show visual impairments [12]. Supplementation of the infant monkeys with  $\alpha$ LNA resulted in an increase in the concentration of DHA in neural tissues and an improvement in visual function [13]. This suggests that a deficit in the availability of  $\alpha$ LNA for conversion to, in particular,

DHA was the principal mechanism underlying the deficiency symptoms.

Increased consumption of oily fish or taking fish oil supplements has been shown to increase plasma, cell and tissue EPA and DHA concentrations which is associated with benefits to health, particularly in relation to cardiovascular and inflammatory diseases [14–17]. However, the efficacy of recommendations to increase EPA and DHA intakes [14, 15, 18–20] may be limited by patterns of food choice and availability of fish stocks to sustain the supply of oily fish or fish oil. If  $\alpha$ LNA can substitute for long chain n-3 PUFA then recommendations to increase its intake could be made. In order to support these, the ability of  $\alpha$ LNA to be converted to longer-chain n-3 PUFA and so to increase plasma, cell and tissue pools with EPA and DHA needs to be determined. Furthermore differences among populations or population subgroups and the reasons for these need to be identified. The purpose of this review is to discuss the extent to which conversion of  $\alpha$ LNA to longer chain n-3 PUFA occurs in adult humans and how this process may differ between groups of individuals.  $\alpha$ LNA metabolism in human infants will not be considered as these represent a distinct group with specific demands for long chain PUFA which differ in magnitude from adults.

## 2. BIOAVAILABILITY AND HANDLING OF $\alpha$ -LINOLENIC ACID FROM THE DIET

The bioavailability of dietary  $\alpha$ LNA for conversion to longer-chain PUFA is determined by the efficiency of absorption across the gastrointestinal tract, uptake and partitioning towards  $\beta$ -oxidation, and incorporation into structural and storage pools.

### 2.1. Absorption of $\alpha$ -linolenic acid

There is very little information regarding the absorption of  $\alpha$ LNA by the human gut.

Measurement of the concentrations of deuterated fatty acids in chylomicron triacylglycerol (TAG) following ingestion of deuterated  $\alpha$ LNA as synthetic TAG showed that the absorption and secretion of oleic acid (18:1n-9), linoleic and  $\alpha$ LNA were similar [21]. Although this does not specifically measure absorption across the gut, it does indicate that the overall bioavailability of  $\alpha$ LNA is similar to that of other unsaturated fatty acids. Measurement of the cumulative concentration of labeled  $\alpha$ LNA in stool collected over 5 days following ingestion of 750 mg [ $U$ - $^{13}$ C] $\alpha$ LNA showed that greater than 96% of the administered dose was absorbed (G.C. Burdge, unpublished observation). Comparable levels of  $\alpha$ LNA uptake have also been reported in patients with ileostomies who were fed 100 g linseed oil [22]. These findings suggest that absorption of  $\alpha$ LNA across the gut and its secretion into the bloodstream are efficient.

### 2.2. Assimilation of $\alpha$ -linolenic acid into adipose tissue

Adipose tissue accounts for approximately 15% of body mass in males and 23% of body mass in females. Thus incorporation of  $\alpha$ LNA into this storage pool represents a potentially important route of disposal of dietary  $\alpha$ LNA and a reserve pool which is available for mobilisation during periods of increased demands.  $\alpha$ LNA accounts for about 0.7% of total fatty acid neutral lipids in adipose tissue in men and women, while DHA concentration is approximately 0.1% and EPA is practically undetectable [23, 24]. Thus, it can be calculated that, in a 75 kg man with a fat mass of 15%, the whole body  $\alpha$ LNA reserve in adipose tissue would be approximately 79 g (roughly equivalent to typical intake over 53 days). Likewise, in a 65 kg woman with a fat mass of 23%, the whole body  $\alpha$ LNA reserve in adipose tissue would be approximately 105 g (roughly equivalent to typical intake over 70 days).

Following ingestion of a meal there is a metabolic drive to store fatty acids which is

facilitated by the insulin-dependent increase in lipoprotein lipase activity in adipose tissue. In the fasting state, plasma non-esterified fatty acids (NEFA) are derived primarily from release of adipose tissue TAG stores by the action of hormone-sensitive lipase. The exchange of  $\alpha$ LNA between the blood and adipose tissue compartments has not been characterised in humans *in vivo*. However, when men consumed [ $U$ - $^{13}$ C]  $\alpha$ LNA, labelled  $\alpha$ LNA was detected in plasma NEFA pool within two hours and reached a peak at six hours [25]. While a proportion of labeled  $\alpha$ LNA detected in the NEFA pool in the early postprandial period probably reflects incomplete entrapment of fatty acids released by hydrolysis of chylomicron TAG [26], at 6 h after consumption of the meal the presence of labeled  $\alpha$ LNA in plasma NEFAs probably reflects mobilisation of recently assimilated fatty acid. The overall effect of rapid release of  $\alpha$ LNA into the NEFA pool, together with the  $\alpha$ LNA pool associated with chylomicron remnant particles, would tend to facilitate supply of  $\alpha$ LNA to the liver.

The concentration of [ $^{13}$ C] $\alpha$ LNA in plasma NEFAs was 2-fold greater in women than in men over 21 days [27]. This suggests gender differences in the metabolism of  $\alpha$ LNA in storage pools and potentially greater short-term availability of  $\alpha$ LNA for supply to the liver in women.

### 2.3. Disposal of $\alpha$ -linolenic acid by $\beta$ -oxidation

$\alpha$ LNA is a substrate for  $\beta$ -oxidation in humans and the proportion of ingested [ $^{13}$ C]  $\alpha$ LNA used in  $\beta$ -oxidation has been estimated from the appearance of labeled  $\text{CO}_2$  in breath. The values reported to date for the amount of labeled  $\alpha$ LNA which is used in  $\beta$ -oxidation probably represent an approximately 30% underestimate of the actual proportion of ingested  $\alpha$ LNA used in energy production due to trapping of  $^{13}\text{CO}_2$  in bicarbonate pools [28]. Since the period

of collection of  $\text{CO}_2$  differs between reports from 9 to 48 h the estimates of partitioning towards  $\beta$ -oxidation differ from 15 to 33% [25, 29–31]. When subjects were studied under comparable conditions, the fractional  $\beta$ -oxidation of  $\alpha$ LNA in women was estimated as 22% of administered dose compared to 33% in men [25, 27]. This may reflect lower muscle mass in women, and the potential overall effect would be to increase availability of  $\alpha$ LNA for conversion to longer chain PUFA in women compared to men. The extent of partitioning of  $\alpha$ LNA towards  $\beta$ -oxidation, when assessed under identical conditions, was almost twice that of palmitic, stearic and oleic acids [29], which may reflect the higher affinity of carnitine palmitoyl transferase-1 for  $\alpha$ LNA [32]. Since  $\alpha$ LNA is essential in the human diet, this finding is somewhat counterintuitive and there is currently no explanation for the preferential use of  $\alpha$ LNA as an energy source. One study has reported the effect of altering the n-3 PUFA content of the background diet on the proportion of ingested [ $^{13}$ C] $\alpha$ LNA recovered as  $^{13}\text{CO}_2$  on breath [33]. Three groups of men matched for body-mass-index, age and fasting plasma TAG concentrations consumed a standard meal containing 700 mg [ $U$ - $^{13}$ C] $\alpha$ LNA and excretion of  $^{13}\text{CO}_2$  on breath was measured over 24 h. Subjects then consumed either a control diet ( $\alpha$ LNA 1.7 g per day, EPA + DHA 0.4 g per day), a diet containing an increased amount of  $\alpha$ LNA ( $\alpha$ LNA 9.6 g per day; EPA + DHA 0.4 g per day) or a diet containing an increased amount of EPA + DHA ( $\alpha$ LNA 1.7 g per day; EPA + DHA 1.6 g per day) for 8 weeks. There was no difference in energy intake between groups. The proportion of ingested labeled  $\alpha$ LNA recovered as  $^{13}\text{CO}_2$  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 labeled  $\alpha$ LNA partitioned towards  $\beta$ -oxidation in the group consuming the control (33.1% vs. 38.9%), increased  $\alpha$ LNA (36.1% vs. 34.3%) or increased EPA + DHA (32.3% vs. 38.3%) diets. This suggests that

the extent of partitioning of  $\alpha$ LNA towards  $\beta$ -oxidation is relatively stable over short periods of time and that altering the amount of either  $\alpha$ LNA or long chain-3 PUFA in the diet does not significantly alter this process.

In addition to conversion to  $\text{CO}_2$  by the activity of Krebs's cycle, carbon in acetyl-CoA generated by fatty acid  $\beta$ -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 [34] and rats [35, 36]. However, there is only one report in humans which describes recycling of carbon released by  $\beta$ -oxidation of  $\alpha$ LNA [37]. Men (35 years of age) and women (28 years of age) consumed 700 mg [ $U$ - $^{13}\text{C}$ ]  $\alpha$ LNA and the concentrations of labeled saturated (SFA) and monounsaturated (MUFA) fatty acids in plasma were measured over 21 days. Labeled 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 to TAG in men and 25-fold greater in plasma PC than TAG in women. The concentrations of SFA and MUFA in plasma are greater in TAG than PC. Thus these data suggest channeling of SFA and MUFA synthesised by the recycling pathway into phospholipids by the liver in contrast to the molecular partitioning of the bulk of the hepatic SFA and MUFA pools towards TAG.

The total concentration of labeled SFA and MUFA in plasma lipids was 20% greater in men compared with women. This is in agreement with greater partitioning of  $\alpha$ LNA towards  $\beta$ -oxidation in men compared to women (see earlier). One overall implication of these findings is that the extent of partitioning of  $\alpha$ LNA towards  $\beta$ -oxidation and carbon recycling may be important in the regulation of the availability of  $\alpha$ LNA for conversion to longer-chain PUFA.

### 3. CONVERSION OF $\alpha$ -LINOLENIC ACID TO LONGER-CHAIN POLY-UNSATURATED FATTY ACIDS

#### 3.1. The pathway for conversion of $\alpha$ -linolenic acid to longer-chain polyunsaturated fatty acids

A pathway for the conversion of the essential fatty acids LA and  $\alpha$ LNA to longer-chain PUFA has been described in rat liver (reviewed in [38]) and is summarized in Figure 1. With the exception of the final reaction which results in the formation of DHA, all reactions occur in the endoplasmic reticulum. Since both n-6 and n-3 PUFA are metabolized by the same desaturation/elongation pathway, there exists potential for competition between these two families of fatty acids. The initial conversion of  $\alpha$ LNA to 18:4n-3 by the action of  $\Delta$ 6-desaturase is the rate limiting reaction of the pathway. The affinity of  $\Delta$ 6-desaturase for  $\alpha$ LNA is greater than for LA [38]. However, the typically higher concentration of LA than  $\alpha$ LNA in cellular pools results in greater conversion of n-6 PUFA. The introduction of a double bond at the  $\Delta$ 6 position is followed by the addition of  $\text{C}_2$  by elongase activity and then by desaturation at the  $\Delta$ 5 position by  $\Delta$ 5-desaturase to form EPA. Docosapentaenoic acid (22:5n-3, DPAn-3) is synthesized from EPA by addition of  $\text{C}_2$ . The conversion of DPAn-3 to DHA has been a matter of controversy and  $\Delta$ 4-desaturase activity has been suggested to be the primary mechanism for DHA synthesis [39]. However, of studies in which subcellular organelles were isolated and then recombined [40] and reports of the action of the specific  $\Delta$ 6-desaturase inhibitor SC-26196 [41] strongly support the suggestion that synthesis of DHA involves desaturation at the  $\Delta$ 6-position as follows. DPAn-3 is elongated to 24:5n-3 which is desaturated at the  $\Delta$ 6-position by the action of  $\Delta$ 6-desaturase activity to form 24:6n-3. It is unclear whether the same enzyme is responsible for desaturation of  $\alpha$ LNA and 24:5n-3 [42, 43]. 24:6n-3 is translocated

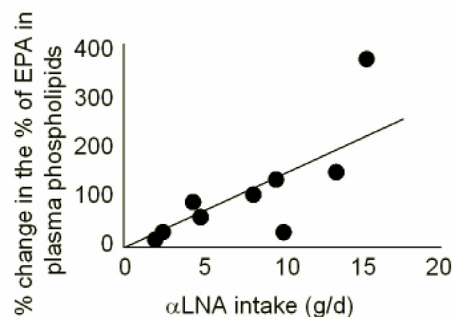
from the endoplasmic reticulum to the peroxisome where the acyl chain is shortened by  $C_2$  by one cycle of the  $\beta$ -oxidation pathway to form DHA. DHA is then translocated back to the endoplasmic reticulum. Although the precise regulation of the translocation steps and limited  $\beta$ -oxidation is not known, it is possible that this represents a locus for metabolic regulation that facilitates control of DHA synthesis independently from the preceding steps of the pathway.

### 3.2. $\alpha$ -Linoleic acid conversion to longer chain n-3 polyunsaturated fatty acids in adult humans

Current understanding of the extent to which humans can convert  $\alpha$ LNA to longer-chain PUFA is based on two types of evidence: the findings of studies reporting the effects of chronic increases in intake of  $\alpha$ LNA on concentrations of n-3 PUFA in plasma, cell and tissue lipid pools and shorter term studies in which subjects consume a bolus of  $\alpha$ LNA labeled with a stable isotope.

#### 3.2.1. Effects of chronically increased $\alpha$ -linolenic acid consumption

A number of studies have reported the effects of consuming increased amounts of  $\alpha$ LNA, usually via inclusion of oils with a high  $\alpha$ LNA content or of products made with those oils (e.g., spreads) in the diet, on the fatty acid composition of plasma or cell lipids (Tabs. II and III). These studies were conducted either in men or in mixed groups of men and women, used intakes of  $\alpha$ LNA ranging from less than one to more than 18  $g \cdot day^{-1}$  and were of a few weeks to many months duration (Tabs. II and III). These studies consistently demonstrate increased proportions of EPA in both plasma and cell lipids when  $\alpha$ LNA intake is increased (Tabs. II and III). Increases in  $\alpha$ LNA intake such that total intake exceeds 4.5  $g \cdot day^{-1}$  appear to result in enhancements in EPA content of plasma phospholipids of between 33 and 370% [44–50]. While the relation-



**Figure 2.** The relationship between  $\alpha$ -linolenic acid intake and the increase in EPA content of plasma phospholipids. Data for mean change in EPA content are taken from the studies described in Table II.

ship between  $\alpha$ LNA intake and EPA incorporation is a significant linear one ( $r = 0.795$ ; Fig. 2), there is some variation in the response between studies which might reflect differences in age and gender mix of the subjects studied, differences in background diet (e.g., habitual long chain n-3 PUFA intake, linoleic acid intake), differences in the way in which  $\alpha$ LNA was provided and differences in analytical procedures used.

Because of competition for metabolism between LA and  $\alpha$ LNA, the LA content of the diet may influence conversion of  $\alpha$ LNA 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  $\alpha$ LNA if LA intake is decreased. A study by Chan et al. [51] demonstrated that this is the case. Subjects consumed diets providing 7  $g \alpha$ LNA  $\cdot day^{-1}$  for 18 days against a background of either 21 or 50  $g$  LA  $\cdot day^{-1}$ . The proportion of EPA was higher after the low compared with the high LA background in plasma PC (0.8 vs. 0.3% of fatty acids), plasma phosphatidylethanolamine (0.9 vs. 0.3% of fatty acids) and platelet PC (0.25 vs. 0.1% of fatty acids).

Several studies also demonstrate increased proportions of DPAn-3 in plasma and cell lipids when  $\alpha$ LNA consumption is increased



**Table II.** Summary of studies investigating the effect of increased  $\alpha$ -linolenic acid consumption on the fatty acid composition of blood lipids in humans.

Reference	Subjects	Country	$\alpha$ LNA intake (g day <sup>-1</sup> ) <sup>a</sup>	LA intake (g day <sup>-1</sup> ) <sup>a</sup>	How $\alpha$ LNA provided	Duration (weeks)	Blood lipid fraction	Change in proportion of total fatty acids from baseline (%)			
								EPA	DPAn-3	DHA	
[44]	M + F	UK	7.8	8 <sup>d</sup>	Flaxseed oil capsules	2	Plasma PC	108 <sup>e</sup>	NA	NA	-14
[45]	M	Australia	13.7	8.4	Flaxseed oil + spread	4	Plasma PL Plasma CE Plasma TAG	143 <sup>e</sup> 150 <sup>e</sup> 200 <sup>e</sup>	NA	NA	14 20 20
[75]	M	USA	20 <sup>d</sup>	15 <sup>d</sup>	Flaxseed oil	8	Total serum	0	20	20	38
[47]	M + F	Canada	9 <sup>b</sup>	NA <sup>c</sup>	Muffins made with flaxseeds	4	Plasma PL Plasma TAG	33 <sup>e</sup> 300 <sup>e</sup>	36 <sup>e</sup> 250 <sup>e</sup>		-3 -33
[48]	M	Australia	15.4	17.4	Flaxseed oil + spread	4	Plasma PL Plasma TAG	367 <sup>e</sup> 300 <sup>e</sup>	50 <sup>e</sup> 50		-5 0
[76]	M + F	Netherlands	6.3	26.3	Spread	52	Serum CE	40 <sup>e</sup>	NA	NA	50
[50]	M	UK	4.7	14.2	Flaxseed oil capsules	12	Plasma PL	60 <sup>e</sup>	NA	NA	3
[51]	M + F	UK	4.5	16.2	Spread	24	Plasma PL	82 <sup>e</sup>	5		-2
[51]	M + F	UK	9.5	13.1	Spread	24	Plasma PL	133 <sup>e</sup>	39 <sup>e</sup>		6
[52]	M + F	Australia	0.75 then 1.5 <sup>b</sup>	7 <sup>d</sup>	$\alpha$ LNA ethyl ester in capsules	4 then 4	Plasma PL	8 then 15	0 then 7	15 then -11	

<sup>a</sup>  $\alpha$ LNA and LA intakes refer to total intakes unless otherwise specified.

<sup>b</sup> Excluding background diet contribution of approximately 1 g per day.

<sup>c</sup> Likely to be about 15 g per day including background diet.

<sup>d</sup> Approximate intake.

<sup>e</sup> Values significantly different from baseline.

$\alpha$ -Linolenic acid ( $\alpha$ LNA); cholesteryl ester (CE); data not available (NA); docosahexaenoic acid (DHA); docosapentaenoic acid (DPAn-3); eicosapentaenoic acid (EPA); female (F); linoleic acid (L-A); male (M); phosphatidylcholine (PC); phosphatidylethanolamine (PE); phospholipid (PL); triacylglycerol (TAG).

**Table III.** Summary of studies investigating the effect of increased α-linolenic acid consumption on the fatty acid composition of circulating cells in humans.

Reference	Subjects	Country	αLNA intake (g·day <sup>-1</sup> ) <sup>a</sup>	LA intake (g·day <sup>-1</sup> ) <sup>a</sup>	How αLNA provided	Duration (weeks)	Cell lipid fraction	Change in proportion of total fatty acids from baseline (%)		
								EPA	DPAn-3	DHA
[44, 76]	M + F	UK	7.8	8 <sup>d</sup>	Flaxseed oil capsules	2	Platelet PL	100 <sup>e</sup>	NA	-3
[78]	M	Canada	8.5	22.5	Canola oil based foods	2.5	Platelet PC Platelet PE	100 133 <sup>e</sup>	0 51 <sup>e</sup>	-27 <sup>e</sup> -5
[79]	M	USA	8% of fatty acids	22% of fatty acids	Canola oil based foods	8	Platelet PL	14 <sup>e</sup>	13	20
[80]	M	Finland	2.1% energy	5.5% energy	Canola oil based foods	3	Total platelet lipid	-8	NA	20
[75]	M	USA	20 <sup>c</sup>	15 <sup>c</sup>	Flaxseed oil	8	Total mononuclear cell <sup>d</sup> lipid	0	45	-36
[45]	M	Australia	13.7	8.4	Flaxseed oil + spread	4	Neutrophil PL	67 <sup>e</sup>	NA	0
[81]	M + F	Australia	18 <sup>c</sup> (8.5% energy)	10 <sup>c</sup> (4.5% energy)	Flaxseed oil	3	Platelet PL	140 <sup>e</sup>	45 <sup>e</sup>	-11
[82]	M	Australia	13.7	8.4	Flaxseed oil + spread	4	Mononuclear cell <sup>d</sup> PL	133 <sup>e</sup>	NA	-9
[47]	M	Australia	15.4	17.4	Flaxseed oil + spread	4	Platelet PL	150 <sup>e</sup>	56 <sup>e</sup>	-10
[53]	M	UK	4.7	14.2	Flaxseed oil capsules	12	Neutrophil PL	30	NA	15
[50]	M + F	Australia	0.75 then 1.5 <sup>b</sup>	7 <sup>c</sup>	αLNA ethyl ester in capsules	4 then 4	Erythrocyte PL	0 then 11	0 then 3	-4 then -11
[83]	M + F	UK	4.5	16.2	Spread	24	Mononuclear cell <sup>d</sup> PL	0	6	-28 <sup>e</sup>
[83]	M + F	UK	9.5	13.1	Spread	24	Mononuclear cell <sup>d</sup> PL	33 <sup>e</sup>	0	-20 <sup>e</sup>

<sup>a</sup> αLNA and LA intakes refer to total intakes unless otherwise specified. <sup>b</sup> Excluding background diet contribution of approximately 1g per day. <sup>c</sup> Approximate intake. <sup>d</sup> Mononuclear cells are a mix of lymphocytes and monocytes (ratio approx. 85:15). <sup>e</sup> Values significantly different from baseline. α-Linolenic acid (αLNA); data not available (NA); docosahexaenoic acid (DHA); docosapentaenoic acid (DPAn-3); eicosapentaenoic acid (EPA); female (F); linoleic acid (LA); male (M); phospholipid (PL); triacylglycerol (TAG).

**Table IV.** Estimated conversion of conversion of  $\alpha$ LNA to longer chain polyunsaturated fatty acids based on stable isotope tracer studies.

Reference		Outcome measures		
		EPA	DPA	DHA
[19]	Absolute and relative AUC concentrations in total plasma lipids	50 $\mu\text{g}\cdot\text{mL}^{-1}$ (8%)	26 $\mu\text{g}\cdot\text{mL}^{-1}$ (4%)	25 $\mu\text{g}\cdot\text{mL}^{-1}$ (4%)
[61]	Peak concentrations in total plasma lipids	57 $\text{ng}\cdot\text{mL}^{-1}$	ND	< 2 $\text{ng}\cdot\text{mL}^{-1}$
[28]	Peak concentrations adjusted for estimated total blood volume	120 $\mu\text{g}$	50 $\mu\text{g}$	$\approx$ 10 $\mu\text{g}$
[23]	Concentrations in plasma TAG, NEFA and PC over 21 days. Fractional conversion estimated from time $\times$ concentration AUC	8%	8%	Not detected

Subjects were either men or mixed groups of men and women. Triacylglycerol (TAG), non-esterified fatty acid (NEFA), phosphatidylcholine (PC), cholesteryl ester (CE). ND = not determined.

(Tabs. II and III). The studies also consistently demonstrate that increased consumption of  $\alpha$ LNA does not result in increased proportions of DHA in plasma or cell lipids (Tabs. II and III). Indeed many studies report a tendency for DHA to decline when  $\alpha$ LNA consumption is markedly increased, although few studies have identified this as a statistically significant effect (Tabs. II and III). Overall, these studies demonstrate that chronically increased consumption of  $\alpha$ LNA 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.

### 3.2.2. Estimates $\alpha$ -linolenic acid conversion from stable isotope tracer studies

Development of sophisticated mass spectrometry techniques, in particular gas chromatography combined with either chemical ionisation or isotope ratio mass spectrometry, and the availability of  $\alpha$ LNA labelled with stable isotopes which avoid the biological hazards associated with radioisotopes have allowed detailed investigations of the metabolic fate of ingested  $\alpha$ LNA in humans. The advantages and limitations of

these techniques in humans have been reviewed recently [52]. While there are advantages in terms of safety, there are 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 gender, 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 [53]. Together these factors have resulted in considerable heterogeneity in the findings of studies of  $\alpha$ LNA metabolism in humans using stable isotope tracers [21, 23, 25, 27, 32, 54–57]. This presents a considerable challenge to any attempt to reach a consensus view on  $\alpha$ LNA metabolism in man. Nevertheless, at present there are no practical alternatives to these stable isotope tracer techniques to study  $\alpha$ LNA metabolism in humans in vivo.

The outcomes of stable isotope tracer studies designed to investigate conversion of  $\alpha$ LNA to longer-chain PUFA in humans are summarized in Table IV. Despite the heterogeneity of the study design and the

mode of expression of results, the consensus of the studies summarised in Table IV is that the proportion of  $\alpha$ LNA entering the desaturation/elongation pathway and converted to EPA is low, possibly in the order of 8% [21, 25]. The extent of conversion of  $\alpha$ LNA to DHA is less clear (Tab. IV). The highest estimated fractional conversion is 4% [21], while most other studies have reported lower estimates of conversion (less than 0.05%) [33] and one study failed to detect significant incorporation of stable isotope into DHA above background [ $^{13}\text{C}$ ] enrichment [25].

Pawlosky et al. [56] 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 deuterated fatty acids in plasma from a mixed group of men and women consuming a beef-based diet. The findings of this study were that the efficiency of conversion of  $\alpha$ LNA to EPA was 0.2%, of EPA to DPAn-3 65% and of DPAn-3 to DHA 37%. This is in general agreement with the studies summarised in Table IV and with the assumption that the first reaction catalysed by  $\Delta 6$ -desaturase is the rate-limiting step of the pathway. Thus the overall efficiency of conversion from  $\alpha$ LNA is 0.2% to EPA, 0.13% to DPA and 0.05% to DHA.

Several studies have reported the effects of modifications to the background diet on the extent of  $\alpha$ LNA conversion to long chain n-3 PUFA determined by stable isotope tracing. In particular, the effect of increased consumption of LA compared to  $\alpha$ LNA has been of interest, as these two fatty acids compete for the rate limiting step of the desaturation/elongation pathway. Likewise the effect of increased consumption of EPA + DHA or DHA alone on this process has been of interest because of the potential for feedback inhibition or inhibition due to competition for  $\Delta 6$ -desaturase. Emken et al. [54] compared the effect of consuming diets either containing < 0.1 g DHA per day or supplemented with 6.5 g·day<sup>-1</sup> purified

DHA. 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 DPAn-3 to DHA following consumption of a fish-based diet (containing EPA + DHA) compared to a beef-based diet in a mixed group of men and women [57]. However, when the fractional conversion of DPAn-3 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 [58]. Consumption of 1.6 g per day EPA + DHA for 8 weeks decreased EPA and DPAn-3, but not DHA, synthesis when  $\alpha$ LNA conversion was compared before and after the intervention in the same individuals [33]. These studies indicate that increased consumption of long chain n-3 PUFA acts to down-regulate their synthesis from  $\alpha$ LNA, although the mechanism by which this occurs is not yet clear. Recently, Hussein et al. [59] have applied kinetic analysis to compare LA and  $\alpha$ LNA conversion in subjects consuming either 17 g per day LA or  $\alpha$ LNA. The effect of these diets was to inhibit the conversion of the alternate series of fatty acids, although the apparent synthesis of DHA was consistently low (< 0.01%) and not influenced by the intakes of these fatty acids. In contrast, one report showed that increased  $\alpha$ LNA intake (8 g per day) decreased EPA, DPA and DHA synthesis [30], although others have not found this [33].

Overall, substantial increases in the intakes of individual fatty acids are able to modify the conversion of  $\alpha$ LNA, although there are inconsistencies in the magnitude of these effects between reports. The relative effects of LA and  $\alpha$ LNA may be explained by competition for  $\Delta 6$ -desaturase. However, the down regulation of  $\alpha$ LNA conversion by DHA or EPA + DHA may have a more complex explanation. Tang et al. [60] have shown recently that the promoter region of  $\Delta 6$ -desaturase contains the response element for the ligand-activated transcription factor peroxisomal proliferator

activated receptor- $\alpha$  (PPAR $\alpha$ ). This study shows that binding of DHA to PPAR $\alpha$  suppresses transcription of  $\Delta 6$ -desaturase and so would be expected to down-regulate conversion of  $\alpha$ LNA to longer-chain PUFA. Furthermore, the absence of effects of altered background diet on DHA synthesis when conversion of  $\alpha$ LNA to EPA and DPAn-3 was decreased supports the suggestion that DHA formation may be regulated independently of other fatty acids in the pathway.

#### 4. THE EFFECT OF GENDER ON $\alpha$ -LINOLENIC ACID METABOLISM

The majority of investigations of  $\alpha$ LNA metabolism in humans have focused on groups of relatively young healthy individuals, either men or mixed groups of men and women. Thus there is relatively little information regarding the effects of gender on this process. Only two reports have specifically studied  $\alpha$ LNA conversion in women of reproductive age. Burdge and Wootton [27] showed that conversion of  $\alpha$ LNA 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 [25]. This finding is strongly supported by kinetic analysis which showed that the rate constant coefficient for the conversion of DPAn-3 to DHA was approximately 4-fold greater in women compared to men [58]. In part, this may reflect greater availability of  $\alpha$ LNA in women than men due in part to lower partitioning towards  $\beta$ -oxidation. However, since the rate constant coefficient for the conversion of DPAn-3 to DHA was greater in women than men, it is likely that there is a gender-related difference in the activity of the desaturation/elongation pathway in addition to differences between men and women in the extent of partitioning of  $\alpha$ LNA towards  $\beta$ -oxidation. One possible explanation for the greater synthesis of EPA and DHA from  $\alpha$ LNA in women compared to men is the action of oestrogen.

DHA synthesis was almost 3-fold greater in women using an oral contraceptive pill containing 17 $\alpha$ -ethynylloestradiol (EE<sub>2</sub>) than in those who were not [27]. 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 resulted in greater plasma dihomogamma-linolenic and arachidonic acid concentrations than before treatment [61]. Furthermore, DHA concentration in the plasma cholesteryl ester fraction has recently been shown to be greater in women (0.53% total fatty acids) compared to men (0.48% total fatty acids) consuming diets controlled for energy and  $\alpha$ LNA content, although DHA is a minor component of this plasma lipid pool [62]. DHA concentration was also greater in women taking oral contraceptives (0.58% total fatty acids) than in those who were not, which is in agreement with the effects of oral contraceptive pill use on  $\alpha$ LNA conversion [27]. Interestingly, administration of EE<sub>2</sub> to male to female transsexuals increased the concentration of DHA in plasma cholesteryl esters by 42%, while testosterone decreased DHA concentration by 22% in female to male transsexuals [62]. Together these data strongly support the suggestion that sex hormones regulate the activity of the desaturation/elongation pathway in humans.

One possible biological role for greater capacity for DHA synthesis in women may be in meeting the demands of the fetus and neonate for this fatty acid. The developing human fetus assimilates at least 400 mg DHA per week during the last trimester [63]. Since this estimate reflects only brain, adipose tissue and liver requirements, the overall demands for DHA are likely to be substantially greater. Since desaturase activities in developing human liver appear to be lower than in adults [64–68], the extent to which the fetus and neonate are able to satisfy the 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 ( $170 \mu\text{mol}\cdot\text{L}^{-1}$ ) and 40 weeks ( $230 \mu\text{mol}\cdot\text{L}^{-1}$ ) gestation [69]. Studies in rats indicate that this is the result of physiological adaptations to hepatic phospholipid [70] and  $\alpha$ LNA [71] metabolism which may serve to facilitate DHA supply to the offspring. When the increase in maternal blood volume during pregnancy is taken into account [72], this adaptation appears to result in 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  $\alpha$ LNA conversion may increase during gestation. If so, one implication would be that the 50% variation among pregnant women in plasma PC DHA concentration at term [69] may reflect differences in  $\alpha$ LNA metabolism in addition to any dietary effects, and that this may influence the supply of DHA to the fetus and subsequent developmental and function of fetal tissues.

Consumption of 10.7 g  $\alpha$ LNA per day by lactating women increased maternal plasma, erythrocyte and breast-milk  $\alpha$ LNA concentration [73]. The effect on breast-milk EPA and DPAn-3 concentrations is less clear, as the difference between baseline DPAn-3 concentration ( $0.19 \pm 0.05\%$ ) and that after 4 weeks of supplementation ( $0.17 \pm 0.02\%$ ) does not support the claim that the DPAn-3 content of milk increased over time [73]. Increased consumption of  $\alpha$ LNA did not alter breast-milk DHA concentration [73]. This is consistent with the finding that newly synthesised arachidonic acid is a minor component of the arachidonic acid content of breast-milk [74]. This suggests the incorporation of PUFA into milk may be dependent upon mobilisation of stores accumulated before conception and during pregnancy. If so, this emphasises the importance of adequate nutrition of women both before and during pregnancy. Since prolactin suppresses oestrogen activity, the activity of the desaturation/elongation pathway may be down-regulated in lactating compared to non-pregnant and pregnant women,

and so these results do not exclude the possibility of increased DHA synthesis during pregnancy.

## 5. CONCLUSIONS

Studies using chronically increased  $\alpha$ LNA intake or using a single bolus of isotopically-labelled  $\alpha$ LNA yield the same conclusion: that conversion of  $\alpha$ LNA to longer-chain PUFA, particularly DHA, in humans appears to be limited. However, there are important differences between men and women in capacity for synthesis of EPA and DHA from  $\alpha$ LNA and this may be affected by physiological state (e.g., pregnancy). If demands for EPA and DHA are modest and primarily serve to support membrane turnover and renewal in adults, then it is possible that in healthy individuals consuming a balanced diet limited capacity for synthesis of EPA and DHA may be sufficient to maintain tissue function. However, in situations where demand for long chain n-3 PUFA, especially DHA, is increased (e.g., during pregnancy and lactation), then synthesis from  $\alpha$ LNA may be insufficient to meet the demand, although there may be physiological mechanisms by which  $\alpha$ LNA conversion is up-regulated. Clearly more research in this area is required before firmer conclusions can be drawn.

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