

## Long-chain *n*-3 PUFA: plant *v.* marine sources

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Increasing recognition of the importance of the long-chain *n*-3 PUFA, EPA and DHA, to cardiovascular health, and in the case of DHA to normal neurological development in the fetus and the newborn, has focused greater attention on the dietary supply of these fatty acids. The reason for low intakes of EPA and DHA in most developed countries (0.1–0.5 g/d) is the low consumption of oily fish, the richest dietary source of these fatty acids. An important question is whether dietary intake of the precursor *n*-3 fatty acid,  $\alpha$ -linolenic acid ( $\alpha$ LNA), can provide sufficient amounts of tissue EPA and DHA by conversion through the *n*-3 PUFA elongation–desaturation pathway.  $\alpha$ LNA is present in marked amounts in plant sources, including green leafy vegetables and commonly-consumed oils such as rape-seed and soyabean oils, so that increased intake of this fatty acid would be easier to achieve than via increased fish consumption. However,  $\alpha$ LNA-feeding studies and stable-isotope studies using  $\alpha$ LNA, which have addressed the question of bioconversion of  $\alpha$ LNA to EPA and DHA, have concluded that in adult men conversion to EPA is limited (approximately 8%) and conversion to DHA is extremely low (<0.1%). In women fractional conversion to DHA appears to be greater (9%), which may partly be a result of a lower rate of utilisation of  $\alpha$ LNA for  $\beta$ -oxidation in women. However, up-regulation of the conversion of EPA to DHA has also been suggested, as a result of the actions of oestrogen on  $\Delta$ 6-desaturase, and may be of particular importance in maintaining adequate provision of DHA in pregnancy. The effect of oestrogen on DHA concentration in pregnant and lactating women awaits confirmation.

### Marine *n*-3 PUFA: Plant *n*-3 PUFA: CVD: *n*-3 PUFA in pregnancy

There is considerable interest in the health benefits of fish and fish oil consumption, because of increasingly strong evidence linking long-chain (LC) *n*-3 PUFA intake with cardiovascular health. This evidence is based on data obtained from prospective epidemiological studies as well as randomised controlled trials of fish and fish oils in individuals with a previous history of myocardial infarction. There is also evidence, based on feeding trials conducted in preterm infants, to suggest beneficial effects of increased LC *n*-3 PUFA intake on the early development of the visual system (Scientific Advisory Committee on Nutrition, 2004). These findings have focused greater attention on estimates of human requirements for LC *n*-3 PUFA, on the major food sources of these fatty acids and on the extent of their biosynthesis in man at different

stages of the life cycle. Consideration of these issues indicates that direct sources of the LC *n*-3 PUFA in the diet via fish and fish oils are limited, and that future sustainability of this source is uncertain. Furthermore, estimates of requirements for DHA for neurological development during pregnancy and early postnatal life suggest an important gap between dietary provision and physiological requirement. The pathway for conversion of the precursor *n*-3 PUFA,  $\alpha$ -linolenic acid ( $\alpha$ LNA), to EPA and DHA has been shown to operate in man (for review, see Burdge & Calder, 2005). However, the capacity of this pathway appears to be extremely limited, although there may be up-regulation of bioconversion during pregnancy. The present paper provides an overview of these issues, and in particular reviews recent evidence concerning the

**Abbreviations:** DPA, docosapentaenoic acid; LC, long-chain;  $\alpha$ LNA,  $\alpha$ -linolenic acid.

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capacity for conversion of  $\alpha$ LNA to EPA and DHA in man and the possible influence of gender on this process.

### **Beneficial effects of oily fish and long-chain *n*-3 PUFA**

Evidence suggests that the consumption of fish, particularly oily fish, may decrease the risk of CVD because of the beneficial effects of the LC *n*-3 PUFA in oily fish on the cardiovascular system. A large body of evidence exists, including human experimental studies, animal experiments and cell-culture studies, to suggest beneficial effects of fish, oily fish and LC *n*-3 PUFA on the molecular, cellular and whole-body pathogenic processes of atherosclerosis and thrombosis (Calder, 2004). Data derived from prospective epidemiological studies and secondary prevention trials conducted in subjects at high risk of CHD also support the conclusion that these fatty acids protect against CHD (for recent review, see Scientific Advisory Committee on Nutrition, 2004). Three of four randomised controlled trials have shown beneficial effects of increased LC *n*-3 PUFA intake on CHD mortality (Burr *et al.* 1989; Singh *et al.* 1997; GISSI-Prevenzione Investigators, 1999), although the fourth study (Burr *et al.* 2003), conducted in men with unstable angina, has found an adverse effect of fish oil consumption on cardiac mortality. More evidence for the benefits of fish consumption comes from studies that have explored the relationships between biomarkers of fish consumption and CHD in men. In a recent analysis of the Physicians Health Study cohort (Albert *et al.* 2002) whole-blood levels of EPA and DHA were found to be lower at baseline in men who subsequently died of sudden cardiac arrest than in controls matched for age and smoking. The relative risk of sudden death in subjects with LC *n*-3 PFA in the highest quartile was found to be 10% of that in men with values in the lowest quartile. The data are very similar to earlier findings from a case-control study (Siscovick *et al.* 1995) that demonstrated a strong inverse association between erythrocyte LC *n*-3 PUFA and risk of sudden cardiac arrest in a population-based analysis. Taken together, these findings are strongly supportive of the conclusion that LC *n*-3 PUFA, found predominantly in human diets in fish, especially oily fish, have beneficial effects on cardiovascular health. The beneficial effects of LC *n*-3 PUFA observed in secondary prevention trials have been found at intakes of approximately 0.85 g/d, which is consistent with the findings of prospective dietary studies that suggest a plateau effect at intakes of approximately 0.9 g/d.

Higher maternal intakes of EPA and DHA have been proposed to have beneficial effects on a number of pregnancy outcomes. High levels of fish consumption, and of EPA and DHA, have been suggested to be responsible for the reported longer gestation rates and higher birth weights of Faroe Islanders than Danes (Olsen *et al.* 1995). A recent prospective cohort study has reported that low consumption of fish is a strong risk factor for preterm delivery and low birth weight (Olsen & Secher, 2002). Randomised controlled trials that have investigated the effects of supplemental intakes of LC *n*-3 PUFA during pregnancy on birth weights and gestational length provide

some support for these observational data. Three of five studies show longer gestational length and/or higher birth weight in the supplemented groups (Olsen *et al.* 1992, 2000; Smuts *et al.* 2003). The two studies that have failed to show effects (Helland *et al.* 2001; Malcolm *et al.* 2003) were conducted in populations with normal birth weights (>3600 g), suggesting that the effects are likely to be greatest in low-birth-weight populations. Intake of DHA in pregnancy and lactation has also been linked with better visual acuity in early life and this outcome would be consistent with the recently-established role for DHA in GTP-dependent signal transduction pathways involved in vision (Jeffrey *et al.* 2001; Mitchell *et al.* 2003). There is evidence from prospective studies to suggest that children whose mothers ate fish during pregnancy have better visual function in early life than those whose mothers did not eat fish (Williams *et al.* 2001), and evidence from a cross-sectional study of breast-fed infants of a positive association between maternal milk DHA levels and visual acuity in the offspring (Jorgensen *et al.* 2001). Nine of ten randomised controlled trials conducted in preterm infants that have included infant formulas with or without LC *n*-3 PUFA have shown beneficial effects of supplemental LC *n*-3 PUFA intake on visual acuity, measured by visual evoked potentials (Scientific Advisory Committee on Nutrition, 2004). Randomised controlled trials conducted in full-term infants show less evidence for benefit, with four of nine studies showing benefit from supplementation with LC *n*-3 PUFA, whilst five of eight studies show breast-feeding to have beneficial effects on visual function of offspring (Scientific Advisory Committee on Nutrition, 2004). Although a number of studies have evaluated the effects of maternal supplemental LC *n*-3 PUFA intake on behavioural outcomes, results have proven to be equivocal, which may be related to the lack of sensitivity of some of the tests employed in the trials to date.

### **Dietary sources of *n*-3 PUFA and long-chain *n*-3 PUFA supply during pregnancy**

Most of the population in the UK consume very little fish; the most recent survey of adults has reported that over two-thirds of respondents did not eat any fish during the period of the survey (Henderson *et al.* 2004). The National Diet and Nutrition Survey data for consumption of fish in UK adults provides values for mean consumption of white fish of 103 g/week and of oily fish of 50 g/week. These levels of intake equate with a mean daily consumption of LC *n*-3 PUFA of 0.18 g/d compared with the recommended value of 0.45 g/d proposed for normal healthy adults as a means of reducing risk of CHD (Scientific Advisory Committee on Nutrition, 2004). The precursor *n*-3 PUFA,  $\alpha$ LNA, is present in widely-distributed and commonly-consumed oils and foods, such as rapeseed and soyabean oils used in many manufactured foods, green leafy vegetables and nuts such as walnuts. Reported intakes of  $\alpha$ LNA in European countries, the USA and Canada range between 0.8 and 2.2 g/d (for review, see Burdge & Calder, 2005). In the UK the intake of  $\alpha$ LNA has risen from a mean of 1.4 g/d in

**Table 1.** Estimated needs for and provision of DHA during pregnancy and 6 months lactation

Estimated additional needs during pregnancy and 6 months lactation (g)		Estimated additional dietary intake and provision from stores during pregnancy and 6 months lactation (g)	
Fetal tissue deposition	10	10% Higher intake last trimester pregnancy	1
Maternal adipose tissue deposition	4	Mobilisation of maternal adipose tissue	4
6 months lactation	13	25% Higher intake during 6 months lactation	4.5
Total additional needs	27	Total additional provision	9.5

1987–8 to 2.1 g/d in the most recent British Adult Diet Survey (Henderson *et al.* 2004).

It is clear that pregnancy and lactation place considerable additional demands on the supply of LC *n*-3 PUFA, particularly DHA, which is required for the normal development of the mammalian brain during pregnancy and early postnatal life. Since the activities of the desaturase enzymes that regulate synthesis of LC PUFA from their precursors appear to be lower in the developing human liver than in adults (de Gomez Dumm & Brenner, 1975; Poisson *et al.* 1993; Carnielli *et al.* 1996; Salem *et al.* 1996), the extent to which the fetus and neonate are able to satisfy the demands for DHA via conversion from  $\alpha$ LNA may be limited. It would appear that the assimilation of DHA by the fetus has to be met primarily by the supply of DHA by the mother. In pregnant women the plasma phosphatidylcholine-DHA concentration increases by approximately 33% between 16 weeks (170  $\mu$ mol/l) and 40 weeks (230  $\mu$ mol/l) of gestation (Postle *et al.* 1995), and when the increase in maternal blood volume during pregnancy is taken into account (Gregersen & Rawson, 1959), this increase indicates that there is an overall doubling of DHA in the circulation during pregnancy.

Despite the size of this response in terms of circulating provision of DHA, there is little information available on the requirements for DHA in human pregnancy, or any detailed understanding of the adaptational responses involved in ensuring adequate provision of DHA to the developing fetus. Additional demands are likely to be highest during the third trimester of pregnancy when the rate of growth of the fetal brain is most rapid. It has been estimated that the fetus accumulates approximately 60–70 mg DHA/d during the last trimester of pregnancy (Clandinin *et al.* 1980, 1981). When placental tissue accumulation and DHA accumulation in fetal as well as maternal adipose tissue stores (approximately 4 g DHA in 4 kg adipose tissue) are accounted for, total accretion of DHA during pregnancy has been estimated to be approximately 14 g DHA. During lactation, when approximately 70–80 mg DHA/d are needed for milk production (Jensen *et al.* 2000; Makrides & Gibson, 2000), a total amount of 12–13 g DHA would be needed to sustain 6 months of breast feeding. Approximately 4 g DHA could be obtained via mobilisation of 4 kg adipose tissue accumulated during pregnancy. Based on these values, estimates of DHA requirements for pregnancy and lactation are therefore approximately 23 g over a 15-month period (Table 1). Assuming that the additional food (and DHA) intake during the last trimester of pregnancy (10%) and during lactation (25%) is in proportion to the increase in energy

intake, there will be additional intake of 4.5 g DHA over the total period. The data suggest a gap between requirement and provision of approximately 17.5 g DHA for each singleton pregnancy (Table 1).

These values suggest that the gap between dietary provision (0.18 g/d) and requirement (0.45 g/d) for EPA and DHA that is evident for the average UK adult is considerably widened for women during pregnancy and lactation. The extent to which the additional demands for pregnancy can be met will depend on adaptational mechanisms that may operate during pregnancy and lactation including: (1) conservation of DHA via reduction in rates of oxidation and losses through other routes; (2) mobilisation of DHA from maternal and neonatal adipose tissue stores laid down in pregnancy (Haggarty, 2004), which are dependent on the ability to lay down fetal and maternal DHA during pregnancy; (3) increased bioconversion of DHA from precursor  $\alpha$ LNA. Although the latter step offers a very plausible adaptational mechanism, evidence that the pathway for conversion of  $\alpha$ LNA to DHA occurs to a marked extent in adult subjects is based on limited data. However, there is preliminary evidence to suggest there are gender differences in the capacity to up regulate this pathway that may be dependent on oestrogen.

### Conversion of $\alpha$ -linolenic acid to longer-chain PUFA

#### *The pathway for conversion of $\alpha$ -linolenic acid to longer-chain PUFA*

The pathway for the conversion of  $\alpha$ LNA to longer-chain PUFA has been described in rat liver (for review, see Sprecher, 2002) and human neonates (Carnielli *et al.* 1996). With the exception of the final reaction that results in the formation of DHA all reactions occur in the endoplasmic reticulum. The initial introduction of a double bond by the action of  $\Delta$ 6-desaturase converts  $\alpha$ LNA to 18:4*n*-3 and is the rate-limiting reaction of the pathway. This step is followed by the addition of C<sub>2</sub> by elongase activity, which is followed by desaturation by  $\Delta$ 5-desaturase to form EPA. Docosapentaenoic acid (22:5*n*-3; DPA) is synthesised from EPA by the addition of C<sub>2</sub>. Although  $\Delta$ 4-desaturase has been suggested to be the mechanism for DHA synthesis from DPA, it is now widely accepted that the final step in the *n*-3 pathway involves a series of reactions that include: (1) the addition of C<sub>2</sub> by elongase to form 24:5*n*-3; (2) desaturation at the  $\Delta$ 6 position to form 24:6*n*-3; (3)  $\beta$ -oxidation with the loss of C<sub>2</sub> to form 22:6*n*-3 (DHA). The 24:6*n*-3 is translocated from the endoplasmic reticulum to the peroxisome where

acyl chain shortening (loss of C<sub>2</sub>) is achieved by one cycle of the  $\beta$ -oxidation pathway to form DHA. DHA is then translocated back to the endoplasmic reticulum. The first step in the pathway involving  $\Delta$ 6-desaturation is shared with linoleic acid (18:2n-6) for its conversion to its longer-chain products. Although the affinity of  $\Delta$ 6-desaturase is higher for  $\alpha$ LNA than for 18:2n-6, the typically higher concentrations of 18:2n-6 in cellular pools result in greater conversion of 18:2n-6 to longer-chain n-6 PUFA. As  $\Delta$ 6-desaturation is the rate-limiting step in the pathway, high dietary intakes of n-6 PUFA have been proposed to be a limiting factor in the conversion of  $\alpha$ LNA to its LC products EPA and DHA.

#### *$\alpha$ -Linolenic acid conversion to longer-chain n-3 PUFA in adult subjects*

Two types of study have been employed to investigate the conversion of  $\alpha$ LNA to EPA and DHA in man: (1) those reporting the effects of chronic increases in intake of  $\alpha$ LNA on concentrations of LC n-3 PUFA in plasma, cell and tissue lipid pools; (2) shorter-term studies in which subjects consume a bolus of  $\alpha$ LNA labelled with a stable isotope to follow incorporation of the label into EPA and DHA.

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

A number of studies have reported the effects of consuming increased amounts of dietary  $\alpha$ LNA on the fatty acid composition of plasma or cell lipids (for review, see Burdge & Calder, 2005). These studies consistently show that intakes of  $\alpha$ LNA ranging from <1 to >18 g/d result in enhancement of EPA in plasma and cell lipids (Sanders & Younger, 1981; Mantzioris *et al.* 1994; Cunnane *et al.* 1995; Li *et al.* 1999; Finnegan *et al.* 2003; James *et al.* 2003; Wallace *et al.* 2003). Many of these studies also demonstrate increased proportions of DPA in plasma and cell lipids when  $\alpha$ LNA consumption is increased, although the enhancement is lower and more variable than that for EPA. While the relationship between  $\alpha$ LNA intake and EPA incorporation has been shown to be linear (Burdge & Calder, 2005) there is some variation in the response between studies, which might reflect differences in the age and gender mix of the subjects studied and variations in background diet (e.g. habitual LC n-3 PUFA intake, linoleic acid intake), as well as differences in the way in which  $\alpha$ LNA was provided (capsules, oils, margarines, prepared foods) and differences in the analytical procedures used.

The studies also consistently demonstrate that increased consumption of  $\alpha$ LNA does not result in increased proportions of DHA in plasma or cell lipids. It is notable that many studies report a tendency for DHA to decline when  $\alpha$ LNA consumption is markedly increased, although few studies have identified this effect as being significant. Overall, these studies demonstrate that although chronically-increased consumption of  $\alpha$ LNA results in conversion to EPA and enhancement of EPA concentration in plasma and cell pools, the extent of

conversion to DHA is insufficient to increase the concentration of this fatty acid.

#### *Estimates of $\alpha$ -linolenic acid conversion from stable-isotope-tracer studies*

The availability of  $\alpha$ LNA labelled with stable isotopes, which avoid the biological hazards associated with radioisotopes, has allowed detailed investigations of the metabolic fate of ingested  $\alpha$ LNA in human subjects. However, interpretation of these data has been controversial because of the relatively small number of studies conducted and unresolved issues surrounding methods, modelling and standardisation (for recent review, see Emken, 2001). These factors, as well as differences in study design and use of different lipid pools as markers of fatty acid metabolism, have resulted in considerable heterogeneity in the findings of studies of  $\alpha$ LNA metabolism in human subjects using stable-isotope tracers (Emken *et al.* 1994, 1999; Salem *et al.* 1999; Vermunt *et al.* 2000; Pawlosky *et al.* 2001, 2003a,b; Burdge *et al.* 2002, 2003; Burdge & Wootton 2002; Goyens *et al.* 2005; Hussein *et al.* 2005; Table 2). This diversity presents a considerable challenge to any attempt to reach a consensus view on  $\alpha$ LNA metabolism in man.

The outcomes of stable-isotope-tracer studies designed to investigate conversion of  $\alpha$ LNA to longer-chain PUFA in human subjects are summarised in Table 2. Despite the heterogeneity of the study design and the mode of expression of results, the consensus of the studies summarised in Table 2 is that the proportion of  $\alpha$ LNA entering the desaturation–elongation pathway and converted to EPA is <10% and possibly approximately 8% (Emken *et al.* 1994; Burdge *et al.* 2002). The extent of conversion of  $\alpha$ LNA to DHA is less clear (Table 2). The highest estimated fractional conversion is 4% (Emken *et al.* 1994), while most other studies have reported lower estimates of conversion (<0.05%; Burdge *et al.* 2003) and one study has failed to detect incorporation of stable isotope into DHA above background <sup>13</sup>C enrichment (Burdge *et al.* 2002).

Pawlosky *et al.* (2001) have suggested estimates for the efficiency of conversion for individual steps in the desaturation–elongation pathway from kinetic analysis based on the concentrations of individual <sup>2</sup>H-labelled fatty acids in plasma from a mixed group of men and women consuming a beef-based diet. The findings of this study are that the overall efficiency of conversion of  $\alpha$ LNA to EPA is 0.2%, of EPA to DPA is 0.13% and of DPA to DHA is 0.05%. This result is in general agreement with the findings of the studies summarised in Table 2 and with the assumption that the first reaction catalysed by  $\Delta$ 6-desaturase is the rate-limiting step of the pathway.

#### **The effect of gender on $\alpha$ -linolenic acid metabolism**

There is relatively little information about the effects of gender on the bioconversion of  $\alpha$ LNA to EPA and DHA. There are no studies reported in the literature in which enhancement of EPA, DPA and DHA in plasma or tissues

Table 2. Estimated conversion of  $\alpha$ -linolenic acid ( $\alpha$ LNA) to longer-chain PUFA

Reference	Subjects	Isotope and dose	Outcome measures			
			EPA	DPA	DHA	
Emken <i>et al.</i> (1994)	M	[ <sup>2</sup> H] $\alpha$ LNA, 3.5 g mixed TAG	50 8	26 4	25 4	
Emken <i>et al.</i> (1999)	M	[ <sup>2</sup> H] $\alpha$ LNA, 3.1 g mixed TAG Subjects consumed 6.5 or <0.1 g DHA/d for 90 d before experiment	Higher DHA consumption decreased EPA and DHA synthesis by 76% and 88% respectively			
Salem <i>et al.</i> (1999)	Adults*	<sup>2</sup> H-labelled ethyl ester of $\alpha$ LNA, 1 g	57†	ND	<2†	
Vermunt <i>et al.</i> (2000)	M + F	U- <sup>13</sup> C-labelled methyl ester of $\alpha$ LNA, 45 mg	120	50	10‡	
Pawlosky <i>et al.</i> (2001)	M + F	<sup>2</sup> H-labelled ethyl ester of $\alpha$ LNA, 1 g	0.2	0.13	0.05	
Burdge <i>et al.</i> (2002)	M	[U- <sup>13</sup> C] $\alpha$ LNA, NEFA, 0.7 g	8	8	ND	
Burdge & Wootton (2002)	F	[U- <sup>13</sup> C] $\alpha$ LNA, NEFA, 0.7 g	21	6	9	
Pawlosky <i>et al.</i> (2003b)	M + F	<sup>2</sup> H-labelled ethyl ester of $\alpha$ LNA, 1 g	Increased fish consumption decreased the rate constant for conversion of DPA to DHA by 70% compared with a beef-based diet			
Burdge <i>et al.</i> (2003)	M	[U- <sup>13</sup> C] $\alpha$ LNA, NEFA, 0.7 g	Increased EPA + DPA, but DHA synthesis reduced by the EPA + DHA-enriched diet, but no effect of increased $\alpha$ LNA consumption			
Pawlosky <i>et al.</i> (2003a)	M v. F	<sup>2</sup> H-labelled ethyl ester of $\alpha$ LNA	Conversion about 2-fold greater in women than men on <i>ad libitum</i> and beef-based diet. Reduced to the same level as men by consuming a fish-based diet			
Hussein <i>et al.</i> (2005)	M	[U- <sup>13</sup> C] $\alpha$ LNA, NEFA	0.29 0.19 0.26	0.05 0.02 0.04	<0.01 <0.01 <0.01	
Goyens <i>et al.</i> (2005)	Adults*	[U- <sup>13</sup> C] $\alpha$ LNA, NEFA, 30 mg bolus plus eight 20 mg daily doses	Of the ingested $\alpha$ LNA 7% was incorporated into plasma PL, of which 99.8% was converted to EPA (6.98% ingested), and 1% each to DPA and DHA (0.07% ingested for each fatty acid)			

M, males; F, females; TAG, triacylglycerol; AUC, area under the curve; PC, phosphatidylcholine; CE, cholesteryl ester; PL, phospholipid; DPA, docosapentaenoic acid; ND, not detected.

\*Gender distribution not disclosed.

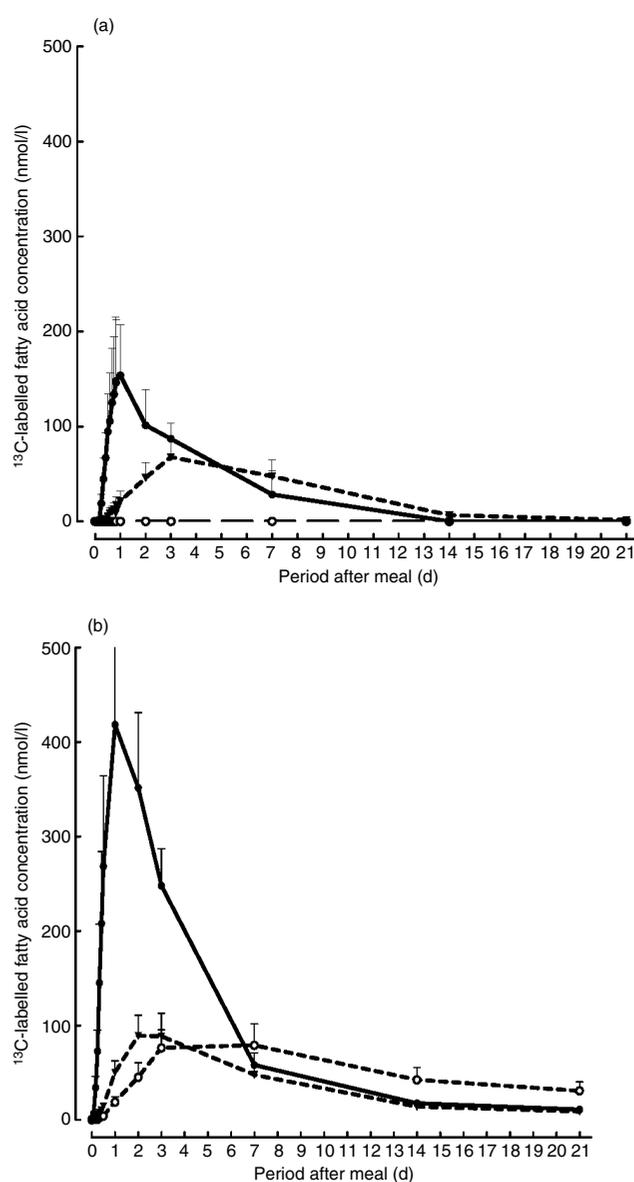
†Peak concentrations.

‡Approximate value.

following increased dietary  $\alpha$ LNA intake has been compared in men and women. In addition, the majority of investigations of stable-isotope-labelled  $\alpha$ LNA in human subjects have focused on groups of relatively young healthy individuals, either men or mixed groups of men and women. There are only two reports of studies that have specifically investigated  $\alpha$ LNA conversion in women of reproductive age. Burdge & Wootton (2002) have shown that conversion of  $\alpha$ LNA to EPA and DHA in women aged about 28 years is substantially greater (2.5-fold and >200-fold respectively) than that observed in a comparable study of men of similar age (Burdge *et al.* 2002; Fig. 1). In part, gender differences may reflect greater availability of  $\alpha$ LNA in women than men as a result of lower partitioning towards  $\beta$ -oxidation. However, since the rate constant coefficient for the conversion of DPA to DHA has been shown to be greater in women than men (Pawlosky *et al.* 2003a), it is likely that there is also a gender-related difference in the activity of the desaturation–elongation pathway.

One possible explanation for the greater synthesis of EPA and DHA from  $\alpha$ LNA in women compared with men is the action of oestrogen. DHA synthesis is almost 3-fold greater in women using an oral contraceptive pill containing 17 $\alpha$ -ethinyloestradiol than it is in those not using the pill (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 post-menopausal women results in greater plasma concentrations of dihomo- $\gamma$ -linoleic acid and arachidonic acid than before treatment (Ottoson *et al.* 1984). Furthermore, the DHA concentration in the plasma cholesteryl ester fraction has 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  $\alpha$ LNA content, although DHA is a minor component of this plasma lipid pool (Giltay *et al.* 2004). DHA concentration is also greater in women taking oral contraceptives (0.58% total fatty acids) than in those who are not taking oral contraceptives, which is in agreement with the effects of oral contraceptive pill use on  $\alpha$ LNA conversion (Burdge & Wootton, 2002). Interestingly, administration of 17 $\alpha$ -ethinyloestradiol to male-to-female trans-sexuals increases the concentration of DHA in plasma cholesteryl esters by 42%, while testosterone decreases DHA concentration by 22% in female-to-male trans-sexuals (Giltay *et al.* 2004). Together these data strongly support the suggestion that sex hormones regulate the activity of the desaturation–elongation pathway in man.

The data provide evidence to support the view that at least part of the adaptational response directed towards increased provision of DHA in pregnancy may involve up-regulation of the  $\alpha$ LNA desaturation–elongation pathway via oestrogen. If so, one implication would be that the 50% variation among pregnant women in plasma phosphatidylcholine-DHA concentration at term (Postle *et al.* 1995) may reflect differences in  $\alpha$ LNA metabolism in addition to any dietary effects, and that this factor may influence the supply of DHA to the fetus and subsequent development and function of fetal tissues. Evidence that



**Fig. 1.** Concentrations of  $^{13}\text{C}$ -labelled EPA (●—●), docosapentaenoic acid (▼—▼) and DHA (○—○) in plasma phosphatidylcholine in men (a) and women (b) following consumption of a meal containing 700 mg  $[\text{U-}^{13}\text{C}]\alpha$ -linolenic acid. Values are means with their standard errors represented by vertical bars for six subjects. (Data from Burdge *et al.* 2002; Burdge & Wootton, 2002.)

this pathway remains up regulated during lactation is less convincing. Although consumption of 10.7 g  $\alpha$ LNA/d by lactating women increases maternal plasma, erythrocyte and breast-milk  $\alpha$ LNA concentrations, the effects of increased dietary  $\alpha$ LNA on breast-milk EPA and DPA concentrations are modest, and there is no effect on breast-milk DHA (Francois *et al.* 2003). This finding suggests that the incorporation of PUFA into milk may be dependent on mobilisation of stores accumulated before conception and during pregnancy, which, if substantiated, 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 women compared with non-pregnant and pregnant women.

### Conclusions

The LC *n*-3 PUFA, EPA and DHA, appear to be important in maintaining optimal cardiovascular health, and most nutritional guidelines now include recommendations for increased intakes of these fatty acids in adult subjects as a means of protecting against CVD (for example, see Scientific Advisory Committee on Nutrition, 2004). Despite this position the consumption of oily fish, the richest dietary source of these fatty acids, remains low in most Westernised diets. Attempts to increase consumption of oily fish appear to be undermined by the lack of taste acceptability and concern about contamination with methyl mercury and organochlorides, although increased consumption has been observed in middle-aged women in the UK in recent years (Scientific Advisory Committee on Nutrition, 2004). Provision of DHA during pregnancy and lactation is of particular importance in order to support optimal rates of neurological development in the fetus and newborn. In preterm infants provision of supplemental DHA has been shown to have beneficial effects on the early development of the visual system, illustrating the importance of DHA supply during late pregnancy.

Although plant sources of *n*-3 PUFA can, in theory, provide EPA and DHA via desaturation–elongation of  $\alpha$ LNA, studies using chronically-increased  $\alpha$ LNA intake, or using a single bolus of isotopically-labelled  $\alpha$ LNA, yield the same conclusion, i.e. conversion of  $\alpha$ LNA to longer-chain PUFA, particularly DHA, in human subjects appears to be limited. 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 this limited capacity for synthesis of EPA and DHA from  $\alpha$ LNA may be sufficient to maintain tissue function. Whether such levels are sufficient to maintain optimal cardiovascular health is uncertain.

Preliminary data suggest that there are important differences between men and women in their capacity for synthesis of EPA and DHA from  $\alpha$ LNA, and that this capacity may be affected by physiological state (e.g. pregnancy). The ability to up regulate this pathway during pregnancy may be one of the adaptational mechanisms by which circulating maternal DHA levels are increased and provision of fetal DHA needs are met. This capacity for adaptation may be of particular importance in vegan pregnancies, where direct dietary provision of DHA is absent, and in multiple and sequential pregnancies, where demand for DHA will be much greater than normal. Clearly, more research in this area is required before firmer conclusions can be drawn.

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