α-Linolenic acid metabolism in men and women: nutritional and biological implications

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Purpose of review

This review critically evaluates current knowledge of α-linolenic acid metabolism in adult humans based on the findings of studies using stable isotope tracers and on increased dietary α-linolenic acid intake. The relative roles of α-linolenic acid and of longer-chain polyunsaturated fatty acids in cell structure and function are discussed together with an overview of the major metabolic fates of α-linolenic acid. The extent of partitioning towards β-oxidation and carbon recycling in humans is described. The use and limitations of stable isotope tracers to estimate α-linolenic acid desaturation and elongation are discussed. A consensus view of the extent of α-linolenic acid conversion to longer-chain fatty acids in humans is presented. The extent to which increasing dietary α-linolenic acid intake alters the concentrations of longer-chain n-3 fatty acids is described. The biological and nutritional implications of these findings are discussed.

Recent findings

Conversion of α-linolenic acid to eicosapentaenoic acid is limited in men and further transformation to docosahexaenoic acid is very low. A lower proportion of α-linolenic acid is used as a substrate for β-oxidation in women compared with men, while the fractional conversion to longer-chain fatty acids is greater, possibly due to the regulatory effects of oestrogen.

Summary

Overall, α-linolenic acid appears to be a limited source of longer-chain n-3 fatty acids in man and so adequate intakes of preformed n-3 polyunsaturated fatty acids, in particular docosahexaenoic acid, may be important for maintaining optimal tissue function. Capacity to upregulate α-linolenic acid transformation in women may be important for meeting the demands of the fetus and neonate for docosahexaenoic acid.

Keywords

α-linolenic acid, docosahexaenoic acid, gender, β-oxidation

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Abbreviations

α-LNA	α-linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
PUFA	polyunsaturated fatty acid

1363-1950

Introduction

The essential fatty acid α-linolenic acid (18:3n-3, α-LNA) is the predominant n-3 fatty acid in the Western diet. Estimated α-LNA consumption is approximately 1.5 g per day, which is about 10-fold lower than linoleic acid (18:2n-6), the equivalent n-6 essential fatty acid [1,2]. Whether the dietary essentiality of α-LNA reflects the activity of α-LNA itself or of longer-chain, more unsaturated fatty acids synthesized from α-LNA, including eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), remains a matter for debate. The concentration of α-LNA in cell membranes and blood lipids in healthy adult humans is typically less than 0.5% of total fatty acids, which suggests that the α-LNA content of these lipid pools is likely to have a limited influence on biological function. In contrast, the longer-chain more unsaturated n-3 fatty acids docosapentaenoic acid (22:5n-3, DPA) and DHA, and to a lesser extent EPA, are present in substantially greater amounts in cell membranes and in the circulation. In particular, DHA accounts for about 20–50% of fatty acids in the brain and in the retina [3]. Perhaps the strongest evidence in support of the suggestion that the principal biological role of α-LNA is as a substrate for synthesis of longer chain polyunsaturated fatty acids (PUFAs) comes from studies in animal models, which show that reduced maternal dietary α-LNA intake during pregnancy adversely affects retinal function in the offspring. This is due to a reduction in accumulation of DHA into photoreceptor cells and reflects impaired rhodopsin activity [4•,5]. Phospholipid DHA concentration also alters the activities of several cellular signalling mechanisms important for neurological function [6]. In patients receiving total parenteral nutrition, visual function was compromised in about 50% of children and 30% of adults and was associated with low plasma α-LNA concentration, although linoleic acid concentration was also reduced [7]. This indicates that there is a continuing requirement for DHA to support retinal function beyond the fetal and neonatal period, probably to support membrane turnover, and that capacity to meet such demands may be limited by α-LNA availability.

While there appears to be a continuing requirement for n-3 fatty acid to support neurological function, and probably that of other tissues, this may be relatively modest. Specific periods during adult life, however, may be associated with increased demands for n-3 fatty acids. Pregnancy represents a physiological challenge in which

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demands on the mother for n-3 fatty acids, in particular DHA, increase in order to meet the needs of the developing fetus. Since there is no selective increase in dietary DHA intake during gestation, the ability of the mother to provide sufficient DHA to the fetus may depend substantially upon capacity for α-LNA conversion.

This review will discuss the findings of recent human studies on the extent of α-LNA conversion in adult humans, the dietary and physiological regulation of α-LNA metabolism and the extent to which this process may be upregulated to meet increased demands.

α-Linolenic acid metabolism

Three principal metabolic fates have been described for α-LNA in humans: incorporation into structural, transport or storage pools, β-oxidation, and carbon chain elongation and further desaturation.

Partitioning of α-linolenic acid towards β-oxidation in humans

α-LNA is a substrate for β-oxidation and so is an energy source in man. Fractional oxidation of [^{13}C]α-LNA in men varies between 24% and 33% of administered dose [8,9,10•,11•]. Vermunt *et al.* [12] reported that 15% of ingested [^{13}C]α-LNA was recovered as $^{13}CO_2$ in a mixed group of men and women. Differences in the values obtained may in part be due to variations in the duration of the sampling period (Table 1). Fractional oxidation of α-LNA in women (22% of administered dose) [13•] is lower than in men (33%) [10•,11•] under identical experimental conditions, which may reflect differences in muscle mass. This is in agreement with previous reports of lower use of fat and a greater use of carbohydrate as an energy source in women compared with men [14,15]. One implication is that the extent of partitioning of α-LNA towards β-oxidation in men compared with women may be an important determinant of the availability of α-LNA for conversion to longer chain PUFAs. This will be discussed in more detail below. When compared directly, fractional recovery of labelled α-LNA as $^{13}CO_2$ on breath was almost twice that of palmitic, stearic and oleic acids in men [9], suggesting some preferential partitioning of α-LNA towards β-oxidation. Although recovery of α-LNA as $^{14}CO_2$ in rats was greater than in humans (approximately 67% of administered [^{14}C]α-LNA [16]), α-LNA was again the preferred substrate for the β-oxidation pathway.

Table 1. Proportion of administered dose of [^{13}C] α-linolenic acid recovered as $^{13}CO_2$ on breath

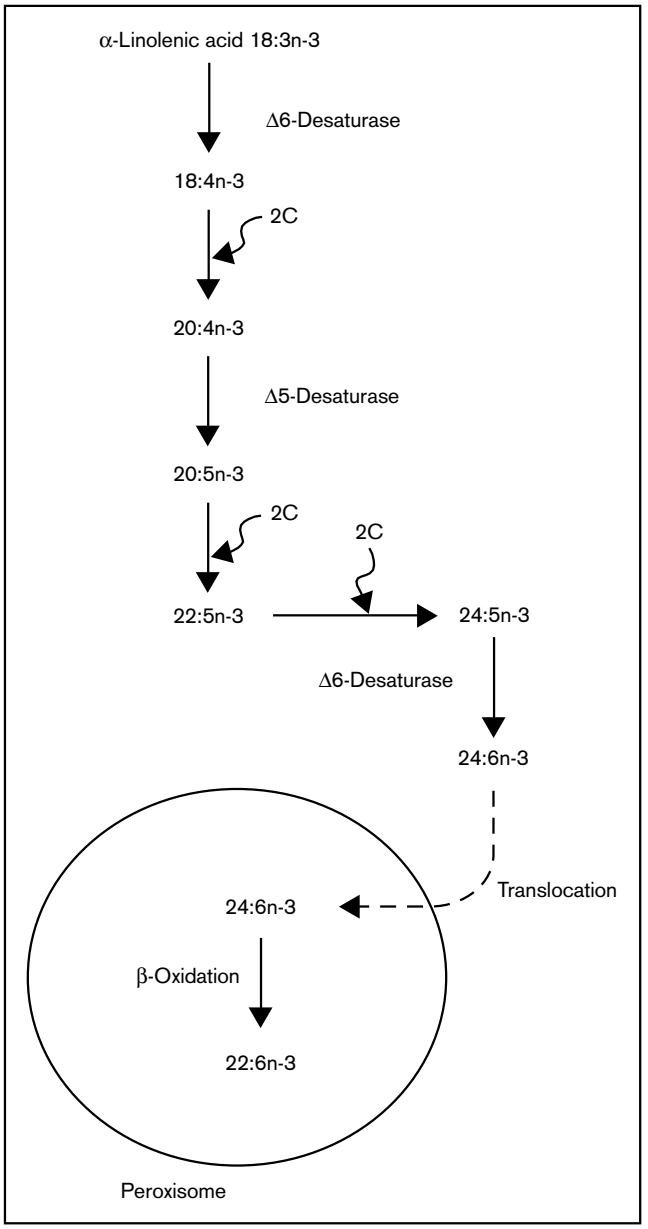
Fractional recovery (% administered dose)	Period of sample collection (h)	Participants	Reference
24	48	Men	[8]
27	9	Men	[9]
33	24	Men	[10•,11•]
11	12	Men and women	[12]
22	24	Women	[13•]

Recycling of carbon produced by β-oxidation of α-LNA into fatty acid and cholesterol biosynthesis *de novo* has been reported in monkeys [17]. This has been suggested to be important for maintaining optimal supply of membrane components [18•] and for meeting fetal demands for fatty acids during pregnancy [17]. Incorporation of [^{13}C] from [*U*-^{13}C]α-LNA into palmitic, palmitoleic, stearic and oleic acids shows that recycling of carbon from β-oxidation of α-LNA occurs in both men and to a lesser extent in women [19•]. In men, this may represent an equally important metabolic fate to conversion to EPA.

The pathway for desaturation and chain elongation of α-linolenic acid

The pathway for conversion of α-LNA to EPA, DPA and DHA has been described in rat liver [20•]. All reactions occur at the endoplasmic reticulum, with the exception of the final reaction to form DHA. The rate limiting reaction is the initial desaturation at the Δ6 position by Δ6-desaturase, followed by addition of C_2 and desaturation at the Δ5 position to form EPA (Fig. 1). Although α-LNA is the preferred substrate for Δ6-desaturase, the excess of linoleic acid typical of the diets of humans and laboratory animals leads to a greater net conversion of linoleic acid compared with α-LNA. EPA is then converted to DPA by addition of C_2. Synthesis of DHA requires further addition of C_2 to form 24:5n-3 and Δ6 desaturation to form 24:6n-4. Whether the same Δ6-desaturase is responsible for both the initial rate-limiting reaction and the second Δ6 desaturation is unclear, although enzyme preparations with both activities have been reported [21,22]. Synthesis of DHA involves translocation of 24:6n-3 from the endoplasmic reticulum to peroxisomes and removal of C_2 by β-oxidation. The role of the second Δ6 desaturation and limited β-oxidation has been challenged in favour or direct conversion from DPA by Δ4 desaturation [23]. However, there is strong evidence from studies involving reconstitution of microsomes and peroxisomes [24], and from the use of the specific Δ6-desaturase inhibitor SC-26196 [25•] to suggest that Δ4-desaturase activity is unlikely to be the major pathway for DHA synthesis. The complexity of the conversion of DPA to DHA may represent a locus of metabolic control [26] and suggests the possibility that DHA synthesis may be regulated separately from that of EPA and DPA.

Figure 1. The general pathway for α-linolenic acid desaturation and chain elongation in mammals



Based on data summarized in [20•,26].

Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids in humans

Development of sophisticated mass spectrometry techniques and the availability of α-LNA labelled with stable isotopes have permitted the metabolic fate of ingested α-LNA, in particular conversion to longer-chain PUFAs, to be followed in humans. The advantages and limitations of using fatty acids labelled with stable isotopes to probe fatty acid metabolism in humans have been reviewed in detail [27]. While there are obvious advantages in terms of safety compared with radioactive tracers, there are unresolved issues regarding standardization of quantification of data (particularly how conversion between fatty acids should be estimated), kinetic modelling, between subject variability and the use of blood data as a marker of fatty acid metabolism within tissues [27]. There is considerable heterogeneity between the reports published to date in the design of the tracer studies using stable isotope-labelled α-LNA [10•,11•,12,13•,28–31,32•] (Table 2). In addition to the issues discussed by Emken [27], the gender and age distribution of the study participants, the macronutrient composition of the meal vehicle used to deliver the labelled α-LNA tracer, the gender mix of the participants, and the timing and duration of sample collection differ markedly between reports. This presents a considerable challenge to any attempt to reach a consensus view on α-LNA metabolism in man. Nevertheless, stable isotope techniques represent the best technology currently available to study α-LNA metabolism in humans *in vivo*.

All of the studies carried out in adult humans have demonstrated conversion of labelled α-LNA to EPA and DPA [10•,11•,12,13•,28–31,32•] (Table 2). However, the proportion of α-LNA entering the desaturation/elongation pathway and converted to EPA is low [10•,11•,12,28–31,32•]. Estimates of the extent of conversion in men are about 8% [10•,28], while the conversion efficiency based on kinetic measurements is about 0.2% [31], which together indicate a constrain in the first steps in the pathway consistent with the rate-limiting role of Δ6-desaturase. Relative DPA synthesis appears to occur at a similar magnitude to that of EPA [10•,11•,12,28] (Table 2). The extent of conversion of α-LNA to DHA is less clear. The highest estimated fractional conversion is 4% [28], while others have either failed to detect significant DHA synthesis [10•] or estimated that less than 0.05% of α-LNA was converted to DHA [11•]. In studies where estimation of fractional DHA synthesis has not been reported, the relative amount of labelled DHA in plasma was 12-fold [12] to 29-fold [30] less than EPA (Table 2). Although the conversion efficiency of DPA to DHA is about 37%, the overall efficiency of DHA synthesis from α-LNA is 0.05% [31]. The consensus of these studies with markedly different designs appears to be that conversion of α-LNA to longer-chain PUFAs in humans is 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 that limited capacity for synthesis of EPA and DHA may be sufficient to maintain tissue function. However, this is not supported by the results of studies in which α-LNA consumption is increased.

Table 2. Estimated conversion of α-linolenic acid to longer chain polyunsaturated fatty acids

			Outcome measures			
Reference	Participants	Isotope and dose		EPA	DPA	DHA
[28]	M	d_2, 3.5 g mixed TAG	Absolute and relative AUC concentrations in total plasma lipids	50 μg/ml (8%)	26 μg/ml (4%)	25 μg/ml (4%)
[29]	M	d_4, 3.1 g mixed TAG. Participants consumed 6.5 g or <0.1 g DHA per day for 90 days prior to experiment	AUC concentrations in total plasma lipids. Higher DHA consumption decreased EPA and DHA synthesis by 76% and 88%, respectively			
[30]	Adults[a]	D_5 ethyl ester, 1 g	Concentrations in total plasma lipids	57 ng/ml[b]	ND	<2 ng/ml[b]
[12]	M + F	[*U*-^{13}C]methyl ester, 45 mg	Peak concentrations adjusted for estimated total blood volume. Consumption of 8 g α-LNA per day for 6 weeks reduced peak labelled EPA (≈60%), DPA (≈50%) and DHA (≈50%) concentrations	120 μg, reference diet	50 μg, reference diet	≈10 μg, reference diet
[31]	M + F	d_5 ethyl ester, 1 g	Mathematical modelling of kinetic parameters following consumption of a beef-based diet. Data expressed here as conversion efficiency from α-LNA (and between successive metabolites)	0.2% (α-LNA to EPA 0.2%)	0.13% (EPA to DPA 63%)	0.05% (DPA to DHA 37%)
[10•]	M	[*U*-^{13}C]α-LNA, free fatty acid, 0.7 g	Concentrations in plasma TAG, NEFA and PC over 21 days. Fractional conversion estimated from time × concentration AUC	8%	8%	ND
[13•]	F	[*U*-^{13}C]α-LNA, free fatty acid, 0.7 g	Concentrations in plasma TAG, NEFA, CE and PC over 21 days. Fractional conversion estimated from time × concentration AUC	21%	6%	9%
[32•]	M + F	d_5 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			
[11•]	M	[*U*-^{13}C]α-LNA, free fatty acid, 0.7 g	Repeated analysis of participants at baseline and after consuming control, α-LNA or EPA + DHA-enriched diets for 8 weeks. Increased EPA + DPA, but not DHA synthesis reduced by the EPA + DHA-enriched diet, but no effect of increased α-LNA consumption			

EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; TAG, triacylglycerol; AUC, area under the curve; ND, not detected; LNA, linolenic acid; NEFA, nonesterified fatty acid; PC, phosphatidylcholine; CE, cholesteryl ester. [a]Gender distribution not disclosed. [b]Peak concentration.

The effect of increased α-linolenic acid consumption on the concentrations of longer-chain fatty acids

A substantial number of studies have reported the effects of feeding α-LNA or oils with a high α-LNA content, typically flaxseed oil, on the fatty acid composition of plasma lipids or membrane phospholipids in circulating cells or platelets. The majority of the reports of α-LNA supplementation in either men or mixed groups of men and women show increased EPA and DPA concentrations [33–44], while the level of DHA either did not change or decreased slightly. Two studies reported increased EPA, DPA and DHA concentrations following α-LNA supplementation using low erucic acid rapeseed oil [45] or perilla oil [46]. The effect of increased α-LNA concentration upon the concentrations of longer-chain PUFAs is reviewed by Gerster [47]. Three recent publications have reported the effect of feeding 150 moderately hyperlipidemic men and women (25–72 years) 4.5 or 9.5 g α-LNA per day, using a linseed and rapeseed oil blend, for 6 months on a variety of cardiovascular risk factors. The higher α-LNA intake was associated with increased plasma phospholipid α-LNA and EPA, but not DPA and DHA

concentrations [48••], indicating constraint in α-LNA conversion beyond EPA. An intake of 9.5 g per day of α-LNA increased EPA, but not DPA, concentration in peripheral blood mononuclear cells, while DHA concentration decreased [49•]. This suggests that availability of DHA was insufficient to maintain DHA levels in these cells, and importantly that α-LNA cannot replace dietary DHA [48••]. This selective change in EPA concentration was unable to alter blood lipid, glucose, insulin concentrations, postprandial lipaemia [48••], blood coagulation or fibrinolytic factors [50•], or markers of immune function [49•]. Overall, these investigations of the effect of α-LNA supplementation support the results of the tracer studies in that increased α-LNA consumption was able to raise both plasma and cellular EPA concentrations, which in itself may potentially be beneficial. It appears, however, that consumption of 1–1.5 g may not be sufficient to meet even modest demands for DHA to support membrane synthesis and turnover. If so, dietary intakes of EPA and DHA are likely to be critical for maintaining tissue function.

Retroconversion of docosahexaenoic acid to eicosapentaenoic acid in humans

EPA and DPA can also be synthesized from DHA by retroconversion due to limited peroxisomal β-oxidation [51]. Supplementation of the background diet of humans with 3.6, 4 or 6 g of purified DHA per day produced a dose-dependent increase (0.5%, 1% and 4%, respectively) in EPA concentration in plasma phospholipids [52–54], although lower intakes of DHA (0.75 g) did not alter circulating EPA concentration [55]. This suggests that although retroconversion is possible in humans, this is limited and unlikely to contribute significantly to EPA synthesis at habitual DHA intakes (0.1 g/day) [1]. This is supported in the one study of DHA metabolism using [^{13}C]DHA in humans [56], which showed overall retroconversion of 1.4%. Therefore, intakes of 0.1 g DHA per day would be expected to generate 1 mg EPA per day.

The effect of altered n-3 fatty acid consumption of α-linolenic acid metabolism

Increased consumption of purified DHA (6.5 g per day) [28] or fish [32•] decreased the extent of conversion of labelled α-LNA to DHA (Table 2). This reflects a decrease in the rate of conversion of DPA to DHA, although DHA turnover was also reduced [32•]. Increased consumption of α-LNA decreased conversion to EPA by about 75%, although the effect on DPA and DHA synthesis was less clear [12]. In contrast, there was no effect of consuming a diet providing 9.5 g unlabelled α-LNA per day on the extent of [^{13}C]α-conversion when measurements were made in the same individuals before and after the dietary intervention [11•]. This was accompanied by an increase in plasma phosphatidylcholine EPA and triacylglycerol EPA and DPA concentrations [11•]. It is unclear how consumption of similar amounts of α-LNA and using comparable food products produced very different effects upon α-LNA metabolism and on the concentration of EPA, DPA and DHA [11•,12]. Neither study showed an effect of increased α-LNA or EPA + DHA consumption on the fractional recovery of labelled α-LNA as $^{13}CO_2$ on breath [11•,12]. The inhibitory effects of EPA and DHA [11•,29,32•] and possibly α-LNA [12] on α-LNA conversion may be due to downregulation of Δ6-desaturase activity by a peroxisomal proliferator-activated receptor-α dependent mechanism [57••].

α-Linolenic acid metabolism in women

The developing human fetus assimilates at least 400 mg DHA per week during the last trimester [58]. Since this estimate reflects only brain, adipose tissue and liver, the overall demands for DHA are likely to be substantially greater. Direct measurement of desaturase activities in human fetal liver in late gestation [59] and conversion of labelled linoleic acid and α-LNA by infants born preterm [60–62] indicate some capacity for synthesis of longer-chain PUFAs. However, this activity appears less than in adults [63], although there are no estimates for the extent of α-LNA conversion in the human fetus. This suggests that assimilation of DHA by the fetus has to be met primarily by supply of pre-formed DHA by the mother. Thus pregnancy represents a substantial burden on the mother to provide sufficient DHA to support the development of the fetus, in particular the brain and retina [3]. In addition, DHA supply in milk may be important for supporting continuing neural development. Plasma phosphatidylcholine DHA concentration increases by approximately 33% between 16 weeks' (170 μmol/l) and 40 weeks' (230 μmol/l) gestation [64], which implies a physiological adaptation to facilitate DHA supply to the fetus. The blood volume of a 70 kg woman is approximately 3.2 l [65]. Thus total plasma phosphatidylcholine DHA content in a nonpregnant woman would be about 180 mg. The increases in DHA concentration per litre and blood volume (50%) during pregnancy would result in a total plasma phosphatidylcholine DHA pool of 360 mg – an overall doubling of DHA in the circulation. In order for maternal blood DHA content to increase during pregnancy, conversion of α-LNA to DHA, mobilization of body stores, or dietary consumption of DHA must increase. While the two metabolic adaptations are possible and are not mutually exclusive, the selective increase in DHA consumption seems an unlikely and nutritionally precarious means of ensuring adequate DHA supply to the fetus.

The estimated conversion of [^{13}C]α-LNA in women of reproductive age was EPA 21%, DPA 6% and DHA 9% [13•], which suggests greater EPA and DHA synthesis

compared with men using the same study design [10•]. This is supported by higher plasma DHA concentrations in women compared with men [66•]. Conversion of [^{13}C]α-LNA to DHA was 62% greater in those who used an oral contraceptive pill compared with those who did not, which implies upregulation of DHA synthesis by the action of oestrogen [13•]. This is consistent with previous reports of increased activity of the desaturation/chain elongation pathway in postmenopausal women receiving oestrogen therapy [67]. Since circulating oestrogen rises during pregnancy, one potential implication is that α-LNA conversion may increase during gestation, which is supported by the increase in Δ6-desaturase activity in the pregnant rat [68•]. This does not exclude the possibly that mobilization of adipose reserves contributes significantly to increasing circulating DHA concentration in pregnant women. One possible implication is that the 50% variation among pregnant women in plasma phosphatidylcholine DHA concentration at term [64] may have a metabolic basis in addition to any dietary effects, and that this may influence both fetal DHA assimilation and subsequent tissue function.

Consumption of 10.7 g α-LNA per day by lactating women increased maternal plasma, erythrocyte and breast milk α-LNA and EPA concentrations. The effect on breast-milk DPA concentration is less clear than the difference between baseline DPA concentration ($0.19 \pm 0.05\%$) and after 4 weeks of supplementation ($0.17 \pm 0.02\%$) does not support the claim that the DPA content of milk increased over time [69•]. However, there was no significant effect of consuming flax seed oil on breast milk DHA concentration. This is consistent with the minor contribution of newly synthesized arachidonic acid to the arachidonic acid content of breast milk [70]. Together, these studies suggest that incorporation of DPA, DHA, and arachidonic acid during lactation may reflect mobilization of maternal stores. If so, this emphasizes 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 downregulated in lactating women compared with nonpregnant and pregnant women. Thus these results do not exclude the possibility of increased DHA synthesis during pregnancy.

Conclusion

Overall, the capacity for conversion of α-LNA to DHA differs markedly between men and women. This has important implications for their nutritional requirements for n-3 PUFAs. It is possible that demands for DHA by individual tissues in men are relatively modest, possibly due to efficient recycling, and can be met by the diet or the low level of α-LNA conversion. Nevertheless, men with a poor DHA intake together with higher partitioning of fatty acids towards β-oxidation would be at greater risk of marginal DHA status than women. There is evidence which suggests oestrogen-mediated upregulation of conversion of α-LNA to DHA in women. Whether there are other physiological or pathological states in which α-LNA or linoleic acid conversion can be upregulated in humans remains to be determined. For example, cells of the immune system contain substantial amounts of PUFAs and so synthesis of cell membranes to support cell proliferation and synthesis of lipid mediator represents a potential increase in PUFA demands. Since alterations in PUFA availability alter immune function [71•], failure to meet such demands may modify the immune response. It would be of interest to ascertain whether upregulation of PUFA synthesis is important for meeting such demands, particularly during chronic inflammation. It is clear that there is considerable potential to extend understanding of the regulation of essential fatty acid metabolism beyond healthy male volunteers and to focus on individuals with differing physiological and pathological states.

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