Metabolism of a-linolenic acid in humans

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Introduction
This study investigates how alpha-linolenic acid (ALA) is metabolized when consumed.

Conclusions

ALA can be used for the generation of cellular energy, but is also used for two other primary purposes - the generation of saturated and monounsaturated fats, as well as the production of omega-3s in the body.

Men tend to use more for metabolism (cell energy), while women tend to use more for the generation of omega-3 fats.

Amendments

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Metabolism of α-linolenic acid in humans

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Abstract

α-Linolenic acid (18:3n-3) is essential in the human diet, probably because it is the substrate for the synthesis of longer-chain, more unsaturated n-3 fatty acids eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) which are required for tissue function. This article reviews the recent literature on 18:3n-3 metabolism in humans, including fatty acid β-oxidation, recycling of carbon by fatty acid synthesis de novo and conversion to longer-chain polyunsaturated fatty acids (PUFA). In men, stable isotope tracer studies and studies in which volunteers increased their consumption of 18:3n-3 show conversion to 20:5n-3 and 22:5n-3, but limited conversion to 22:6n-3. However, conversion to 18:3n-3 to 20:5n-3 and 22:6n-3 is greater in women compared to men, due possibly to a regulatory effect of oestrogen, while partitioning of 18:3n-3 towards β-oxidation and carbon recycling was lower than in men. These gender differences may be an important consideration in making dietary recommendations for n-3 PUFA intake.

1. Introduction

α-Linolenic acid (18:3n-3) is one of the two polyunsaturated fatty acids (PUFA) which are essential in the human diet. It is difficult to ascribe specific functional effects directly to 18:3n-3 and the low concentrations of this fatty acid in most mammalian cell membranes, with the exception of skin, suggest that it is unlikely to exert direct effects on cell and tissue function [1]. However, the essentiality of 18:3n-3 may lie primarily in it being a substrate for the synthesis of the long-chain, more unsaturated PUFA eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) [1]. This review will focus primarily on current knowledge about the metabolic disposal of 18:3n-3 in adult humans (Fig. 1), in particular the extent to which dietary 18:3n-3 is a source of 20:5n-3 and 22:6n-3.

2. Methods for measuring α-linolenic acid metabolism in humans

Most of the information about the metabolic fate of dietary 18:3n-3 in humans has been derived from two experimental approaches. Studies in which relatively few volunteers have ingested 18:3n-3 labelled with a stable isotope tracer, either [^{13}C] or deuterium, and the appearance of labelled metabolites then measured in different pools by mass spectrometry and studies in which the amount of 18:3n-3 consumed in the background diet is increased over a period of time and the concentration of 18:3n-3 and its metabolites measured in various body pools, mainly blood. Despite some limitations in experimental design, particularly of the tracer studies (which will be discussed in greater detail below), these methodologies provide complimentary information about the metabolic fate of 18:3n-3.

3. Bioavailability of ingested α-linolenic acid and incorporation into adipose tissue

There is very little information regarding the efficiency of absorption of 18:3n-3 by the human gut. Direct assessment of the absorption of 18:3n-3 requires measurement of the output in stool compared to consumption of a known amount in a meal. Measurement of the cumulative concentration of labelled 18:3n-3 in stool collected over 5 days following ingestion of

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1. Alpha-linolenic acid (ALA) is one of two essential fats we need to consume in our diet - without them, we die. It is believed that its main function is to be converted/used for the generation of longer fat molecules known as omega-3 fats (ALA is an 18 carbon chain and the two main omega-3s are 20 and 22 carbons long).

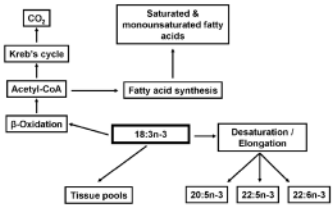

Fig. 1. Summary of the metabolic fates of 18:3n-3 in humans.

750 mg [U-^{13}C] 18:3n-3 by a single individual showed that greater than 96% of the administered dose was absorbed (G.C. Burdge, unpublished observation). This is comparable to the uptake of 18:3n-3 in patients with ileostomies who were fed 100 g linseed oil [2]. These findings suggest that absorption of 18:3n-3 across the gut is efficient in humans.

Since adipose tissue accounts for approximately 15% of body mass in men and 23% of body mass in women incorporation of 18:3n-3 into this storage pool represents a potentially important route of disposal of dietary 18:3n-3 and a reserve pool which is available for mobilisation during periods of increased demands. Together, 18:3n-3 accounts for about 0.7% of total fatty acids in neutral lipids in adipose tissue in men and women [3,4]. Thus, it can be estimated that in a 75 kg man the whole body 18:3n-3 reserve in adipose tissue would be approximately 79 g (roughly equivalent to typical intake over 53 days) and in a 65 kg woman the whole body 18:3n-3 reserve in adipose tissue would be approximately 105 g (roughly equivalent to typical intake over 70 days).

The exchange of αLNA between the blood and adipose tissue compartments has not been characterised in detail in humans in vivo. When men consumed [U-^{13}C] 18:3n-3, labelled 18:3n-3 was detected in plasma non-esterified fatty acid (NEFA) pool within 2 h and reached a peak at 6 h [5]. One possible interpretation is that during the early postprandial period labelled 18:3n-3 which is detected in the NEFA pool probably reflects incomplete entrapment of fatty acids released by hydrolysis of chylomicron triacylglycerol (TAG) [6]. At later time points, the incorporation of labelled 18:3n-3 into the plasma NEFA pool probably reflects mobilisation of recently assimilated fatty acid. The rapid release of 18:3n-3 into the NEFA pool, would tend to facilitate supply of 18:3n-3 to the liver. McCloy et al. [7] estimated that 15–81% of the administered dose of [^{13}C]18:3n-3 was present in adipose tissue at 6 h after the ingestion of the tracer.

4. Partitioning of α-linolenic acid towards β-oxidation

Partitioning of [^{13}C] 18:3n-3 towards β-oxidation in men accounts for between 15% and 33% of administered dose [5,8–11], although the variation in the values reported may be due in part to differences in the duration of breath was collection. The values probably represent an approximately 30% underestimate of the actual proportion of ingested 18:3n-3 used in energy production due to trapping of $^{13}CO_2$ in bicarbonate pools [12]. Fractional oxidation of [^{13}C] 18:3n-3 in women (22% of administered dose) [13] was less than reported in men (33%) over the same sampling period [5]. This may reflect differences between men and women in the mass of tissues, which have highly active fatty acid β-oxidation pathways such as skeletal and cardiac muscle, liver and kidney. 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 to men [14,15]. One implication is that the extent of partitioning of 18:3n-3 towards β-oxidation in men compared to women may be an important determinant of the availability of 18:3n-3 for conversion to longer-chain PUFA. The fractional recovery of ingested [^{13}C] 18:3n-3 as $^{13}CO_2$ on breath was almost twice that of palmitic, stearic and oleic acids in men [9] (Fig. 2). This is in agreement with preferential use of 18:3n-3 for β-oxidation in rats [16]. One possible explanation for the preferred use of 18:3n-3 for β-oxidation is the greater affinity of carnitine: palmitoyl transferase-1, the rate limiting enzyme in mitochondrial fatty acid β-oxidation, for 18:3n-3 compared to other unsaturated fatty acids such as 18:1n-9 and 18:2n-6 (Fig. 3) [17].

One study has reported the effect of altering the n-3 PUFA content of the background diet on the proportion of ingested [^{13}C] 18:3n-3 recovered as $^{13}CO_2$ on breath [10]. There was no significant difference in the fractional recovery of $^{13}CO_2$ on breath when men consumed [^{13}C] 18:3n-3 before and after consuming diets containing 1.7 g 18:3n-3 per day, equivalent to habitual 18:3n-3 intake in the UK [18] or 9.6 g 18:3n-3/day for 8 weeks. This

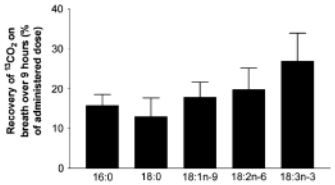

Fig. 2. Fractional recovery of $^{13}CO_2$ on breath following ingestion of [^{13}C]-labelled fatty acids differing in chain length and degree of unsaturation in men over 9 h as described in [9].

2. ALA that is consumed in the diet is absorbed into the body extremely efficiently with over 95% being absorbed (weak data substantiating this).

   ALA can be stored in the fat cells, but makes up less than 1% of the fat molecule content. A 75 kilogram man with about 15% body fat would have almost 80 grams of ALA stored - enough for a 53 day supply. A woman would have more fat, so they'd have enough for 70 days supply.

   When consumed, ALA levels in the blood stream were already detected in 2 hours and peaked at 6 hours.

3. About 30% of ALA goes to beta-oxidation (metabolism), with higher levels in men, but that may be due to the fact that women's cells preferentially use blood sugar for energy.

suggests that the extent of partitioning of 18:3n-3 towards β-oxidation is relatively stable over short periods of time and that increasing the amount of 18:3n-3 in the diet does not significantly alter this process. It is possible that the excess 18:3n-3 in the group that consumed the diet with the higher 18:3n-3 content may have been incorporated into storage pools as there was no evidence for increased synthesis of other metabolites.

5. Incorporation of carbon from α-linolenic acid into saturated (SFA) and monounsaturated fatty acids (MUFA)

Carbon released from fatty acids by β-oxidation enters the cellular acetyl-CoA pool, and may then enter Kreb's cycle or be used in fatty acid synthesis de novo. The recycling of carbon from 18:3n-3 into SFA and MUFA has been suggested to be important as a source of fatty acids in pregnant and foetal monkeys [19] and rats [20]. There is one report, which describes recycling of carbon released by β-oxidation of 18:3n-3 in adult humans [21]. When men (35 years of age) and women (28 years of age) consumed 700 mg [U–^{13}C] 18:3n-3, labelled 16:0, 18:0, 16:1n-7 and 18:1n-9 were detected in plasma phosphatidylcholine (PC) and TAG, but not other plasma lipid pools, in both men and women. The total proportion of label recovered in plasma PC was 6-fold greater than in TAG in men and 25-fold greater in plasma PC than in TAG in women (Fig. 4A). These data suggest preferential channelling by the liver of SFA and MUFA synthesised by carbon recycling into PC compared to TAG. The total concentration of labelled SFA and MUFA in plasma lipids was 20% greater in men compared with women [21] is in agreement with greater partitioning of 18:3n-3 towards β-oxidation in men compared to women [5,13]. Comparison of the amount of [^{13}C] recycled into SFA and MUFA with that incorporated by desaturation and elongation of 18:3n-3 into longer-chain PUFA in plasma over 21 days showed marked differences between men and women (Fig. 4B). In men, carbon recycling into SFA and MUFA greatly exceeded conversion to longer-chain PUFA, while in women the amount of carbon recycled into SFA and MUFA in women was about 1/5 of that recovered in n-3 PUFA. One implication of these findings is that the extent of partitioning of 18:3n-3 towards β-oxidation and carbon recycling may be an important control point in the regulation of the availability of 18:3n-3 for conversion to longer-chain PUFA and that the relative partitioning between these metabolic fates differs markedly between men and women.

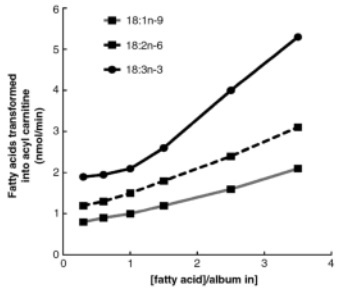

Fig. 3. Rat liver palmitoyl: carnitine acyltransferase activity towards 18 carbon fatty acids differing in the number and positions of *cis* double bonds as described in [17].

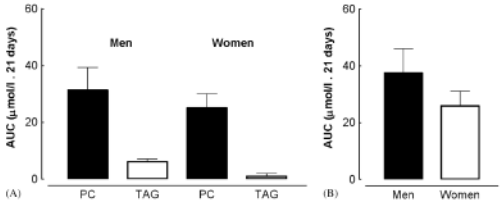

Fig. 4. Recycling of carbon from [^{13}C] 18:3n-3 in men and women over 21 days as described in [21]. (A) Concentration of total labelled saturated and monounsaturated fatty acids in plasma phosphatidylcholine (PC) and triacylglycerol (TAG) in men and in women. (B) Plasma total labelled saturated and monounsaturated fatty acid concentrations in men and in women.

4. ALA can undergo beta-oxidation (be metabolized) for energy (Krebs Cycle) or be recycled for the generation of other fats (fat synthesis de novo). The latter function manifests as an important role for the generation of saturated and unsaturated fats, but men tend to exhibit this far more than women while women use their ALA to generate longer fats (omega-3s).

6. The general pathway for conversion of α-linolenic acid to longer-chain PUFA

A pathway for the conversion of linoleic acid (18:2n-6) and 18:3n-3 to longer-chain PUFA has been described in rat liver (reviewed in [22]) (Fig. 5). The activity of the desaturation/elongation pathway in the liver is the most important in terms of supply of 18:3n-3 metabolites to other tissues. All reactions occur in the endoplasmic reticulum with the exception of the final reaction, which results in the formation of 22:6n-3. The initial conversion of 18:3n-3 to 18:4n-3 by the action of Δ6 desaturase is the rate-limiting reaction of the pathway. The introduction of a double bond at the Δ6 position is followed by the addition of two carbons by elongase activity and then by desaturation at the Δ5 position by Δ5 desaturase to form 20:5n-3. Docosapentaenoc acid (22:5n-3) is synthesised from 20:5n-3 by the further addition of two carbons. The conversion of 22:5n-3 to 22:6n-3 has been a matter of controversy, although evidence from organelle reconstitute studies [23], experiments with specific Δ6 desaturase inhibitors [24] and from patients with Zellweger's syndrome [25] strongly support the formation of the 24 carbon intermediates 24:5n-5 and 24:6n-3 by a second desaturation at the Δ6 position and limited peroxisomal β-oxidation rather than by Δ4 desaturase activity as has been suggested [26]. 22:6n-3 is then translocated back to the endoplasmic reticulum. Although the precise regulation of the translocation steps and limited β-oxidation is not known, it is possible that this represents a locus for metabolic regulation that facilitates control of 22:6n-3 synthesis independently from the preceding steps of the pathway [22].

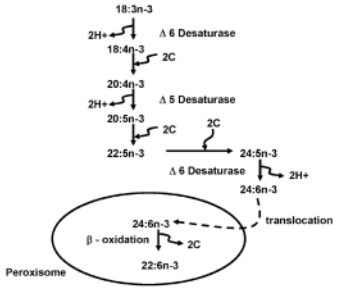

Fig. 5. The general essential fatty acid desaturation/elongation pathway as described in [22].

7. α-Linolenic acid conversion to longer-chain n-3 PUFA in adult humans

7.1. Effects of chronically increased α-linolenic acid consumption

A number of studies have reported the effects of consuming increased amounts of 18:3n-3, usually via consumption of oils with a high 18:3n-3 content or of products made with those oils (for example spreads), on the fatty acid composition of plasma or cell lipids (for a detailed review see [27]). These studies were conducted either in men or in mixed groups of men and women, used intakes of 18:3n-3 ranging from less than one to 20 g/day, and were of a few weeks to many months duration. Overall these studies consistently demonstrate increased proportions of 18:3n-3 in both plasma and cell lipids when 18:3n-3 intake is increased [27]. The relationship between increased 18:3n-3 intake and increased 20:5n-3 concentration in plasma phospholipids is a significant linear one ($r = 0.846$, $P = 0.004$) Fig. 6). However, none of these studies showed a significant increase in 22:6n-3 concentration and in some, for example Finnegan et al. [28], 22:6n-3 concentration decreased during the period of increased 18:3n-3 intake. This suggests that provision of 22:6n-3 either from the diet or from 18:3n-3 conversion was not sufficient to meet the demands of the body. Several studies also demonstrate increased proportions of 22:5n-3 in plasma and cell lipids when αLNA consumption is increased [27]. Overall, these studies demonstrate that chronically increased consumption of 18:3n-3 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.

One study has investigated the effect of increased consumption of important intermediates in the synthesis of 22:6n-3 from 18:3n-3 as pure ethyl esters [29]. 18:3n-3 which is the initial substrate for the pathway, 18:4n-3 which bypasses the initial rate limiting reaction catalysed by Δ6 desaturase and 20:5n-3 which bypasses the reactions catalysed by Δ6 desaturase and Δ5 desaturase. The findings of this study showed that 20:5n-3 and 22:5n-3 concentrations increased in plasma phospholipids when subjects consumed any of the dietary supplements (Fig. 7). However, there was no significant change in 22:6n-3 concentration. This suggests that the constraint in 22:6n-3 synthesis occurs after the formation of 22:5n-3 and may reflect the activity of the reactions that convert 22:5n-3 to 22:6n-3 via the 24-carbon intermediates (Fig. 5).

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

A growing number of studies using 18:3n-3 labelled with either [^{13}C] or deuterium have reported estimates of

5. Increased consumption of ALA leads to increased amount in the blood plasma as well as in the cells of the body, as well as increased production of longer fat molecules like eicosapentanoic acid (EPA, 22 carbon omega-3 dat).

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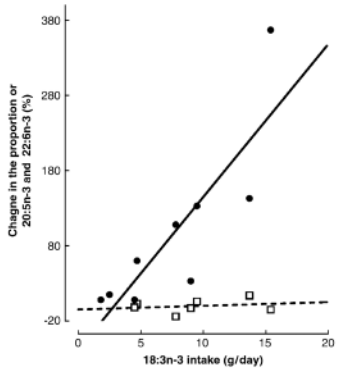

Fig. 6. The effect of consuming different amount of 18:3n-3 in the background diet on the change in the concentrations of 20:5n-3 and 22:6n-3 from baseline. Here, 20:5n-3 is indicated by solid circles and solid regression line and 22:6n-3 is indicated by open squares and dashed line. Each data point represents the mean value from a single study. Data are derived from [27].

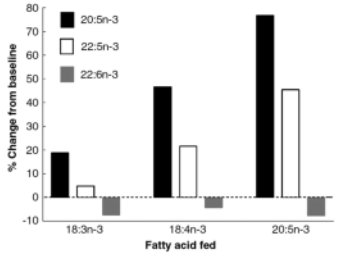

Fig. 7. The effect of consuming purified ethyl esters of 18:3n-3, 18:4n-3 and 20:5n-3 on the concentrations of 20:5n-3, 22:5n-3 and 22:6n-3 in plasma phospholipids as described in [29].

the extent of conversion to longer-chain PUFA in humans [5,10,11,13,30–36]. The advantages and limitations of these techniques in have been reviewed recently [37]. There remain unresolved issues regarding standardisation of quantification of data 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. It should be noted that even sophisticated kinetic modelling analysis based on the concentrations of labelled metabolites in blood only provides a proxy measure of the actual efficiency with which conversion of 18:3n-3 occurs in tissues. How close this approximation is to the actual conversion efficiency is not known. Thus the findings of tracer studies must be interpreted cautiously as reflecting the net result of the partitioning of individual 18:3n-3 between different metabolic fates within liver, the major source in blood of PUFA synthesised de novo, selectivity in incorporation of fatty acids into secretory pools, regulation of VLDL secretion and composition, and differential turnover of individual lipid classes and fatty acids in the blood compartment. The findings of individual studies have been described in detail in a number of recent reviews [27,38] and will not be reproduced here. Overall, if the concentrations of labelled 18:3n-3 metabolites in blood are assumed to reflect the extent of 18:3n-3 conversion, the general consensus of these tracer studies is that conversion of 18:3n-3 to 20:5n-3 (estimates are from 0.2% to about 6%) and 22:5n-3 (estimates are from 0.13% to 6%) in men is limited and the synthesis of 22:6n-3 is highly constrained (0.05% or less). One exception is the findings reported in [30] which showed substantial synthesis of 22:6n-3 (4%). Thus, the findings of stable isotope studies are in good agreement with those of dietary supplementation studies.

7.3. The effect of gender on conversion of 18:3n-3 to longer-chain PUFA

The majority of investigations of 18:3n-3 metabolism in humans have focused on groups of relatively young healthy individuals, either men or mixed groups of men and women. There are only two reports that have specifically studied 18:3n-3 in women of reproductive age. Burdge and Wootton [13] showed that conversion of 18:3n-3 to 20:5n-3 and 22:6n-3 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 [5]. This finding is supported by kinetic analysis which showed that the rate constant coefficient for the conversion of 22:5n-3 to 22:6n-3 was approximately 4-fold greater in women compared to men [39]. Although this may reflect in part greater availability of 18:3n-3 for conversion in women than in men because of lower partitioning towards β-oxidation in women, the difference between men and women in the rate constant

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6. Women tend to convert more ALA to longer fat molecules (omega-3s), because they have a greater ability to spit the molecule out of the endoplasmic reticulum (an organelle in the cell) to move it to the peroxisome (another organelle in the cell), where the final step of fat molecule elongation occurs. Omega-3 generation by women is also much higher with elevated estrogen.

   Its also believed women have a greater capacity, because fetal liver has markedly lower levels of the enzymes necessary for the generation of omega-3s.

coefficient for the conversion of 22:5n-3 to 22:6n-3 [39] suggests a gender-related difference in the activity of the desaturation/elongation pathway. It appears that the part of the pathway that involves to translocation of 24:6n-3 between the endoplasmic reticulum and peroxisome, which was shown by the findings of James et al. [29] to be constrained in men, is up-regulated in women. This further supports the suggestion that 22:6n-3 synthesis may be regulated independent of the rest of the desaturation/elongation pathway.

One possible explanation for the greater synthesis of 20:5n-3 and 22:6n-3 from 18:3n-3 in women compared to men is the action of oestrogen. 22:6n-3 synthesis was almost 3-fold greater in women using an oral contraceptive pill containing 17α-ethylnyloestradiol than in those who did not [13]. The suggestion that oestrogen may increase the activity of the desaturation/elongation pathway is consistent with the findings that oestrogen-based hormone replacement therapy in postmenopausal increases the concentration of arachidonic acid (20:4n-6) in plasma phospholipids [40], and 20:4n-6 and 22:6n-3 concentrations in plasma cholesteryl esters [41]. We have also found that individuals consuming their habitual diet women (aged 18–35 years) have a higher concentration of 22:6n-3 in plasma PC, NEFA and TAG compared to men of similar age and body mass index (>20 and <30 kg/m^2) (Bakewell, Burdge and Calder; unpublished observations). Together these data strongly support the suggestion that sex hormones, in particular oestrogen, regulate the activity of the desaturation/elongation pathway in humans.

One possible biological role for greater capacity for 18:3n-3 conversion in women may be in meeting the demands of the foetus for 22:6n-3 which has been estimated as at least 400 mg DHA per week during the third trimester (reviewed in [42]). Since desaturase activities in developing human liver appear to be lower than in adults [43–47] assimilation of 22:6n-3 by the foetus has to be met primarily by supply of 22:6n-3 by the mother. In pregnant women, plasma PC 22:6n-3 concentration increases by approximately 33% between 16 weeks (170 μmol/l) and 40 weeks (230 μmol/l) gestation [48] which reflects an approximate doubling of the total blood 22:6n-3 pool when the increase in maternal blood volume during pregnancy is taken into account [49]. Circulating oestrogen concentration rises during pregnancy due to synthesis and secretion by the placenta. Thus it is possible that the increasing oestrogen concentration may up-regulate 18:3n-3 conversion during gestation. This is supported by studies in rats that indicate that the increase in plasma PC 22:6n-3 concentration during pregnancy is the result of physiological adaptations to hepatic phospholipid [50] and 18:3n-3 [51] metabolism. If true in women, one implication would be that variations in capacity for 22:6n-3 synthesis from 18:3n-3 may influence the supply of 22:6n-3 from the mother to her foetus.

8. Overall conclusions

The conversion of 18:3n-3 to its longer-chain derivatives, especially 22:6n-3, is limited in humans, although conversion is greater in women than men. The limited extent to which 18:3n-3 is converted to its longer-chain metabolites in men may explain the relative lack of effectiveness of increased consumption of 18:3n-3 in raising the concentration of 22:6n-3 in blood. Whether young women, who possess greater capacity for conversion to 20:5n-3 and 22:6n-3, are more effective at raising the concentration of 22:6n-3 in blood in response to increased 18:3n-3 consumption remains to be determined. Such gender differences in n-3 PUFA metabolism should be considered in dietary recommendations about the type and amount of n-3 PUFA consumed by men and by women.

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