**Is essential fatty acid interconversion an important source of polyunsaturated fatty acids in humans?**

Graham C Burdge

Human Health and Development, Faculty of Medicine, University of Southampton, Southampton, UK.

Correspondence to:- Professor GC Burdge, Human Health and Development, Faculty of Medicine, University of Southampton, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK. Telephone: +44(0)2381205259

email:g.c.burdge@soton.ac.uk

Running title: Polyunsaturated fatty acid biosynthesis

Key words: Desaturase; Elongase; FADS; liver; lymphocyte; fatty acid

*Abstract*

Humans can obtain preformed long chain polyunsaturated fatty acids (PUFA) from the diet, but are also able to convert essential fatty acids (EFAs) to longer chain PUFA. The metabolic pathway responsible for EFA interconversion involves alternating desaturation and carbon chain elongation reactions, and carbon chain shortening by peroxisomal β-oxidation. Studies using stable isotope tracers or diets supplemented with EFAs show that capacity for PUFA synthesis is limited in humans such that docosahexaenoic acid (22:6n-3) synthesis in men is negligible. PUFA synthesis is greater in women of reproductive age than men. However, the magnitude of the contribution of hepatic PUFA synthesis to whole body PUFA status remains unclear. A number of extra-hepatic tissues have been shown to synthesise PUFA or to express genes for enzymes involved in this pathway. The precise function of extra-hepatic PUFA synthesis is largely unknown, although in T lymphocytes PUFA synthesis is involved in the regulation of cell activation and proliferation. Local PUFA synthesis may also be important for spermatogenesis and fertility. One possible role of extra-hepatic PUFA synthesis is that it may provide PUFA in a timely manner to facilitate specific cell functions. If so, this may suggest novel insights into the effect of dietary PUFA and / or polymorphisms in genes involved in PUFA synthesis on health and tissue function.

**Introduction**

Polyunsaturated fatty acids (PUFA) are important components of cell membranes. Variations in the proportions of individual PUFA can alter cell function by modulating the fluidity of the phospholipid bilayer thus influencing the activity of integral membrane proteins(1). PUFA can also act as substrates for cell signalling processes. Changes in the relative proportions of individual PUFA may modify cell function by changing the nature of lipid second messengers, including eicosanoids(2), diacylglycerol (DAG) and phosphatidic acid(3; 4). For example, activated macrophages produce 2-series prostaglandins (PG) from arachidonic acid (20:4n-6) through the activity of cyclooxygenase, but synthesise less biologically potent 3-series PG from eicosapentaenoic acid (20:5n-3)(2). Protein kinase (PK) Cα has been shown to be preferentially activated by 1,2-dipalmitoylDAG (DPD) compared to 1-stearoyl, 2-docosahexaenoylDAG (SDD). However, activation of PKCγ by SDD is greater compared to DPD(5). PUFA can modify cell function at the level of gene transcription by acting as ligands for peroxisome proliferator-activated receptors (PPARs)(6) and recent findings suggest that PUFA can modify epigenetic processes (7). Thus maintenance of the phospholipid composition of cell membranes and, consequently, normal cell activity requires an adequate, timely supply of specific PUFA. These can be obtained preformed from the diet via the blood stream. However, this may be a precarious strategy for maintaining tissue function because dietary choice and temporal variation in intake may limit the capacity of the diet to supply individual PUFA against changing demands; for example during an immune response to infection.

 Mammals can convert the essential fatty acids (EFAs) linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) to longer chain, more unsaturated fatty acids that are required for cell function. The purpose of this review is to assess whether hepatic and extra-hepatic PUFA synthesis are important for meeting demands for PUFA, primarily in humans.

*Hepatic polyunsaturated fatty acid synthesis in mammals other than humans*

Mammalian cell membranes contain C18, C20 and C22 PUFA, which are classified as n-6, n-3 or n-9 depending on the position of the first double bond from the methyl end of the hydrocarbon chain. The monounsaturated fatty acid oleic acid (18:1n-9) can be synthesised *de novo* from non-lipid precursors by the activity of the fatty acid synthase complex followed by insertion of a double bond at Δ9 position by stearoyl-CoA desaturase(8). During dietary EFA deficiency, 18:1n-9 can be converted to mead acid (20:3n-3) by the sequential activities of Δ6 desaturase, elongase 5 and Δ5 desaturase(8) (Figure 1). EFA deficiency and 20:3n-9 synthesis are rare in humans and occur primarily in patients receiving artificial nutrition(9). However, the precise biological role of conversion of 18:1n-9 to 20:3n-9 is not known. One possibility is that 20:3n-9 may, at least in part, compensate for decreased membrane fluidity due to reduced availability of PUFA for incorporation into the phospholipid bilayer. Alternatively, synthesis of 20:3n-9 involves, in part, enzymes that catalyse PUFA synthesis (see below, Figure 1) and so conversion of 18:1n-9 to 20:3n-9 may be an artefact of reduced flux of EFAs through the PUFA synthesis pathway.

 Mammals do not express Δ12 or Δ15 desaturases which catalyse conversion of 18:1n-9 to 18:2n-6 and 18:2n-6 to 18:3n-3, respectively. Consequently mammals are dependent upon consumption of either pre-formed ≥ 20 carbon PUFA in their diet or conversion of 18:2n-6 and 18:3n-3 to longer chain, more unsaturated fatty acids to meet their PUFA requirements. The general pathway for conversion of 18:2n-6 and 18:3n-3 to longer chain, more unsaturated PUFA was elucidated by studies carried out using rodent liver in the 1960s(10; 11) (Figure 1). The first desaturation reaction, catalysed by Δ6 desaturase, is followed by malonyl-CoA -dependent carbon chain elongation by elongase 5, and then Δ5 desaturation to form 20:4n-6 and 20:5n-3 from 18:2n-6 and 18:3n-3, respectively (Figure 1). 20:4n-6 can be converted to 22:4n-6 and 20:5n-3 to 22:5n-3 by addition of two carbons by either elongase 5 or 2 (Figure 1). Although the first reaction catalysed by Δ6 desaturase is generally assumed to be rate limiting, the chain elongation reactions that follow formation of 22:5n-3 and 22:4n-6 have also been proposed as metabolic control points(12). Synthesis of 22:5n-6 and 22:6n-3 was originally proposed to involve Δ4 desaturation(13). However, this view was superseded because of the apparent absence in rat liver microsomes of an enzyme with Δ4 desaturase activity and the demonstration that synthesis of 22:5n-6 and 22:6n-3 involves further chain elongation by elongase 2 to form the intermediates 24:4n-6 and 24:5n-3, followed by Δ6 desaturation and subsequent translocation of the products, 24:5n-6 and 24:6n-3, to peroxisomes. 22:5n-6 and 22:6n-3 are formed by removal of 2 carbons by single cycle of β-oxidation(14; 15; 16). However, Park *et al*. have shown that the MCF7 human breast cancer cell line, which does not express Δ6 desaturase activity, expresses Δ4 desaturase activity when transfected with FADS2 and FADS1, which encode Δ6 and Δ5 desaturases, respectively(17). However, Δ4 desaturase activity remains to be demonstrated in wild type, untransformed primary cells.

*Hepatic polyunsaturated fatty acid biosynthesis in humans*

In marked contrast to rodents, humans have limited capacity for conversion of EFAs to longer chain PUFA(18; 19). Studies using stable isotope tracer technology to assess whole body, in essence hepatic, interconversion of 18:3n-3 in men or mixed groups of men and women have shown consistently that conversion to 20:5n-3 is limited to less than 10% of the administered labelled 18:3n-3, and that only trace amounts of 22:6n-3 are formed (less than 0.05% of ingested 18:3n-3)(18; 19; 20). Moreover, conversion of [13C]18:3n-3 to 22:6n-3 has been shown to be independent of relative intakes of 18:3n-3 and 18:2n-6(20). Thus the contribution of PUFA synthesis to 22:6n-3 formation appears to be negligible, although one study in men estimated conversion of 18:3n-3 to 22:6n-3 to be approximately 4% of the administered dose(21). It is not known why 22:6n-3 synthesis was greater in this study compared to others. Conversion of [13C]18:2n-6 to 20:4n-6 in men has also been shown to be extremely limited (approximately < 0. 2% of ingested labelled fatty acid)(20).

 There are major challenges in interpreting the concentrations of stable isotope-labelled fatty acids in blood in terms of EFA interconversion, which have been reviewed elsewhere(19). However, such estimates are consistent with the effect of increased 18:3n-3 intake (between 4 g and 20 g/day) on the proportions of 20:5n-3, 22:5n-3 and 22:6n-3 in blood and cell lipids in men or in mixed groups of men and post-menopausal women(22; 23). These studies showed that greater 18:3n-3 intake was associated with increased proportions of 20:5n-3 and 22:5n-3. Based on data summarised in Burdge and Calder (2006)(23), 18:3n-3 intake predicted 66% of the variation in the proportion of 20:5n-3 in plasma phospholipids such that doubling 18:3n-3 intake approximately doubled the change in 20:5n-3 status. However, there was no significant increase in the proportion of 22:6n-3 and in 4 of 9 studies 22:6n-3 status decreased during 18:3n-3 supplementation to below the baseline level(23). In addition, dietary supplementation with 18:3n-3, 18:4n-3 or 20:5n-3 increased the proportions of 20:5n-3 and 22:5n-3 in blood, but was accompanied by a statistically non-significant decrease in 22:6n-3 status(24). One possible explanation for the reduction in 22:6n-3 status is that the rate of 22:6n-3 synthesis was less than the rate of turnover.

 Women and females of other species appear to have greater capacity for synthesis and higher 22:6n-3 status than their male counterparts. Conversion of [13C]18:3n-3 to 20:5n-3 in women of reproductive age has been estimated to be 21% (compared to < 10 % in men) and conversion to 22:6n-3, 9% (compared to less than 0.05% in men)(25; 26). This observation is supported by the findings of a systematic review of 51 observational studies that showed the proportions of 20:4n-6 and 22:6n-3 in blood phospholipids were typically 20% higher in women than men(27) and by a recent kinetic modelling study in overweight women(28). Female rodents(29; 30) and great tits (*Parus major*)(31) also have higher 22:6n-3 status than males which suggests this difference in PUFA status and metabolism between sexes may have been conserved during evolution. Oestrogen is an agonist for PUFA synthesis(25; 32) and hence may be responsible for greater PUFA synthesis in females than males. However, studies in postmenopausal women, in primary human hepatocytes and in HepG2 hepatocarcinoma cells suggest that progesterone may also be involved(33; 34).

 Dietary supplementation with 9.5g/ day 18:3n-3 induced a greater increase in the proportion of 20:5n-3 in women (age 53.5 yrs) compared to men (age 50.5 yrs), but there was no difference between sexes in the proportion of 22:6n-3 at the end of the study(35). Furthermore, the change in plasma 20:5n-3 status was associated negatively with age in women, but not men(35). These findings suggest that, at least in women of post-reproductive age, the contribution of PUFA biosynthesis to plasma PUFA status is similar to men. Whether this pathway is more important for meeting the demands for PUFA of younger women has yet to be determined.

*Hepatic polyunsaturated fatty acid biosynthesis in pregnancy*

 Pregnancy involves increased demands on the mother for PUFA, in order to meet both her requirements and those of the fetus. The human fetus accumulates 22:6n-3, beginning in the second trimester, particularly into the central nervous system(13; 36) and deficits in 22:6n-3 incorporation into developing brain have been associated with cognitive deficits in non-human primates(37). The concentration of 22:6n-3, but not 20:4n-6, has been shown to increase in plasma phosphatidylcholine (PC) during the second trimester of pregnancy, specifically an increase in PC16:0/22:6(38), which precedes the period of prenatal 22:6n-3 accumulation into fetal brain(36). One interpretation is that such adaptation of maternal liver lipid metabolism may facilitate adequate supply of 22:6n-3 to the fetal brain(39). Total plasma 22:6n-3 concentration has also been shown to increase between 18 and 29 days post-conception in women undergoing assisted reproduction(40). This change in plasma PC composition has been shown in rats to involve changes to the composition of the maternal hepatic diacylglycerol pool destined for PC synthesis and decreased acyl remodelling of *sn*-1 16:0 to *sn*-1 18:0 PC molecular species(41) and to be accompanied increased liver *Fads1* and *Fads2* mRNA expression(29; 42), possibly by the action of progesterone(42).

 Despite evidence of coordinated changes in maternal hepatic PUFA metabolism, the contribution, if any, of such adaptations to meeting maternal and fetal demands for PUFA is not known. In pregnant women, maternal plasma PC 22:6n-3 concentration tracks during gestation such that the level in early pregnancy (11 – 17 weeks) predicts 21% of the variation in late pregnancy (35 weeks)(43). Therefore, it may be expected that position in the rank order of maternal 22:6n-3 status, which is a proxy for 22:6n-3 availability to the developing fetus, would influence the cognitive function of the child. However, maternal plasma 22:6n-3 concentration during pregnancy has not been associated with IQ or markers of executive cognitive function at ages 4 years or 6 – 7 years(43; 44), cognition at 4 years(45) or 7 years(46) years, nor with problem behaviour at 7 years(47). Moreover, 22:6n-3 concentration has been shown to be approximately 32% lower in umbilical cord blood and 62% lower in breast milk from vegetarian pregnancies compared to omnivores(48) which suggests that metabolic adaptations that increase 22:6n-3 concentration in maternal blood during pregnancy are insufficient to compensate for low intake of 22:6n-3 in vegetarians(49). One possibility is that maternal adaptations to hepatic PUFA metabolism may be involved in the adaptation of the mother’s tissues to pregnancy rather than contributing directly to the PUFA supply and development of the fetus. For example, in the rat increased maternal plasma PC16:0/22:6 concentration occurs in late pregnancy and may facilitate supply of 22:6n-3 for incorporation into milk(41).

*Extra-hepatic polyunsaturated fatty acid biosynthesis*

 Tissues can acquire preformed ≥ C20 PUFA from blood which are derived from the diet or from hepatic synthesis. These PUFA are assimilated primarily by hydrolysis of triacylglycerol and uptake of fatty acids at the surface of the vascular endothelium via the actions of lipoprotein lipase and CD36, or uptake via CD36 from the non-esterified fatty acid pool(50). The sodium-dependent transporter Mfsd2a has also been shown to transport lysoPC containing 22:6n-3 or 18:1n-9 selectively to the brain(51; 52), skin fibroblasts(53), retina(54) and placenta(55). At least some extra-hepatic tissues express key genes involved in PUFA synthesis or have been shown to convert EFAs to longer chain PUFA. One possible explanation for local intracellular PUFA synthesis is that the pool of pre-formed ≥ 20 carbon PUFA in blood is insufficient to meet the demands of specific tissues, possibly in terms of quantity, composition and / or timing, and that obtaining specific PUFA is closely associated with fundamental processes in the function of those cells.

 *FADS2* mRNA expression has been detected in human heart, brain, lung, liver, skeletal muscle, kidney, pancreas and placenta, while *FADS1* was found to be expressed primarily in liver, lung, brain and heart with only trace expression in pancreas, kidney, skeletal muscle and placenta(56). However, the relative expression of *FADS2* to other genes involved in the pathway differs between reports(56; 57). In general, human brain and liver expressed the highest level of *FADS2* mRNA, followed by heart > placenta ≡ lung > kidney > skeletal muscle > spleen, but was essentially undetectable in pancreas(56; 57). *FADS1*, *FADS2*, *ELOVL5* and *ELOVL4* have also been shown to be expressed in peripheral blood mononuclear cells (PBMCs)(58; 59). Moreover, PUFA biosynthesis has been reported in femoral artery(60), vascular smooth muscle cells (VSM)(61), testis(62), umbilical vein(63), leukocytes(59; 64; 65; 66), and a number of neoplastic and non-cancerous mammary epithelial cells lines(67; 68). There is no evidence that extra-hepatic cells secrete newly synthesised PUFA. Thus the expression of genes involved in PUFA synthesis and / or PUFA synthesis in extra-hepatic tissues suggests that capacity for EFA interconversion may serve a different purpose to that in the liver. However, relatively few studies have investigated the role of PUFA synthesis in cell function. Nevertheless, emerging findings discussed below suggest that local PUFA synthesis is required for the normal function of at least some cell types.

*1. Polyunsaturated fatty acid biosynthesis in leukocytes*

Leukocytes can convert 18:3n-3 and 18:2n-6 to longer chain, more unsaturated PUFA, although the extent of such conversion appears to differ between types of immune cells and with the activation state of the cells. Radiolabelled 18:2n-6 can be converted to 20:2n-6 in quiescent murine macrophages without detectable further desaturation or elongation(69). Incubation of murine peritoneal macrophages with either the calcium ionophore A23187, phorbol myristate acetate, or zymosan did not induce further interconversion of 20:2n-6(69). Furthermore, [14C]18:3n-6 can be converted to 20:3n-6, but not to 20:4n-6, in stimulated murine macrophages(66). Absence of detectable 20:4n-6 suggests that murine macrophages lack Δ5 desaturase activity.

In contrast to macrophages, mitogen stimulation increased the proportions of 18:1n-9, 22:5n-3 and 22:6n-3 and decreased the proportion of n-6 PUFA in T cell membranes(70). This suggests remodelling of membrane phospholipids and/ or increased synthesis or uptake of specific fatty acids including longer chain n-3 PUFA. Mitogen stimulation of human peripheral blood mononuclear cells (PBMCs) increased Δ9, Δ6 and Δ5 desaturase activities which was associated with increased interconversion of [14C]18:0 to 18:1n-9, and of [14C]18:2n-6 and [14C]18:3n-3 to triene and tetriene PUFA(71). The authors noted that it was uncertain whether such capacity was sufficient to account for the changes in membrane composition that accompany T cell activation. Moreover, incubation of human PBMCs with 18:2n-6 or 18:3n-3 followed by mitogen stimulation increased the incorporation of these fatty acids into the cells(71). Incubation of mitogen-stimulated rat lymph node lymphocytes with 18:3n-3 increased the proportions of 18:3n-3, 20:5n-3 and 22:5n-3, but not 22:6n-3 in membrane phospholipids(72) which suggests that at least some of the changes in membrane composition in activated T cells may involve endogenous PUFA synthesis, while other, such as increased 22:6n-3 content, may represent selective uptake from their environment. Although incubation with 18:2n-6 increased the proportion of this fatty acid in T cell phospholipids, there was only a small, non-significant increase in the 20:4n-6 content(72). The apparent inconsistency in the conversion of 18:3n-3 and 18:2n-6 was not explained by the authors. These findings suggest that, in contrast to macrophages, human and rat lymphocytes express Δ6 and Δ5 desaturase, and that elongase expression is up-regulated in activated cells. Increased membrane fluidity induced by increasing the proportion of unsaturated fatty acids in membrane phospholipids has been suggested a possible mechanism by which unsaturated fatty acids could inhibit immune cell function by modifying membrane protein activity(72). Moreover, increasing the synthesis of PUFA substrates may facilitate the synthesis of pro-resolving mediators and hence attenuate the immune response(73).

 A recent study of PUFA biosynthesis in human PBMCs confirmed that mitogen stimulation induced increased incorporation of [13C]18:3n-3 into cell lipids and conversion to longer chain, more unsaturated fatty acids(59). This was accompanied by marked up-regulation of *FADS2*, *FADS1*, *ELOVL5* and *ELOVL4* mRNA expression(59). The first two reactions were chain elongation of 18:3n-3 to 20:3n-3 by an unidentified elongase followed by Δ8 desaturation of 20:3n-3 to 20:4n-3, essentially the reverse of the initial reactions of the pathway reported in liver(14; 34) (Figure 2). The first Δ6 desaturation in liver has been shown to be rate limiting(74) and to preferentially catalyse conversion of 18:3n-3 compared to 18:2n-6(74). Thus, one implication of the apparent substitution of the initial Δ6 desaturation with elongase activity is that the regulation of the PUFA synthesis pathway in PBMCs may differ from that of other tissues. For example, elongases have been shown to differ in substrate preference in terms of chain length and level of unsaturation such that elongase 5 catalyses elongation of 18 and 20 carbon PUFA, while elongase 2 exhibits marked preference of 20 and 22 carbon PUFA(12). Both of these enzyme preferentially catalyse conversion of n-3 compared to equivalent n-6 PUFA(12), If so, this may have implications for understanding the influence of dietary fatty acids on immune function. The protein product of the baboon *Fads2* gene has been associated with Δ8 desaturase activity when transfected into yeast cells, although Δ6 desaturase activity predominated(75). Thus it is possible that Δ8 desaturase activity in PBMCs was also associated with the protein encoded by the *FADS2* gene, although no Δ6 desaturase activity was detected(59). Moreover, Δ4-desaturase activity has been demonstrated in MCF7 breast cancer cells transfected with *FADS2* that do not normally express this gene(17). Thus the desaturase activity of the *FADS2* protein product may depend upon the cellular environment in which it is expressed. *ELOVL2* was not expressed in PBMCs and the PUFA synthesis pathway was truncated after synthesis of 22:5n-3(59).

 *ELOVL4* catalyses conversion of ≥ C20 PUFA, but not 22:6n-3, to PUFA with chain length C24 – C38(76; 77; 78; 79) which are involved in the formation and structure of lipid rafts required for T cell signalling(80). Thus it has been suggested that truncation of the PUFA synthesis pathway after 22:5n-3 and up-regulation of ELOVL4 may provide very long chain PUFA required for T cell activation(59). Moreover, a lipoxygenase-derived metabolite of 20:3n-9 can modulate leukotriene B4 synthesis, and hence neutrophil activation, via inhibition of leukotriene A hydrolase(81). It is not known whether 20:3n-3 can also be converted into bioactive metabolites, but demonstration of such activity may suggest an additional mechanism to link Δ8 desaturation to immune function. Of potential importance to understanding the role of PUFA synthesis in stimulated T cells, pharmacological inhibition of the *FADS2* protein reduced mitogen-induced T cell proliferation(59). Thus PUFA synthesis appears to be involved in the regulation of T cell proliferation, possibly by providing substrates for the assembly of lipid microdomains and/or through the formation novel lipid mediators (Figure 2).

*2. Polyunsaturated fatty acid synthesis in cancer cells*

PUFA synthesis has been described in several cancer cells types. One recent study compared PUFA synthesis in Jurkat T cell leukaemia cells with PBMCs(59). The findings showed that interconversion of [13C]18:3n-3 was constitutive and 17-fold greater in Jurkat cells than in mitogen-stimulated PBMCs. *FADS2*, *FADS1*, *ELOVL4*, and *ELOVL5* expression was also up to 17-fold greater in Jurkat cells than in stimulated PBMCs and *ELOVL2* was expressed which is consistent with conversion of 18:3n-3 to 22:6 in Jurkat cells. These findings suggest that oncogenic transformation may disrupt PUFA synthesis at the level of transcription at several points in the pathway. The major product of PUFA synthesis in Jurkat cells was 22:5n-3 rather than 20:3n-3, which was the major fatty acid synthesised from 18:3n-3 in PBMCs. Jurkat cells expressed both Δ8 and Δ6 desaturase activities, while PBMCs only expressed Δ8 desaturase activity. Furthermore, inhibition of the *FADS2* protein did not significantly alter Jurkat cell proliferation(59). Stimulation of PBMCs induced hypermethylation of a 600bp region of the *FADS2* promoter. In contrast, this putative regulatory region was hypomethylated in Jurkat cells(59). Together, these findings suggest that PUFA synthesis and its role in regulating lymphocyte cell proliferation is disrupted in Jurkat cells.

 Δ6 desaturase activity and *FADS2* mRNA expression were shown to be substantially greater in murine xenografts of B16 melanoma and Lewis lung carcinoma (LLC) cells compared to normal tissue(82). *FADS2* RNAi knockdown inhibited proliferation and reduced tumour size in both B16 and LLC tumours(82) which suggests that PUFA synthesis may be involved in the regulation of mitosis in these cells. The ratio of the sum of n-6 PUFA to 18:2n-6 in primary breast cancer tissue, a proxy marker of PUFA synthesis, was greater than in uninvolved tissue(83), and higher in more aggressive estrogen receptor negative (ER-) tumours compared to ER+ tumours(83).

 The absence of *FADS2* expression in MCF7 breast carcinoma cells results in synthesis by Δ5 desaturase of 5,11,14-20:3 and 5,11, 14, 17-20:4 which lack the 8-9 double bond of their eicosanoid substrate counterparts 20:4n-6 and 20:5n-3(84). This suggests a mechanism by which deletion of *FADS2* during malignant transformation may lead to disruption of cellular signalling and the production of novel second messengers(84). Furthermore, activation of the c-Ha-ras oncogene, but not the c-Ha-ras pro-oncogene, in spontaneously-immortalised MCF10A mammary epithelial cells resulted in loss of capacity for Δ6 and Δ4 desaturation(85).

 Together these studies suggest that altered regulation of PUFA synthesis is a characteristic of at least some types of cancer which may be linked to their proliferative capacity. This is consistent with the findings of studies that show reduction in proliferation and/or induction of apoptosis of cancer cells treated with 22:6n-3(86; 87; 88; 89).

*3. Polyunsaturated fatty acid synthesis in spermatogenesis*

Human sperm is highly enriched in 22:6n-3. Morphologically normal sperm cells contain approximately 35% 22:6n-3, while the proportion of 20:4n-6 is approximately 10.5%(90). The presence of 22:6n-3 in sperm membrane phospholipids has been associated with cell motility, membrane fusion and the synthesis of second messengers, and is consistent with the presence of high levels of antioxidant defence mechanisms(90). Experimental EFA deficiency in rats can induce degeneration of seminiferous tubules, and reduced sperm formation and fertility(91). Moreover, feeding male *Fads2* null mice a diet containing 18:3n-3 and 18:2n-6, without longer chain PUFA, induced 74% reduction in the proportion of 22:6n-3 and 92% decrease in the relative amount of 20:4n-6 (92%) in testis(92). This was accompanied by arrested spermatogenesis and degradation of seminiferous tissue(92). Dietary supplementation of *Fads2* null mice with 22:6n-3 restored spermatogenesis and fertility to levels equivalent to wild type mice(92). This suggests that PUFA synthesis is important for fertility in mice fed a diet without longer chain PUFA, However, because the knockout was not tissue specific it is not possible to conclude whether the activity of the PUFA synthesis pathway in testis is involved. However, there is some evidence for local PUFA synthesis in testis. Microsomes from human testis can convert [14C]18:2n-6 to 20:3n-6 (88%) and 20:4n-6 (12%), but not 18:3n-6(62) indicating that the initial reactions were elongation of 18:2n-6 to 20:2n-6, followed by Δ8 desaturation to form 20:3n-6 followed by Δ5 desaturation to 20:4n-6. This pathway has also been reported in rat testis(93). Moreover, the proportion of 22:6n-3 in testis from humans and from Rhesus macaques increases during puberty and remains essentially unchanged during adulthood(94; 95). One possible mechanism is that testicular 22:6n-3 synthesis increases during puberty in order to meet requirements for 22:6n-3 during spermatogenesis. If so, this suggests that regulation of PUFA synthesis in testis differs from hepatocytes in which testosterone has no significant effect on 22:6n-3 synthesis(35). However, the contribution of testicular PUFA synthesis to male fertility has yet to be determined.

*4. Polyunsaturated fatty acid synthesis in arteries*

Bovine aortic endothelial cells have been shown to retroconvert 22:6n-3 to 22:5n-3 and 20:5n-3(96), and 22:4n-6 to 20:4n-6(97). Elongation of [14C]20:5n-3 to 22:5n-3 and of [14C]20:4n-6 to 22:4n-6 has also been shown in human vascular endothelial cells(63), although the role of PUFA synthesis in endothelial cell function has not been reported. Phenylephrine (Pe) stimulation of isolated rat aortae increased *Fads1* and *Fads2* mRNA expression compared to unstimulated vessels(60). Moreover, treatment of isolated rat aortae or human femoral artery with the *FADS2* protein inhibitor SC26196 or rat aortae with the Δ5 desaturase inhibitor sesamin reduced Pe-induced vasoconstriction and secretion by rat aortae of pro-constriction eicosanoids PGF2α, PGE2 and thromboxane A2(60). Thus PUFA synthesis, which was localised to vascular smooth muscle (VSM) rather than the endothelium(60), appears to be involved in regulating vasoconstriction. Mouse aortae, immortalised murine VSM (MOVAS) cells and human primary aortic VSM cells express *Fads1*, *Fads2* and *Elovl5*, but not *Elovl2*(61). This is consistent with the apparent absence of conversion of 22:4n-6 to 22:5n-6, in MOVAS cells(61). Since *Elovl2* was also not expressed in PBMCs, it is possible that suppression of *Elovl2* transcription is a characteristic of at least some excitable tissues although the precise function may differ. The absence of *Elovl2* expression did not appear to involve hypermethylation of the promoter(61). Inhibition of Δ6 or Δ5 desaturase significantly reduced Pe-mediated calcium release and secretion of PGE2 and PGF2α in MOVAS cells(57). The findings suggest that α1-adrenergic receptor-mediated vasoconstriction involves activation of PUFA synthesis acting via regulation of endogenous Ca2+ release and secretion of specific vaso-active eicosanoids. One possible mechanism that has been suggested to explain the link between PUFA synthesis and vasoconstriction is activation of PKCζ and inhibition of myosin light chain phosphorylase by newly synthesised 20:4n-6(61).

*Polymorphisms in genes involved in polyunsaturated fatty acid biosynthesis*

The region of human chromosome 11 that contains the *FADS1* and *FADS2* genes in a head to head orientation (11q12.2 – q13.1), together with FADS3(98), contains 4391 variants, of which 217 can result in amino acid changes(99) and hence potentially alter enzyme activity. This region has been identified as a cancer ‘hotspot locus’(84; 100). Several genome-wide association studies have reported associations between polymorphisms in genes associated with PUFA synthesis and the concentrations of PUFA in blood. A meta-analysis of 8,866 individuals from five cohorts showed that minor alleles of SNPs in *FADS2* and *FADS1* were associated with a higher level of 18:3n-3 and a lower level of 20:5n-3, 22:5n-3 and 22:6n-3, while minor alleles of *ELOVL2* were associated with higher proportions of 20:5n-3 and 22:5n-3 and a lower proportion of 22:6-3(101). The variation in the proportions of n-6 PUFA in plasma phospholipids explained by 11 locus haplotypes has been shown to be 18:2n-6, 9.2%; 18:3n-6, 7.9%; 20:2n-6, 12.3%; 20:3n-6, 10.8% and 20:4n-6, 28.5%(97). In contrast, there were only limited associations with n-3 PUFA (18:3n-3, 5.4%; 20:5n-3, 6.9%; 22:5n-3, 5.1%; 22:6n-3, 2.9%)(98). However, whether these polymorphisms alter tissue function is not known. Moreover, interpretation of such associations may be confounded by contributions of hepatic phospholipid biosynthesis by the CDP-choline and phosphatidylethanolamine (PE) *N*-methylation pathways to the fatty acid composition of plasma phospholipids because human(102) and rat(41) liver selectively synthesise 20:4n-6 and 22:6n-3 -containing PC by PE *N*-methylation. One possible explanation for the greater effect of *FADS* polymorphisms on the proportions of n-6 PUFA for n-3 PUFA is the typically greater intake of 18:2n-6 than 18:3n-3(103) and consequently greater flux of n-6 PUFA through the PUFA synthesis pathway than 18:3n-3. Women carrying *FADS1* and *FADS2* minor alleles had lower proportions of 20:4n-6 and 22:5n-3 in their plasma and in breast milk, although the effect on the development of their infants and children was not assessed(104). Thus mutations in genes involved in PUFA synthesis are associated with variations in plasma PUFA status which suggests that hepatic PUFA synthesis can contribute to the levels of circulating PUFA. However, these findings have not been used to estimate the magnitude of this contribution. Carriers of the minor allele (deletion) of a 22 bp *FADS2* insertion-deletion mutation had lower expression of *FADS1* in lymphocytes(105) and lower 20:4n-6 status compared to the major insertion allele(106). The frequency of the insertion/insertion allele was greater in populations with a tradition of vegetarian diet compared to western omnivorous populations who had a higher frequency of the deletion/deletion genotype. This has been suggested as a possible adaption to low intakes of preformed longer chain PUFA(106).

 To date, the effect of polymorphisms in genes involved in PUFA synthesis on tissue function have not been investigated directly, although such studies may provide novel insights into tissue function and disease aetiology. However, there is some evidence that individuals carrying minor *FADS1* - *FADS2* alleles that were associated with lower 20:4n-6 status have a lower prevalence of allergic disease which may be attributable to a reduction in the availability of 20:4n-6 for the synthesis of pro-inflammatory eicosanoids(98). However, since PUFA synthesis can regulate T cell activation and proliferation(59) it is also possible that reduced desaturase activity may constrain the activity of the PUFA synthesis pathway thus limiting cell proliferation and the immune response.

*Conclusions*

Humans have limited capacity for hepatic conversion of EFAs to longer chain, more unsaturated fatty acids(18; 19). Current knowledge about the contribution of EFA interconversion to meeting demands for PUFA in humans is based primarily on the results of whole body tracer studies that are difficult to interpret in terms of net contribution of PUFA status and on dietary intervention trials that use amounts of EFAs that exceed those found in typical western omnivorous diets(18; 19). The relatively few studies that report PUFA status in individuals consuming vegetarian diets that exclude preformed ≥ C20 PUFA suggest that hepatic PUFA synthesis is unable to supply sufficient longer chain PUFA to maintain status equivalent to that achieved by consuming an omnivorous diet(49). However, whether the levels of PUFA found in omnivores are optimal or whether they exceed requirements for maintaining health is uncertain. It is possible that humans can function adequately with PUFA status found in vegetarians, which primarily reflects PUFA synthesis from EFAs, as may be implied by the health benefits of such diets(49). If so, it may be appropriate to question whether recommendations for intake of omega-3 PUFA are higher than needed for optimal tissue function as opposed to pharmacological intakes that may prevent specific lifestyle-associated diseases. Overall, the contribution of PUFA synthesis to meeting the demands for ≥ C20 PUFA of individuals is uncertain and requires further consideration.

 A number of extra-hepatic tissues express genes involved in PUFA synthesis or have been shown to be capable of converting EFAs to longer chain PUFA. The details of such processes differ between cell types that suggest extra-hepatic PUFA synthesis is involved in and tailored to a particular cell phenotype. However, there are important gaps in knowledge regarding the precise role of PUFA synthesis in each cell type. Nevertheless, the findings of experiments that have attempted to address this problem suggest PUFA synthesis can be involved in key regulatory processes such as cell proliferation and that oncogenic transformation may result in disruption of the link between PUFA synthesis and cell function.

 In conclusion, the biological importance of extra-hepatic PUFA biosynthesis remains unclear and there are major gaps in knowledge about PUFA synthesis in humans. Moreover, it may be that the primary role of PUFA biosynthesis within specific tissues lies in local intracellular provision of specific PUFA in a timely manner to meet the demands of specific cell functions.

**Acknowledgements**

Thank you to Dr Elizabeth Miles for her helpful comments and suggestions during the preparation of this manuscript.

**Financial support**

None

**Declarations**

GCB has received research funding from Nestle, Abbott Nutrition and Danone. He is a member of the Scientific Advisory Board of BASF and a member of the BASF Asia-Pacific Grant Award Panel. At the time the manuscript was submitted, we was Editor-in-Chief of the British Journal of Nutrition, but was excluded completely from the peer review process. GCB declares no conflicts of interest with the content of this article.

**References**

1. Calder PC, Yaqoob P, Harvey DJ *et al.* (1994) Incorporation of fatty acids by concanavalin A-stimulated lymphocytes and the effect on fatty acid composition and membrane fluidity. *Biochem J*  **300 ( Pt 2)**, 509-518.

2. Wada M, DeLong CJ, Hong YH *et al.* (2007) Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products. *J Biol Chem* **282**, 22254-22266.

3. Heung YM, Postle AD (1995) The molecular selectivity of phospholipase D in HL60 granulocytes. *FEBS Lett* **364**, 250-254.

4. Heung YM, Postle AD (1995) Substrate selectivity of phospholipase D in HL60 granulocytes: effects of fatty acid supplementation. *Biochem Soc Trans* **23**, 276S.

5. Kamiya Y, Mizuno S, Komenoi S *et al.* (2016) Activation of conventional and novel protein kinase C isozymes by different diacylglycerol molecular species. *Biochem Biophys Rep* **7**, 361-366.

6. Forman BM, Chen J, Evans RM (1997) Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci U S A* **94**, 4312-4317.

7. Burdge GC, Lillycrop KA (2014) Fatty acids and epigenetics. *Curr Opin Clin Nutr Metab Care* **17**, 156-161.

8. Ichi I, Kono N, Arita Y *et al.* (2014) Identification of genes and pathways involved in the synthesis of Mead acid (20:3n-9), an indicator of essential fatty acid deficiency. *Biochim Biophys Acta* **1841**, 204-213.

9. Barr LH, Dunn GD, Brennan MF (1981) Essential fatty acid deficiency during total parenteral nutrition. *Ann Surg* **193**, 304-311.

10. Klenk E, Mohrhauer H (1960) Studies on the metabolism of polyenoic fatty acids in the rat. *Hoppe Seylers Z Physiol Chem* **320**, 218-232.

11. Mead JF (1968) The metabolism of the polyunsaturated fatty acids. *Prog Chem Fats and other Lipids* **9**, 161-192.

12. Gregory MK, Gibson RA, Cook-Johnson RJ *et al.* (2011) Elongase reactions as control points in long-chain polyunsaturated fatty acid synthesis. *PLoS One* **6**, e29662.

13. Innis SM (1991) Essential fatty acids in growth and development. *Prog lipid Res* **30**, 39-103.

14. Voss AC, Sprecher H (1988) Regulation of the metabolism of linoleic acid to arachidonic acid in rat hepatocytes. *Lipids* **23**, 660-665.

15. Voss AC, Sprecher H (1988) Metabolism of 6,9,12-octadecatrienoic acid and 6,9,12,15-octadecatetraenoic acid by rat hepatocytes. *Biochim Biophys Acta* **958**, 153-162.

16. Voss A, Reinhart M, Sankarappa S *et al.* (1991) The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *J Biol Chem* **266**, 19995-20000.

17. Park HG, Park WJ, Kothapalli KS *et al.* (2015) The fatty acid desaturase 2 (FADS2) gene product catalyzes Delta4 desaturation to yield n-3 docosahexaenoic acid and n-6 docosapentaenoic acid in human cells. *FASEB J* **29**, 3911-3919.

18. Plourde M, Cunnane SC (2007) Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Appl Physiol Nutr Metabol* **32**, 619-634.

19. Burdge G (2004) Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care* **7**, 137-144.

20. Hussein N, Ah-Sing E, Wilkinson P *et al.* (2005) Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. *J Lipid Res* **46**, 269-280.

21. Emken EA, Adlof RO, Gulley RM (1994) Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochim Biophys Acta* **1213**, 277-288.

22. Baker EJ, Miles EA, Burdge GC *et al.* (2016) Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog Lipid Res* **64**, 30-56.

23. Burdge GC, Calder PC (2006) Dietary alpha-linolenic acid and health-related outcomes: a metabolic perspective. *Nutr Res Rev* **19**, 26-52.

24. James MJ, Ursin VM, Cleland LG (2003) Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. *Am J Clin Nutr* **77**, 1140-1145.

25. Burdge GC, Wootton SA (2002) Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* **88**, 411-420.

26. Burdge GC, Jones AE, Wootton SA (2002) Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr* **88**, 355-363.

27. Lohner S, Fekete K, Marosvolgyi T *et al.* (2013) Gender differences in the long-chain polyunsaturated fatty acid status: systematic review of 51 publications. *Ann Nutr Metab* **62**, 98-112.

28. Lin YH, Hibbeln JR, Domenichiello AF et al. (2018) Quantitation of Human Whole-Body Synthesis-Secretion Rates of Docosahexaenoic Acid and Eicosapentaenoate Acid from Circulating Unesterified α-Linolenic Acid at Steady State. *Lipids* **53**, 547-558.

29. Burdge GC, Slater-Jefferies JL, Grant RA *et al.* (2008) Sex, but not maternal protein or folic acid intake, determines the fatty acid composition of hepatic phospholipids, but not of triacylglycerol, in adult rats. *Prostaglandins Leukot Essent Fatty Acids*  **78**, 73-79.

30. Childs CE, Romeu-Nadal M, Burdge GC *et al.* (2010) The polyunsaturated fatty acid composition of hepatic and plasma lipids differ by both sex and dietary fat intake in rats. *J Nutr* **140**, 245-250.

31. Isaksson C, Hanson, M.A., Burdge, G.C. (2014) The effects of spatial and temporal ecological variation on fatty acid compositions of wild great tits Parus major. *J Avian Biol* **46**, 245-253.

32. Giltay EJ, Gooren LJ, Toorians AW *et al.* (2004) Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr* **80**, 1167-1174.

33. Giltay EJ, Duschek EJ, Katan MB *et al.* (2004) Raloxifene and hormone replacement therapy increase arachidonic acid and docosahexaenoic acid levels in postmenopausal women. *J Endocrinol* **182**, 399-408.

34. Sibbons CM, Brenna JT, Lawrence P *et al.* (2014) Effect of sex hormones on n-3 polyunsaturated fatty acid biosynthesis in HepG2 cells and in human primary hepatocytes. *Prostaglandins Leukot Essent Fatty Acids* **90**, 47-54.

35. Childs CE, Kew S, Finnegan YE *et al.* (2014) Increased dietary alpha-linolenic acid has sex-specific effects upon eicosapentaenoic acid status in humans: re-examination of data from a randomised, placebo-controlled, parallel study. *Nutr J* **13**, 113.

36. Kuipers RS, Luxwolda MF, Offringa PJ *et al.* (2012) Fetal intrauterine whole body linoleic, arachidonic and docosahexaenoic acid contents and accretion rates. *Prostaglandins Leukot Essent Fatty Acids* **86**, 13-20.

37. Reisbick S, Neuringer M, Hasnain R *et al.* (1994) Home cage behavior of rhesus monkeys with long-term deficiency of omega-3 fatty acids. *Physiol Behav* **55**, 231-239.

38. Postle AD, Al MD, Burdge GC *et al.* (1995) The composition of individual molecular species of plasma phosphatidylcholine in human pregnancy. *Early Hum Dev* **43**, 47-58.

39. Postle AD, Burdge GC, Al MD (1994) Molecular species composition of plasma phosphatidylcholine in human pregnancy. *World Rev Nutr Diet* **75**, 109-111.

40. Meyer BJ, Onyiaodike CC, Brown EA *et al.* (2016) Maternal Plasma DHA Levels Increase Prior to 29 Days Post-LH Surge in Women Undergoing Frozen Embryo Transfer: A Prospective, Observational Study of Human Pregnancy. *J Clin Endocrinol Metabo* **101**, 1745-1753.

41. Burdge GC, Hunt AN, Postle AD (1994) Mechanisms of hepatic phosphatidylcholine synthesis in adult rat: effects of pregnancy. *Biochem J* **303 ( Pt 3)**, 941-947.

42. Childs CE, Hoile SP, Burdge GC *et al.* (2012) Changes in rat n-3 and n-6 fatty acid composition during pregnancy are associated with progesterone concentrations and hepatic FADS2 expression. *Prostaglandins Leukot Essent Fatty Acids*  **86**, 141-147.

43. Crozier SR, Sibbons CM, Fisk HL *et al.* (2018) Arachidonic acid and DHA status in pregnant women is not associated with cognitive performance of their children at 4 or 6-7 years. *Br J Nutr* **119**, 1400-1407.

44. Brouwer-Brolsma EM, van de Rest O, Godschalk R *et al.* (2017) Associations between maternal long-chain polyunsaturated fatty acid concentrations and child cognition at 7 years of age: The MEFAB birth cohort. *Prostaglandins Leukot Essent Fatty Acids* **126**, 92-97.

45. Ghys A, Bakker E, Hornstra G *et al.* (2002) Red blood cell and plasma phospholipid arachidonic and docosahexaenoic acid levels at birth and cognitive development at 4 years of age. *Early Human Dev* **69**, 83-90.

46. Bakker EC, Ghys AJ, Kester AD *et al.* (2003) Long-chain polyunsaturated fatty acids at birth and cognitive function at 7 y of age. *Eur J Clin Nutr* **57**, 89-95.

47. Krabbendam L, Bakker E, Hornstra G *et al.* (2007) Relationship between DHA status at birth and child problem behaviour at 7 years of age. *Prostaglandins Leukot Essent Fatty Acids* **76**, 29-34.

48. Sanders TA, Reddy S (1992) The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. *J Pediatr* **120**, S71-77.

49. Burdge GC, Tan SY, Henry CJ (2017) Long-chain n-3 PUFA in vegetarian women: a metabolic perspective. *J Nutr Sci* **6**, e58.

50. Goldberg IJ, Eckel RH, Abumrad NA (2009) Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways. *J Lipid Res* **50 Suppl**, S86-90.

51. Guemez-Gamboa A, Nguyen LN, Yang H *et al.* (2015) Inactivating mutations in MFSD2A, required for omega-3 fatty acid transport in brain, cause a lethal microcephaly syndrome. *Nat Genet* **47**, 809-813.

52. Nguyen LN, Ma D, Shui G *et al.* (2014) Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature* **509**, 503-506.

53. Moore S A, Hurt E, Yoder E, Sprecher H, Spector A A(1995). Docosahexaenoic acid synthesis in human skin fibroblasts involves peroxisomal retroconversion of tetracosahexaenoic acid. *J Lipid Res* **36**, 2433-2443.

54. Wong BH, Chan JP, Cazenave-Gassiot A *et al.* (2016) Mfsd2a Is a Transporter for the Essential omega-3 Fatty Acid Docosahexaenoic Acid (DHA) in Eye and Is Important for Photoreceptor Cell Development. *J Biol Chem* **291**, 10501-10514.

55. Prieto-Sanchez MT, Ruiz-Palacios M, Blanco-Carnero JE *et al.* (2017) Placental MFSD2a transporter is related to decreased DHA in cord blood of women with treated gestational diabetes. *Clinl Nutr* **36**, 513-521.

56. Cho HP, Nakamura M, Clarke SD (1999) Cloning, expression, and fatty acid regulation of the human delta-5 desaturase. *J Biol Chem* **274**, 37335-37339.

57. Cho HP, Nakamura MT, Clarke SD (1999) Cloning, expression, and nutritional regulation of the mammalian Delta-6 desaturase. *J Biol Chem* **274**, 471-477.

58. Chisaguano AM, Montes R, Perez-Berezo T *et al.* (2013) Gene expression of desaturase (FADS1 and FADS2) and Elongase (ELOVL5) enzymes in peripheral blood: association with polyunsaturated fatty acid levels and atopic eczema in 4-year-old children. *PLoS One* **8**, e78245.

59. Sibbons CM, Irvine NA, Pérez-Mojica JE *et al.* (2018) Polyunsaturated Fatty Acid Biosynthesis Involving Δ8 Desaturation and Differential DNA Methylation of FADS2 Regulates Proliferation of Human Peripheral Blood Mononuclear Cells. *Front Immunol* **9**, 432.

60. Kelsall CJ, Hoile SP, Irvine NA *et al.* (2012) Vascular dysfunction induced in offspring by maternal dietary fat involves altered arterial polyunsaturated Fatty Acid biosynthesis. *PLoS One* **7**, e34492.

61. Irvine NA, Lillycrop KA, Fielding B *et al.* (2015) Polyunsaturated fatty acid biosynthesis is involved in phenylephrine-mediated calcium release in vascular smooth muscle cells. *Prostaglandins Leukot Essent Fatty Acids* **101**, 31-39.

62. Albert DH, Rhamy RK, Coniglio JG (1979) Desaturation of eicosa-11,14-dienoic acid in human testes. *Lipids* **14**, 498-500.

63. Rosenthal MD, Garcia MC, Jones MR *et al.* (1991) Retroconversion and delta 4 desaturation of docosatetraenoate (22:4(n-6)) and docosapentaenoate (22:5(n-3)) by human cells in culture. *Biochim Biophys Acta* **1083**, 29-36.

64. Chapkin RS, Miller CC, Somers SD *et al.* (1988) Utilization of dihomo-gamma-linolenic acid (8,11,14-eicosatrienoic acid) by murine peritoneal macrophages. *BiochimBiophysActa* **959**, 322-331.

65. Chapkin RS, Miller CC (1990) Chain elongation of eicosapentaenoic acid in the macrophage. *Biochim Biophys Acta* **1042**, 265-267.

66. Chapkin RS, Coble KJ (1991) Utilization of gammalinolenic acid by mouse peritoneal macrophages. *Biochim Biophys Acta* **1085**, 365-370.

67. Grammatikos SI, Subbaiah PV, Victor TA *et al.* (1994) Diversity in the ability of cultured cells to elongate and desaturate essential (n-6 and n-3) fatty acids. *Ann New York Acad Sci* **745**, 92-105.

68. Grammatikos SI, Subbaiah PV, Victor TA *et al.* (1994) n-3 and n-6 fatty acid processing and growth effects in neoplastic and non-cancerous human mammary epithelial cell lines. *Br J Cancer* **70**, 219-227.

69. Chapkin RS, Somers SD, Erickson KL (1988) Inability of murine peritoneal macrophages to convert linoleic acid into arachidonic acid. Evidence of chain elongation. *J Immunol* **140**, 2350-2355.

70. Anel A, Naval J, Gonzalez B *et al.* (1990) Fatty acid metabolism in human lymphocytes. I. Time-course changes in fatty acid composition and membrane fluidity during blastic transformation of peripheral blood lymphocytes. *Biochim Biophys Acta* **1044**, 323-331.

71. Anel A, Naval J, Gonzalez B *et al.* (1990) Fatty acid metabolism in human lymphocytes. II. Activation of fatty acid desaturase-elongase systems during blastic transformation. *Biochim Biophys Acta* **1044**, 332-339.

72. Calder PC, Yaqoob P, Harvey DJ *et al.* (1994) Incorporation of fatty acids by concanavalin A-stimulated lymphocytes and the effect on fatty acid composition and membrane fluidity. *Biochem J* **300 ( Pt 2)**, 509-518.

73. Halade GV, Black LM, Verma MK (2018) Paradigm shift - Metabolic transformation of docosahexaenoic and eicosapentaenoic acids to bioactives exemplify the promise of fatty acid drug discovery. *Biotechnol Adv* **36**, 935-953.

74. Rodriguez A, Sarda P, Nessmann C *et al.* (1998) Delta6- and delta5-desaturase activities in the human fetal liver: kinetic aspects. *J Lipid Res* **39**, 1825-1832.

75. Park WJ, Kothapalli KS, Lawrence P *et al.* (2009) An alternate pathway to long-chain polyunsaturates: the FADS2 gene product Delta8-desaturates 20:2n-6 and 20:3n-3. *J Lipid Res* **50**, 1195-1202.

76. Agbaga MP, Brush RS, Mandal MN *et al.* (2008) Role of Stargardt-3 macular dystrophy protein (ELOVL4) in the biosynthesis of very long chain fatty acids. *Proc Natl Acad Sci USA* **105**, 12843-12848.

77. Mandal MN, Ambasudhan R, Wong PW *et al.* (2004) Characterization of mouse orthologue of ELOVL4: genomic organization and spatial and temporal expression. *Genomics* **83**, 626-635.

78. Vasireddy V, Uchida Y, Salem N *et al.* (2007) Loss of functional ELOVL4 depletes very long-chain fatty acids (>= C28) and the unique omega-O-acylceramides in skin leading to neonatal death. *Human Mol Gen* **16**, 471-482.

79. Suh M, Clandinin MT (2005) 20:5n-3 but not 22:6n-3 is a preferred substrate for synthesis of n-3 very-long- chain fatty acids (C24-C36) in retina. *Curr Eye Res* **30**, 959-968.

80. Iwabuchi K, Nakayama H, Iwahara C *et al.* (2010) Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction. *FEBS Lett* **584**, 1642-1652.

81. Cleland LG, James MJ, Proudman SM *et al.* (1994) Inhibition of human neutrophil leukotriene B4 synthesis in essential fatty acid deficiency: role of leukotriene A hydrolase. *Lipids* **29**, 151-155.

82. He C, Qu X, Wan J *et al.* (2012) Inhibiting delta-6 desaturase activity suppresses tumor growth in mice. *PLoS One* **7**, e47567.

83. Pender-Cudlip MC, Krag KJ, Martini D *et al.* (2013) Delta-6-desaturase activity and arachidonic acid synthesis are increased in human breast cancer tissue. *Cancer Sci* **104**, 760-764.

84. Park WJ, Kothapalli KS, Lawrence P *et al.* (2011) FADS2 function loss at the cancer hotspot 11q13 locus diverts lipid signaling precursor synthesis to unusual eicosanoid fatty acids. *PLoS One* **6**, e28186.

85. Grammatikos S, Harvey M, Subbaiah P *et al.* (1995) Loss of Fatty-Acid delta(6) desaturating ability in human mammary epithelial-cells that express an activated C-ha-ras oncogene. *Int J Oncol* **6**, 1039-1046.

86. Liu J, Ma DW (2014) The role of n-3 polyunsaturated fatty acids in the prevention and treatment of breast cancer. *Nutrients* **6**, 5184-5223.

87. Skender B, Vaculova AH, Hofmanova J (2012) Docosahexaenoic fatty acid (DHA) in the regulation of colon cell growth and cell death: a review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **156**, 186-199.

88. Yousefnia S, Momenzadeh S, Seyed Forootan F *et al.* (2018) The influence of peroxisome proliferator-activated receptor gamma (PPARgamma) ligands on cancer cell tumorigenicity. *Gene* **649**, 14-22.

89. Burdge GC, Rodway H, Kohler JA *et al.* (2000) Effect of fatty acid supplementation on growth and differentiation of human IMR-32 neuroblastoma cells in vitro. *J Cell Biochem* **80**, 266-273.

90. Lenzi A, Picardo M, Gandini L *et al.* (1996) Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy. *Human Reprod Update* **2**, 246-256.

91. Leath WMF, Northop,C.A. Harrison, F.A.; Cox, R.W. (1983) Effect of linoleic acid and linolenic acid on testicular developments in the rat. *Q J Exp Physiol* **68**, 221-231.

92. Roqueta-Rivera M, Stroud CK, Haschek WM *et al.* (2010) Docosahexaenoic acid supplementation fully restores fertility and spermatogenesis in male delta-6 desaturase-null mice. *J Lipid Res* **51**, 360-367.

93. Albert DH, Coniglio JG (1977) Metabolism of eicosa-11,14-dienoic acid in rat testes. Evidence for delta8-desaturase activity. *Biochim Biophys Acta* **489**, 390-396.

94. Coniglio JG, Grogan WM, Jr., Rhamy RK (1975) Lipid and fatty acid composition of human testes removed at autopsy. *Biol Reprod* **12**, 255-259.

95. Connor WE, Lin DS, Neuringer M (1997) Biochemical markers for puberty in the monkey testis: desmosterol and docosahexaenoic acid. *J Clinl Endocrinol Metabol* **82**, 1911-1916.

96. Hadjiagapiou C, Spector AA (1987) Docosahexaenoic acid metabolism and effect on prostacyclin production in endothelial cells. *Arch Biochem Biophys* **253**, 1-12.

97. Mann CJ, Kaduce TL, Figard PH *et al.* (1986) Docosatetraenoic acid in endothelial cells: formation, retroconversion to arachidonic acid, and effect on prostacyclin production. *Arch Biochem Biophys* **244**, 813-823.

98. Schaeffer L, Gohlke H, Muller M *et al.* (2006) Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Human Mol Gens* **15**, 1745-1756.

99. Minihane AM (2016) Impact of Genotype on EPA and DHA Status and Responsiveness to Increased Intakes. *Nutrients* **8**, 123.

100. Marquardt A, Stohr H, White K *et al.* (2000) cDNA cloning, genomic structure, and chromosomal localization of three members of the human fatty acid desaturase family. *Genomics* **66**, 175-183.

101. Lemaitre RN, Tanaka T, Tang W *et al.* (2011) Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genetics* **7**, e1002193.

102. Pynn CJ, Henderson NG, Clark H *et al.* (2011) Specificity and rate of human and mouse liver and plasma phosphatidylcholine synthesis analyzed in vivo. *J Lipid Res* **52**, 399-407.

103. Burdge GC, Calder PC (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* **45**, 581-597.

104. Xie L, Innis SM (2008) Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. *J Nutr* **138**, 2222-2228.

105. Reardon HT, Zhang J, Kothapalli KS *et al.* (2012) Insertion-deletions in a FADS2 intron 1 conserved regulatory locus control expression of fatty acid desaturases 1 and 2 and modulate response to simvastatin. *Prostaglandins Leukot Essent Fatty Acids* **87**, 25-33.

106. Kothapalli KS, Ye K, Gadgil MS *et al.* (2016) Positive Selection on a Regulatory Insertion-Deletion Polymorphism in FADS2 Influences Apparent Endogenous Synthesis of Arachidonic Acid. *Mol Biol Evol* **33**, 1726-1739.

Figure 1. The pathway for synthesis of longer chain n-9 fatty acids overlaps in part with metabolic reactions involved in n-3 and n-6 polyunsaturated fatty acid biosynthesis. FAS, fatty acid synthase.

Figure 2. The proposed pathway for polyunsaturated fatty acid biosynthesis linked to proliferation of T lymphocytes(57). Dashed arrows indicate putative reactions.

