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REVIEW

Elevated Production of Docosahexaenoic Acid in Females: Potential Molecular Mechanisms

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Abstract Observational evidence suggests that in populations consuming low levels of n-3 highly unsaturated fatty acids, women have higher blood levels of docosahexaenoic acid (DHA; 22:3n-6) as compared with men. Increased conversion of alpha-linolenic acid (ALA; 18:3n-3) to DHA by females has been confirmed in fatty acid stable isotope studies. This difference in conversion appears to be associated with estrogen and some evidence indicates that the expression of enzymes involved in synthesis of DHA from ALA, including desaturases and elongases, is elevated in females. An estrogen-associated effect may be mediated by peroxisome proliferator activated receptor-α (PPARα), as activation of this nuclear receptor increases the expression of these enzymes. However, because estrogens are weak ligands for PPARα, estrogen-mediated increases in PPARα activity likely occur through an indirect mechanism involving membrane-bound estrogen receptors and estrogen-sensitive G-proteins. The protein kinases activated by these receptors phosphorylate and increase the activity of PPARα, as well as phospholipase A_2 and cyclooxygenase 2 that increase the intracellular concentration of PPARα ligands. This review will outline current knowledge regarding elevated DHA production in females, as well as highlight interactions between estrogen signaling and PPARα activity that may mediate this effect.

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Abbreviations

ALA	Alpha-linoleic acid
AMPK	AMP-activated protein kinase
Ca^{2+}-PLA_2	Calcium-dependent phospholipase A_2
CE	Cholesteryl esters
COX	Cyclooxygenase
CREB	cAMP response element binding
D5D	Delta-5 desaturase
D6D	Delta-6 desaturase
DHA	Docosahexaenoic acid
DPAn-3	Docosapentaenoic acid, n-3
ELOVL	Elongase of very long chain fatty acids
EPA	Eicosapentaenoic acid
ER	Estrogen receptor
ERK-MAPK	Extracellular receptor kinase-mitogen activated protein kinase
FABP	Fatty acid binding proteins
GPR30	G-protein receptor 30
HUFA	Highly unsaturated fatty acids
LNA	Linoleic acid
MAPK	Mitogen-activated protein kinase
PC	Phosphatidyl choline
PE	Phosphatidyl ethanolamine
PL	Phospholipids
PLA_2	Phospholipase A_2
PPAR	Peroxisome proliferator activated receptor
PPRE	Peroxisome proliferator response element
PS	Phosphatidyl serine
PUFA	Polyunsaturated fatty acids

Introduction

The dietary intake and blood content of omega-3 polyunsaturated fatty acids (n-3 PUFA, ≥18 carbons, ≥2 double bonds), particularly the highly unsaturated fatty acids (HUFA, ≥20 carbons, ≥3 carbon–carbon, double bonds) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:3n-3), are associated with a reduced risk of sudden cardiac death when incorporated into cardiac membranes as phospholipids or free fatty acids [1–4]. Also, the incorporation of DHA into brain phospholipids is associated with improved neurological development [5] and performance on spatial tasks [6]. EPA and DHA can either be obtained directly from the diet or produced within the body from dietary precursors such as alpha-linolenic acid (ALA, 18:3n-3). As reviewed recently, it appears that in humans, increasing ALA intakes can significantly increase levels of EPA and n-3 docosapentaenoic acid (DPAn-3), but not DHA in various blood measures [7]. However, even limited production of EPA, DPAn-3 and DHA from dietary ALA may be clinically relevant. The anti-arrhythmic benefit of EPA and DHA is estimated to have a very steep dose–response curve that plateaus at an EPA + DHA consumption of 750 mg/day [8], much higher than the estimated North American intakes of EPA + DHA of approximately 100 mg/day [9–12]. It has recently been suggested that 250–500 mg/day of EPA + DHA should be established as a dietary reference intake [13]. Therefore, even a small contribution to tissue EPA and DHA through ALA conversion could have a significant impact on sudden cardiac death risk reduction.

Dietary ALA intakes are inversely associated with sudden cardiac death in women [14] but not in men [15] in North American populations. North American intakes of ALA are approximately 1,500 mg/day [9–12]. Increased ALA conversion to EPA and DHA has been observed in women consuming <200 mg EPA + DHA/day as compared with men and women consuming >500 mg EPA + DHA/day [16, 17] and may partially explain the inverse association with sudden cardiac death. Increased conversion of ALA to DHA [16, 18, 19] and higher levels of DHA in various lipid fractions of liver, erythrocytes, plasma and whole blood [16, 20–24] have been observed in females consuming typical Western diets as compared with males.

An increased capacity to biosynthesize DHA in women is possibly an evolutionary adaptation to attempt to provide a supply of DHA for maternal–fetal transport during the fetal brain growth spurt [7, 12, 25]. Observational results suggest that pregnant women undergo metabolic adaptations to maintain DHA in blood for placental transport [12, 26–28]. Changes in n-3 HUFA have also been associated with both endogenous and exogenous changes in circulating estrogen [21, 29–32], however, the potential mechanisms for this enhanced biosynthesis has not been elucidated.

The biosynthesis of DHA is mediated by elongases and desaturases in the endoplasmic reticulum and acyl-CoA oxidase and multifunctional protein 2 in the peroxisome. Estrogen-response elements have not been associated with the genes of any of these enzymes to date despite our efforts (unpublished observations). This is despite the fact that estrogen response elements are well characterized [33] and can be identified using a variety of tools. Peroxisome-proliferator receptor α (PPARα), a nuclear receptor that increases the expression of desaturases [34, 35] is a potential target for an estrogen mediated effect, but it also lacks an estrogen response element [36]. However, PPARα activity may be increased by indirect estrogen mediated phosphorylation. Several of the protein kinase systems activated by membrane-bound estrogen receptors, including mitogen-activated protein kinase (MAPK) and protein kinase A, phosphorylate and increase the activity of PPARα [37–39]. We presently review evidence supporting sex differences in the conversion of ALA to DHA and suggest a potential mechanism involving interaction between PPARα and estrogen signaling.

Dietary Sources of DHA

Preformed DHA

Blood levels of DHA are strongly correlated with the dietary intake of DHA [40]. In North America, salmon is a dominant source of dietary EPA and DHA due to its high popularity (ranked 4th in consumption frequency among marine foods after shrimp, tuna and breaded fish, respectively), and a high content of EPA and DHA [8, 9, 41]. Salmon provides 1,100–2,100 mg EPA + DHA/100 g as compared with 130–860 mg from tuna, 320–550 mg from shrimp, and 0–210 mg from breaded fish (per 100 g cooked) [42]. Alternative sources that can provide significant amounts of dietary n-3 HUFA include fish oil capsules and novel EPA and DHA enriched functional foods [43, 44]. All these strategies are largely dependent on fish stocks and fish-farming practices that may not be sustainable [45]. Non-fish sources of preformed dietary DHA include fat extraction from species of microalgae such as *Crypthecodinium cohnii* and *Schizochytrium* [46, 47]. The production of DHA by microalgae varies with some species relying on alternating desaturases and elongases while others utilize polyketide synthase systems [48, 49]. There are ongoing efforts to develop genetically modified organisms capable of producing n-3 HUFA [50, 51].

Dietary Precursors of DHA

The primary dietary precursor of long chain n-3 PUFA that is consumed in North America is ALA, which is found in a variety of plant foods and oils, particularly in flaxseed oil. Canola and soybean oil also contain significant amounts of ALA, but also typically contain relatively higher amounts of linoleic acid (LNA, 18:2n-6). Supplementing with relatively low levels of dietary ALA (2.4 g ALA/day in flaxseed oil) can increase erythrocyte EPA [52] with higher doses of ALA resulting in further increases in blood levels of EPA and DPAn-3, but often not DHA as reviewed extensively [7]. There is no evidence that ALA interventions result in increased DHA in blood, however these studies have been done predominantly with males only [52–55] or with mixed sex groups without specific sex group analyses [56–59]. Recently, we demonstrated that DHA levels in free living females was significantly higher than free living males, and that increases in DHA status is much slower than changes in EPA with fish oil supplementation [24]. Therefore, acute intervention studies may not be appropriate to detect DHA biosynthesis and accumulation in human blood. There are some longer ALA intervention studies that suggest DHA blood measures may possibly increase (for example, 52 weeks) [60]. It is also important to note that blood measures of EPA, DPAn-3, and DHA may only reflect hepatic ALA conversion, while there is evidence suggesting tissue specific ALA conversion with brain capable of synthesizing DHA from ALA, while the heart appears to only convert ALA to EPA and DPAn-3 [61].

Mammalian DHA Biosynthesis

The discovery that dietary ALA was the precursor of DHA was made in 1950 by Widmer and Holman [62] by feeding fat-deficient rats isolated ALA and observing the tissue deposition of DHA. Klenk and Mohrhauer [63] later elucidated the pathway of DHA formation from ALA to be: 18:3n-3 → 18:4n-3 → 20:4n-3 → 20:5n-3 → 22:5n-3 → 22:6n-3. It was then determined that the pathway took place in the endoplasmic reticulum [64]. Testing of the assumption that a delta-4 desaturase was responsible for the conversion of 22:5n-3 into 22:6n-3 revealed no microsomal formation of 22:6n-3, but rather two novel fatty acids were microsomally produced: 24:5n-3 and 24:6n-3 [65]. It was later determined that 22:6n-3 was formed by peroxisomal β-oxidation of the microsomally produced 24:6n-3 (Fig. 1) [66].

The first enzyme in the conversion of ALA to longer chain n-3 HUFA is delta-6 desaturase (D6D) [67]. D6D catalyzes the desaturation of both n-6 and n-3 PUFA, and also appears to act on both 18- and 24-carbon PUFA, representing a significant metabolic "bottle-neck" [68]. A D6D knockout mouse has been demonstrated to lack the ability to make arachidonic acid (20:4n-6) from LNA and to lack the ability to make docosapentaenoic acid (22:5n-6) from dietary arachidonic acid [69]. In addition, the competition between ALA and 24:5n-3 for D6D results in EPA accumulation and limited synthesis and accumulation of DHA in Hep-G2 phospholipids [70]. As a result, the expression and activity of D6D, and substrate competition are considered primary determinants of DHA production rate. However, the large induction of both D6D and delta-5 desaturase (D5D), but not elongases, observed in HepG2 cells in response to essential fatty acid deficiency suggests that HUFA production is dependent on the concentration of both of these enzymes [71]. Additionally, these two genes are located only 11 kb apart on chromosome 11 in humans (chromosome 19 in mice) in a head-to-head orientation, suggesting that the transcription of these two genes may be regulated similarly [67]. A thorough review of the desaturases is available [67].

Sex Differences in DHA Status and Metabolism

Several studies have observed sex differences in blood and tissue DHA content in humans and rats (summarized in Table 1). While consuming a habitual diet, women possess significantly higher levels of DHA in total plasma lipids, as well as plasma non-esterified fatty acids, triacylglycerols, and phosphatidyl choline [23]. Sex specific responses to fish-oil supplementation have been observed including higher levels of DHA in erythrocytes and whole blood at baseline and after 8 weeks washout post-supplementation in women as compared with men [24]. Also, female sex has been significantly associated with increased DHA in studies examining various lifestyle and physiological parameters on fingertip prick whole blood in Italians [72] and serum phospholipids and cholesteryl esters in New Zealanders [22]. Higher levels of DHA in the blood of women as compared with men could be the result of differences in DHA mobilization and partitioning, dietary intake and male–female differences in body mass rather than differences in biosynthesis. However, increased ALA to DHA conversion rates in women relative to men have been determined using fatty acid stable isotope tracer studies [16, 18, 19].

The net fractional conversion of an orally ingested bolus of $U^{13}C$-ALA into EPA and DHA in women capable of bearing children has been observed to be approximately 21 and 9.1%, respectively [18] as compared with conversion rates in men of 7.9% for EPA and DHA production that was undetectable by the investigators means [19]. In these

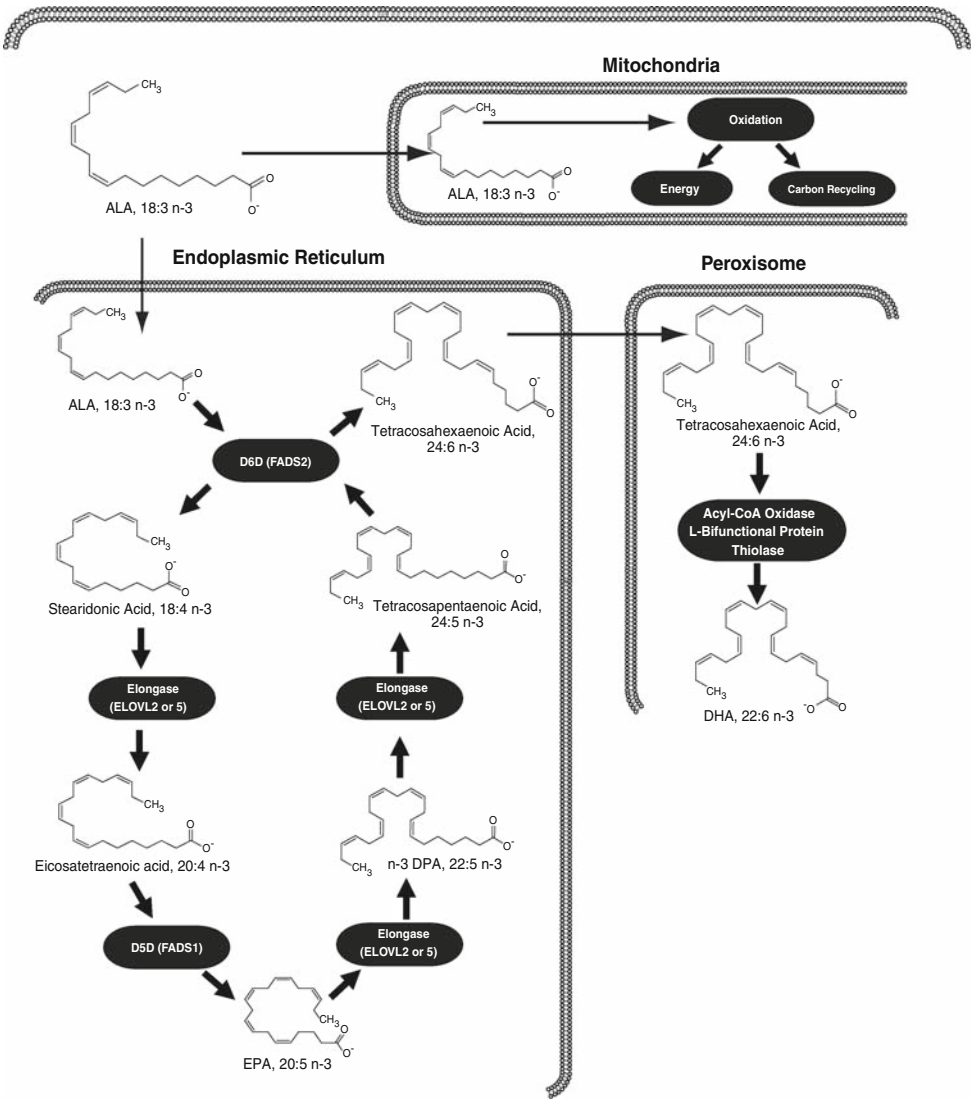

Fig. 1 Two main metabolic fates of alpha-linolenic acid: conversion to longer-chain n-3 polyunsaturated fatty acids in the endoplasmic reticulum and peroxisomes, and mitochondrial oxidation. *ALA* alpha-linolenic acid, *D6D* delta-6 desaturase, *FADS* fatty acid desaturase, *ELOVL* elongase of very long chain fatty acids, *EPA* eicosapentaenoic acid, *D5D* delta-5 desaturase, *DPA* docosapentaenoic acid, *DHA* docosahexaenoic acid

studies, ^{13}C-EPA and DHA primarily appeared in plasma phosphatidyl choline. Labelled U^{13}C-DHA has also been detected in plasma triacylglycerol and phospholipid fractions in women (no men comparison group) after treatment with dietary U^{13}C-ALA [73]. Compartmentalized modeling of ^{2}H-ALA metabolism through examining ^{2}H-HUFA appearance in plasma total lipids has isolated the sex difference to the conversion of labeled 22:5n-3 to labeled 22:6n-3, and that this sex difference was present only when participants consumed a beef-based diet low in n-3 HUFA and not a fish-based diet [16, 17]. Additionally, there may be increased partitioning of ALA towards DHA synthesis in women, as oxidation and carbon-recycling of ALA into saturated and monounsaturated fatty acids are much lower as compared with men [18, 19, 74]. In animal models, elevated DHA has also been observed in the liver phosphatidyl choline and phosphatidyl ethanolamine of mature female relative to male rats [20] and feeding male rats a high-ALA diet containing no EPA or DHA results in elevated liver and heart phospholipid EPA and DPAn-3, but not DHA [75].

In a model of dietary n-3 repletion, increased hepatic expression of D5D and D6D was observed in female rats as compared with male rats that corresponded to higher D5D protein content [76]. An increased expression of D5D only [20] and no differences in D5D and D6D [77] have also been reported. We have observed increased D5D and D6D mRNA expression in females as compared with males at 14 week of age on a standard chow diet (Kitson and Stark, unpublished observations). These results tend to suggest a greater capacity for DHA biosynthesis in females as compared with males. Sexual maturity may contribute to some of the disparity in these studies but this has not been elucidated.

Age may influence DHA accumulation [78]. There are reports of an association between age and DHA levels in

Table 1 Summary of studies investigating sex differences in DHA content or metabolism

Author [references]	Number of subjects	Dietary fatty acid treatment	Results
Rat studies			
Burdge et al. [20]	Wistar, male and female ($n = 24$ for each)	Maternal = 5.9 g ALA/kg diet	Increased DHA in PC and PE (% total fatty acids) in females
		Lactation/weaning = 0.7 g ALA/kg diet	Increased hepatic D5D expression in females.
Extier et al. [76]	Wistar male and female ($n = 6$ for each sex/time point)	Maternal = 0.05 g ALA/kg diet.	Increased DHA in plasma PC and liver PC, PS, and PE in females
		Weanling = 0.2 mg ALA/kg diet.	Increased D5D and D6D and lower PPARα and FABP7 hepatic expression in females
			Increased hepatic D5D protein content in females
			No DHA or gene expression differences in cerebral cortex
Childs et al. [77]	Wistar male and female ($n = 6$ for each sex/diet group)	Low soybean = 1.6 g ALA/kg diet	Increased DHA in plasma PC, liver PC and PE in females in all diets
		High fat soybean = 9.1 g ALA/kg diet	No differences in gene expression
		High fat linseed = 50.2 g ALA/kg diet	
Human studies			
Burdge and Wooton [18]	Women ($n = 6$)	Habitual	Fractional appearance of ^{13}C-labelled fatty acids in plasma was ALA: 63.7%, EPA: 21.1%, n-3 DPA: 5.9%, DHA: 9.2%.
		700 mg of [U-^{13}C] ALA administered	^{13}C recovered in breath was 22.2% of dose.
Burdge et al. [19]	Men ($n = 6$)	Habitual	Fractional appearance of ^{13}C-labelled fatty acids in plasma was ALA: 84%, EPA: 7.9%, n-3 DPA: 8.1%, DHA: n.d.
		700 mg of [U-^{13}C] ALA administered	^{13}C recovered in breath was 33.2% of dose
Pawlosky et al. [16, 17]	Men ($n = 5$)	Ad libitum	Increased conversion of 22:5n-3 to DHA in females within beef-based diet
	Women ($n = 5$)	Fish-based	
		Beef-based	
Giltay et al. [21]	Men ($n = 72$)	Controlled diet, free from fish	Increased DHA in serum CE in females
	Women ($n = 103$)		
Bakewell et al. [23]	Men ($n = 13$)	Habitual intakes	Increased DHA in plasma TG, NEFA, PC, and total lipids in females
	Women ($n = 23$)		
Crowe et al. [22]	Men ($n = 1,246$)	Habitual intakes	Increased DHA in serum PL and CE in females
	Women ($n = 1,547$)		Increased EPA in PL and CE and increased n-3 DPA in PL in men
Marangoni et al. [72]	Men ($n = 47$)	Habitual intakes	Increased n-3 DPA in whole blood in males
	Women ($n = 61$)		
Metherel et al. [24]	Men ($n = 10$)	Baseline habitual diet	Increased DHA and decreased n-3 DPA in whole blood and erythrocytes of females at baseline.
	Women ($n = 10$)	4 week supplementation of 4.8 g/day of EPA + DHA,	Increased DHA:EPA ratio in women in various blood fractions disappeared with supplementation
		8-week washout on habitual diet	

ALA alpha-linolenic acid, *PC* phosphatidyl choline, *PE* phosphatidyl ethanolamine, *D5D* delta-5 desaturase, *D6D* delta-6 desaturase, *DHA* docosahexaenoic acid, *CE* cholesteryl esters, *PL* phospholipids, *EPA* eicosapentaenoic acid, *DPA* docosapentaenoic acid, *PS* phosphatidyl serine, *FABP* fatty acid binding protein, *PPAR* peroxisome proliferators activated receptor, *n.d.* not detected

adult human blood. Although dietary intake of DHA is a potential confounder, age was positively associated with DHA in plasma phospholipids [79, 80], serum phospholipids and cholesteryl esters [22], plasma total lipids [40] and erythrocytes [40, 81] after controlling for DHA intakes. However, no differences in DHA content between subjects over 75 years of age and control subjects between 20 and 48 years of age were observed in plasma phospholipids,

non-esterified fatty acids, triacylglycerides, cholesteryl esters, and erythrocytes [82]. To our knowledge, a direct examination of the effect of age and sex on biosynthesis and accumulation of DHA has not been completed.

Role of Estrogen in DHA Biosynthesis

Sex differences in DHA levels appear to be mediated by circulating sex hormones, particularly estrogen (studies investigating hormone status or hormone manipulations, and DHA are summarized in Table 2). Elevated DHA content of female blood is possibly an evolutionary adaptation meant to provide fetal tissues with sufficient DHA for neural development. During pregnancy, the concentration of DHA in maternal blood is significantly elevated in the third trimester, when circulating levels of estrogen are highest [12, 26, 83]. Presumably, this increase in maternal DHA supports the fetal brain growth spurt in the third trimester, as the fetus' capacity to produce DHA from ALA may be insufficient to meet the extreme demand [25, 84]. With postpartum, maternal estrogen and circulating maternal DHA levels decrease [12, 26].

Blood levels of DHA have been associated with exogenous sex hormone interventions. Postmenopausal women have been observed to have decreased DHA in erythrocyte phospholipids [85], and postmenopausal women undergoing hormone replacement strategies including both direct hormone replacement and selective estrogen-receptor modulators have increased circulating DHA relative to postmenopausal women not using hormone therapies [86, 87]. Women taking an estradiol-based contraceptive pill also have elevated blood and erythrocyte DHA levels as compared to women not taking oral contraceptives [21, 88]. Similarly, DHA increased in the plasma cholesteryl esters of male-to-female transsexuals receiving oral ethinyl estradiol, while plasma cholesteryl ester DHA decreased in the blood of female-to-male transsexuals receiving testosterone treatment [21].

In rats, EPA and DHA in plasma phosphatidyl choline correlate strongly with female sex hormones (estrogen and progesterone), and plasma, liver, and adipose EPA and DHA content vary inversely with testosterone [89]. DHA in liver phospholipids and plasma phosphatidyl choline have been observed to be higher in females as compared with males after n-3 deficiency/repletion [76]. Higher DHA has also been seen in female rat plasma and liver phospholipids in different diets containing varying levels of total fat and ALA [77]. Decreased erythrocyte DHA has been observed after ovariectomization as compared with sham-operated controls [90]. In addition, neuroblastoma cells incubated with ALA and estradiol exhibit higher EPA and DPAn-3 in phosphatidyl ethanolamine and increased expression of D5D as compared with cells incubated with ALA alone [91, 92]. Interestingly, treating neuroblastoma cells with dehydrotestosterone decreases EPA and DHA in phosphatidyl ethanolamine, and decreases D5D expression [91].

Based on current evidence, it appears that sex influences the concentration of circulating DHA, resulting in higher levels in females. This difference is associated with estrogen, and appears to involve increased conversion of ALA to DHA, in particular the conversion of 22:5n-3–22:6n-3, and only during low dietary intake of n-3 HUFA. It is possible that estrogen may increase the concentration or activity of DHA synthesis enzymes, including desaturases, elongases, and peroxisomal β-oxidation enzymes resulting in enhanced DHA biosynthesis.

Estrogen Signalling Mechanisms

Estrogen exerts many effects on a variety of different tissue types, and its actions are mediated by both direct interaction with the genome and nongenomic mechanisms [93]. The effects of estrogen are mediated primarily by estrogen receptors (ERα and ERβ in mammals) which exhibit a variety of subcellular localizations depending on tissue type [94, 95]. For example, in endometrial cells, ERα is primarily found at the cell membrane, whereas in breast cancer cells it is in the nucleus [95].

In direct genomic estrogen signaling, the binding of estrogen to estrogen receptors results in dimerization with another estrogen receptor (ERα or ERβ) followed by binding to an estrogen response element in the promoter of a target gene, causing altered transcription of that gene. In "nongenomic" estrogen signaling, estrogen binds to and activates estrogen receptors anchored to the plasma membrane [96], or a G-protein known as G-protein receptor 30 (GPR30) [97]. Dimerization to another estrogen receptor can also occur with this mechanism [98], however the signal transduction that results from the activation of these membrane bound receptors involves a number of second messenger protein kinases and calcium signaling mechanisms, rather then direct genomic interaction (some are presented below, and are extensively reviewed in [93]).

The observational link between circulating estrogen and DHA production suggests that estrogen increases the expression of the enzymes involved in DHA production from shorter-chain n-3 PUFA. However, to our knowledge, no estrogen response elements have been identified to date that influence the expression of any of the genes involved in DHA synthesis (D6D, D5D, acyl-CoA oxidase), suggesting that the estrogen dependent induction of these genes occurs through indirect mechanisms. Several reliable techniques exist for determining the presence of estrogen

Table 2 Summary of studies investigating effects of exogenous estrogen and menopause on DHA content or metabolism

Author [reference]	Cell/subjects	Treatment	Effects of gender or steroids on measurements
Cell culture			
Alessandri et al. [92]	SH-SY5Y neuroblastoma ($n = 3$ for each condition)	10 nM 17β-estradiol 30 μM of ALA alone 10 nM 17β-estradiol with 30 μM of ALA	Increased EPA and n-3 DPA in PE with 17β-estradiol treatment
Extier et al. [91]	SH-SY5Y neuroblastoma (n = 4 for each condition)	7 μM ALA, LA, or ALA/LA, 10 nM 17β-estradiol, dehydrotestosterone, progesterone or control.	Increased EPA and n-3 DPA and increased D5D expression with ALA, and 17β-estradiol treatment Decreased EPA and DHA and decreased PPARα and D5D expression with ALA and dehydrotestosterone treatment Decreased D6D expression with progesterone treatment
Human			
Stark et al. [29]	Females (43–69 years)	Premenopausal ($n = 19$) Postmenopausal taking hormone therapy ($n = 40$) Postmenopausal not taking hormone therapy ($n = 34$)	Increased DHA and n-3 DPA in plasma PL in postmenopausal women not taking hormone therapy
Sumino et al. [87]	Postmenopausal women (43-63 years)	Taking conjugated equine estrogen and medroxyprogesterone acetate ($n = 59$) Not taking hormone therapy ($n = 45$)	Increased plasma DHA and EPA total lipids in women taking hormones
Giltay et al. [21]	Male-to-female transsexuals (eugonadal)	Cyproterone acetate alone ($n = 16$) Cyproterone acetate with oral ethinyl estradiol ($n = 15$) Transdermal 17β-estradiol ($n = 15$)	Increased DHA in serum CE with cyproterone acetate with oral ethinyl estradiol
	Female-to-male transsexuals (ovariectomized)	Testosterone esters plus anastrozole ($n = 16$) Placebo ($n = 14$) Testosterone esters alone ($n = 17$)	Decreased DHA in serum CE with testosterone esters alone
	Females	Using oral contraceptives ($n = 32$) No oral contraceptive use ($n = 71$) Controlled diet, free from fish	Non-significant increase in DHA in serum CE with oral contraceptive use ($P = 0.08$)
Giltay et al. [86]	Postmenopausal females (47–59 years) Males (60–70 years)	Females 60 mg raloxifene/day ($n = 23$) Females 150 mg raloxifene/day ($n = 20$) Females conjugated equine estrogen with medroxyprogesterone acetate ($n = 17$) Females placebo ($n = 23$). Males 120 mg raloxifene/day ($n = 15$) Males placebo ($n = 15$)	Increased DHA in plasma CE in post-menopausal women taking 150 mg/day raloxifene and in women taking equine estrogens with medroxyprogesterone acetate at 24 months compared to baseline
Stark and Holub [30]	Postmenopausal females (45–70 years)	Postmenopausal taking hormone therapy ($n = 18$) Postmenopausal not taking hormone therapy ($n = 14$) 2.8 g algal DHA vs. placebo in crossover design	Increased estimates of retroconversion of DHA to EPA in plasma phospholipids of women not taking hormone therapy

PLs phospholipids, *DHA* docosahexaenoic acid, *DPA* docosapentaenoic acid, *CE* cholesteryl esters, *ALA* alpha-linolenic acid, *LA* linoleic acid, *D5D* delta-5 desaturase, *D6D* delta-6 desaturase, *PPAR* peroxisome proliferators activated receptor, *EPA* eicosapentaenoic acid, *PE* phosphatidyl ethanolamine

response elements on any gene on a genome-wide basis [99, 100], however genome-wide screening for these elements in DHA-producing genes has not been reported.

Peroxisome Proliferator-Activated Receptor α (PPARα)

The structure, ligand-binding, DNA-binding and metabolic actions of PPARs have been reviewed [101, 102]. PPARs are ligand-activated transcription factors belonging to the nuclear steroid receptor superfamily. Inactive PPARs are bound to corepressor proteins which are released upon ligand binding to the PPAR through a conformational change to the PPAR. This conformational change allows the PPAR to bind to the retinoid-x-receptor and co-activator proteins that facilitate binding of the complex to DNA through a number of different mechanisms including histone acetylase activity [103]. This activated complex then interacts with peroxisome proliferator response elements (PPRE; nucleotide regions with an imperfect direct repeat-1 motif) in the promoter region of target genes, and gene transcription is modulated.

Three distinct PPARs have been discovered with varying tissue expression, genomic targets, and ligands: PPARα, PPARγ (PPARγ$_1$ and PPARγ$_2$ are produced from the same gene by different promoters), and PPARβ/δ. All three subtypes bind to the direct repeat-1 motif, however, each PPAR subtype distinguishes its targets by differences in the region directly upstream of this motif [101].

PPARα is expressed in metabolically active tissues such as liver, heart, kidney, skeletal muscle and brown adipose tissue [104, 105] and is known to regulate the expression of genes involved in PUFA desaturation (D6D, D5D) [35, 106], peroxisomal β-oxidation (acyl-CoA oxidase, D-bifunctional protein) [107, 108], and fatty acid transport (cytosolic fatty acid binding protein, fatty acid transport protein, acyl-CoA binding protein) [109, 110], all of which are involved in DHA formation (a review of PPARα responsive genes is available [111]). PPARα plays a large role in lipid homeostasis by increasing peroxisomal and mitochondrial β-oxidation rates, and producing energy and acetyl units for ketone body formation in periods of fasting and/or low carbohydrate intake [112].

Natural ligands for PPARα include PUFA (n-3 and n-6), monounsaturated fatty acids, and eicosanoids such as leukotriene B4 and hydroxyeicosatetraenoic acids [36]. Fibrates such as clofibrate, fenofibrate, and bezafibrate are synthetic ligands for PPARα and are effective lipid-lowering agents in humans, while in rodents fibrates cause increased hepatic peroxisome number, hepatomegaly, and carcinogenesis at higher doses [111].

PPARα can be phosphorylated at multiple sites, resulting in increased activity (reviewed in [113]). The mechanism by which phosphorylation increases PPARα activity has not been elucidated, however, some evidence suggests that phosphorylation of PPARα results in a decreased affinity for corepressor proteins [114]. This finding is significant, as it suggests that phosphorylated PPARα is more likely to be activated at a biological concentration of PPARα ligands, such as PUFA.

Estrogen and PPARα

There is strong evidence that estrogen signaling interacts with PPARα-dependent gene transcription particularly with regards to lipid metabolism. For example, mice deficient in aromatase, and therefore unable to synthesize estrogen, succumb to hepatic steatosis resulting from elevated hepatic lipid accumulation and deficient β-oxidation [115]. However, estrogen supplementation prevents this effect by increasing mitochondrial and peroxisomal β-oxidation rates via increased expression of mitochondrial and peroxisomal β-oxidation enzymes (acyl-CoA oxidase, medium chain acyl-CoA dehydrogenase) [115, 116], similar to the effect of PPARα activation. Additionally, the expression of stearoyl-CoA desaturase 1, a PPARα-induced gene, is significantly increased in female mouse livers and results in elevated hepatic oleate production [117]. Also, mitochondrial β-oxidation is increased in women but not in men fed a high oleate diet (31.4% of energy), indicating a sex difference in the metabolic response to oleate, a weak PPARα ligand [118]. Treating ovariectomized rats with 17β-estradiol increases PPARα content and expression of PPARα-dependent lipid oxidation genes in red gastrocnemius muscle [119]. Significant increases in the expression and transcriptional activity of PPARβ/δ, and an increase in the expression of lipid oxidation genes under transcriptional control of PPARα, independent of an increase in PPARα expression in liver, muscle, and adipose tissue has been demonstrated using a similar approach [120]. Reduced fat accumulation in female but not male mice has also been observed after treatment with phytol, a peroxisome proliferator [121].

The expression of fatty acid binding proteins (FABP), which is increased following PPARα activation [122], is also much higher in females than in males [123, 124]. FABP transports fatty acids to the nucleus and has been observed to co-localize with and possibly increasing the activity of PPARα in fibroblasts and primary mouse hepatocytes [125–127]. However, transient transfection of fibroblasts with FABPs decreased tetradecylthioacetic acid stimulated PPAR activity [128].

Similarly, estrogen administration causes peroxisome proliferation in the uropygial glands of male and female mallard ducks, accompanied by an increase in the peroxisome-dependent production of 3-hydroxy fatty acid

diesters [129]. In humans, the rate of DHA retroconversion to EPA, a process dependent on peroxisomal activity, is increased in postmenopausal women receiving hormone replacement therapy as compared with postmenopausal women not receiving this treatment [30]. Additionally, phospholipids and long chain PUFA in the brain are increased in female but not male PPARβ knockout mice suggesting sexual dimorphisms and a role of PPARβ in paroxysmal acyl-CoA use in the brain [130]. Considerable differences exist in the response of PPARα and PPARβ/δ to some natural ligands (particularly 8(*S*)-HETE) [131], and that generalizing in vivo results between different PPARs should be done with caution. Nonetheless, it seems that sex differences exist in other PPARs, as well as PPARα.

In a transgenic luciferase-$^{5\times}$PPRE mouse, it was shown that liver exhibited significantly lower PPARα transcriptional activity in females as compared with males in response to oral fibrate administration, food withdrawal, and reversal of feeding schedule [132]. These sexual dimorphisms remained despite ovariectomization and castration that suggests gonadal hormones are not involved. These observations may also be a dependent on the presence of the 5 PPREs in this model as similar observations have not been confirmed in mice with intact genomes. However, this transgenic model does support the notion that sex differences exist in PPARα-dependent gene transcription. Similarly, male as compared with female mice express more PPARα-dependent genes in response to 2 weeks of daily oral trichloroethylene treatment, suggesting a sex specific response to exogenous activators of PPARα [133].

Observations in PPARα-null mice strongly illustrate the interaction between estrogen and PPARα. Normally, ovariectomization results in significant gains in adipose tissue mass, while subsequent treatment with exogenous estradiol relieves this effect. However, in PPARα-null mice, no significant changes in adipose tissue are observed upon ovariectomization and subsequent estradiol administration, suggesting that the effect estrogen has on fat oxidation is dependent on the presence of PPARα [134]. Circulating leptin concentrations decrease in PPARα-null mice as compared with control mice, with a sex-specific effect in the leptin response to feeding as leptin was increased in female PPARα-null mice as compared with male PPARα-null mice [135].

The evidence presented above infers that a significant biological interaction exists between estrogen signaling and PPARα activity, and is present in several animal models. Accordingly, understanding the mechanism by which estrogen regulates PPARα may provide a better understanding of observations of sexual dimorphisms in n-3 PUFA metabolism. Interestingly, in vitro ligand binding analysis has shown that 17-β estradiol is a weak direct activator of PPARα, suggesting that it is not a PPARα ligand [36]. However, many interactions exist between estrogen signaling and PPARα, including protein kinases and other mechanisms, which suggest that estrogen acts on PPARα indirectly.

Estrogen Increases PPARα Activity by Phosphorylation

It is known that the phosphorylation (and corresponding activity) of PPARα is enhanced by several of the protein kinase systems activated by membrane bound estrogen receptors. By phosphorylating PPARα, estrogen will enhance DHA formation by increasing the transcription of enzymes involved in this pathway (Fig. 2).

Estrogen binding to membrane bound estrogen receptors activates extracellular receptor kinase-mitogen activated protein kinase (ERK-MAPK) in a variety of tissue types/cell lines. Injection of 17-β-estradiol increases ERK-MAPK activity in several rat brain structures [38] and the addition of estrogen to male derived hypothalamic nuclei in vitro results in significant elevations in the activity of MAPK [39]. Furthermore, the addition of estrogen to cultured muscle cells [136] and breast cancer cells [137] also results in elevated ERK-MAPK activity, illustrating that this effect is shared by a variety of tissues.

Phosphorylation and increased activity of PPARα resulting from MAPK-mediated signal transduction has been well documented in a variety of cellular processes. Hepatic PPARα is known to be phosphorylated at two serine residues in response to insulin signaling via the ERK-MAPK system, resulting in increased PPARα-dependent gene transcription [114, 138]. The incubation of cultured myotubes with adiponectin results in elevated activity of ERK-MAPK and corresponding phosphorylation of PPARα, resulting in increased target gene expression and lipid catabolism [139]. Similarly, phosphatidyl inositol supplementation also increases PPARα activity via ERK-MAPK-dependent phosphorylation in human hepatocyte cell lines [140], resulting in PPARα-dependent production and secretion of apolipoprotein A-1.

Estrogen also activates protein kinase A phosphorylation systems. In vitro research with hippocampal neurons indicates that estrogen causes significant protein kinase A activation that is dependent on the presence of membrane-bound estrogen receptors [37]. Estrogen has been observed to cause protein kinase A dependent phosphorylation of *N*-methyl-D-aspartic acid receptors in rat spinal neurons, lessening the perception of pain by the animal [141]. In rat liver tissue, estrogen interaction with GPR30 results in activated protein kinase A signal transduction and prevention of apoptosis following organ injury [142]. Activation of protein kinase A signaling by cholera toxin has also been shown to increase the phosphorylation and transcriptional

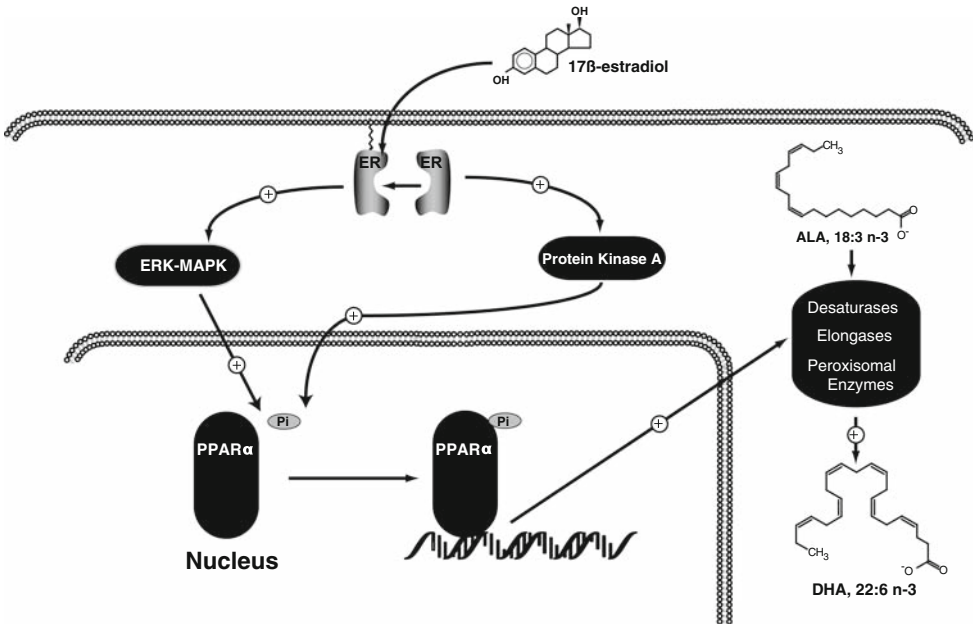

Fig. 2 Proposed mechanism of increased peroxisome-proliferator activated receptor α phosphorylation by estrogen. *ER* estrogen receptor, *ERK-MAPK* extracellular receptor kinase-mitogen activated protein kinase, *Pi* inorganic phosphate, *PPARα* peroxisome-proliferator activated receptor α, *ALA* alpha-linolenic acid, *DHA* docosahexaenoic acid

activity of PPARα [143]. Protein kinase A phosphorylates several enzymes in lipid-utilization systems and metabolism, including diacylglycerol lipase in the brain [144].

Phosphorylation of 5′AMP-activated protein kinase has also been observed in response to 17β-estradiol supplementation in the skeletal muscle of ovariectomized rats. Lipid oxidation in white adipose tissue [145] and skeletal muscle [146] is increased during AMP-activated protein kinase (AMPK) activation, and it has been found that siRNA inhibition of PPARα prevents this response [146].

Phosphorylation of PPARα via activation of membrane bound estrogen receptors is likely similar to the estrogen mediated phosphorylation and increased activity of cAMP response element binding (CREB) protein. Activation of CREB protein results in the increased expression of anti-apoptotic proteins, including the Bcl-2 family [147]. It has been well established that estrogen binding to membrane bound estrogen receptors results in the phosphorylation and increased activity of CREB protein. The precise protein kinase that mediates this phosphorylation varies by tissue, as protein kinase A is responsible in ZR-75 breast cancer cells [148], and protein kinase B/Akt and ERK-MAPK mediate this response in neuronal cells [149]. The increased CREB protein activity that results from phosphorylation has anti-apoptotic effects in all cell types, indicating increased CREB protein-dependent transcription.

Increased Concentration of Intracellular PPARα Ligands

Despite not being a PPARα ligand, estrogen may increase the intracellular concentrations of effective PPARα ligands, particularly PUFA and eicosanoids (Fig. 3). PUFA are most often found in the inner membrane leaflet of cells, in the *sn-2* position of phosphatidyl ethanolamine [150]. Hydrolytic release of PUFA from this position for cell signaling or eicosanoid synthesis is catalyzed by phospholipase A_2 (PLA_2). Upon phosphorylation, the activity and a calcium-dependent isoform of PLA_2 (Ca^{2+}-PLA_2) is increased, and the enzyme becomes localized in the cell membrane and nuclear envelope regions. By localizing in these regions, Ca^{2+}-PLA_2 functions to release PUFA from biological membranes for cell signaling and eicosanoid synthesis.

Similarly to PPARα, activation of the ERK-MAPK signaling cascade has been observed to phosphorylate Ca^{2+}-PLA_2 in HeLa cells [151, 152]. Because estrogen has been observed to activate the ERK-MAPK system [38], as well as IP_3-dependent calcium signaling cascades [153–155], estrogen would be expected to increase the activity of Ca^{2+}-PLA_2. Indeed, in pregnant rats uterine Ca^{2+}-PLA_2 activity is elevated near gestation in response to increased estradiol concentrations [156]. Estradiol-administration also enhanced the activity of Ca^{2+}-PLA_2 of mussel (*Mytilus galloprovincialis*) blood cells [157].

Eicosanoids are known to be involved in atherosclerosis, bronchial asthma, and many other inflammatory conditions [158]. Prostaglandins are produced in most tissue types, and are known to activate PPARα [36]. These cytokines are produced by enzymes known as cyclooxygenases (COX) of which there are two types: COX-1, which is constitutively expressed in most tissue types, and COX-2, the expression of which is induced during periods of inflammation. With regards to estrogen, increased COX-2 expression has been observed in response to elevated estrogen in human

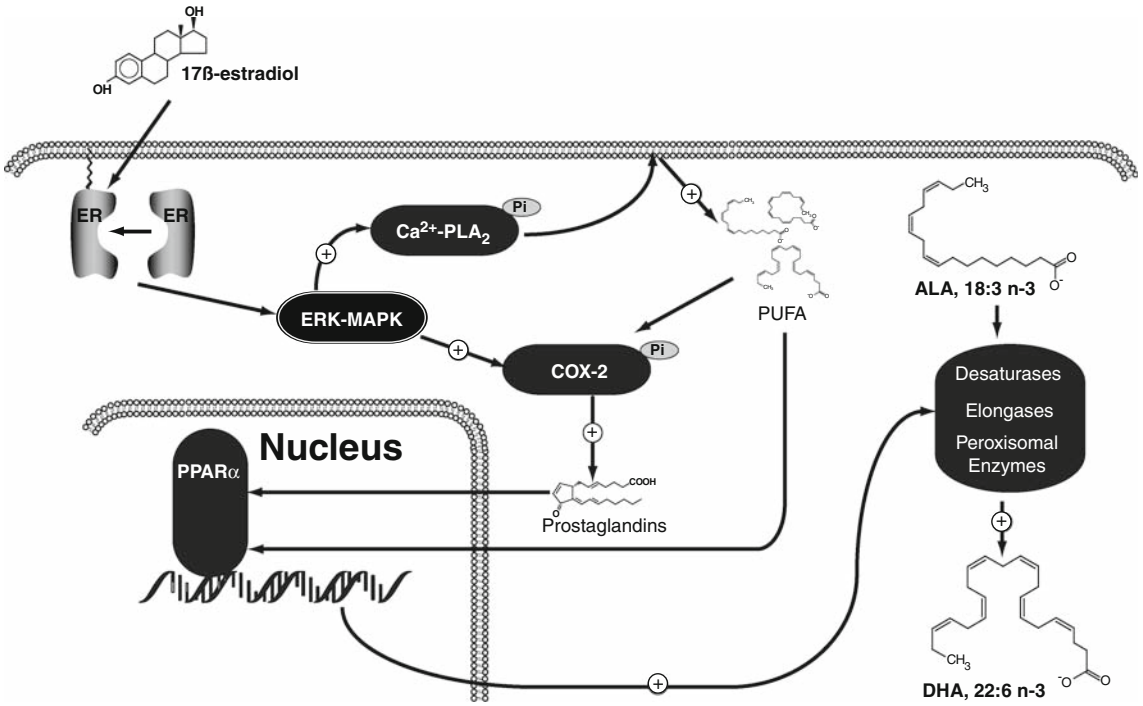

Fig. 3 Proposed mechanism by which estrogen increases intracellular concentration of ligands for peroxisome-proliferator activated receptor α. *ER* estrogen receptor, *ERK-MAPK* extracellular receptor kinase-mitogen activated protein kinase, *Ca^{2+}-PLA_2* calcium-dependent phospholipase A_2, *COX-2* cyclooxygenase-2, *Pi* inorganic phosphate, *PUFA* polyunsaturated fatty acids, *PPARα* peroxisome-proliferator activated receptor α, *ALA* alpha-linolenic acid, *DHA* docosahexaenoic acid

amnion, resulting in elevated prostaglandin E_2 production [159].

Conclusions

The current knowledge of sex differences in n-3 PUFA metabolism is presently outlined and various likely mechanisms mediating the elevated n-3 HUFA synthesis observed in females have been indicated. Women have higher circulating concentrations of DHA as compared with men that is associated with estrogen, and is a result of increased conversion of ALA into DHA. This increased conversion likely involves the increased expression/activity of DHA synthesis enzymes, highlighting possible interaction between estrogen signaling mechanisms and the expression of enzymes responsible for the synthesis of DHA. Two likely mechanisms by which estrogen increases PPARα activity and subsequent DHA formation are presented: (1) estrogen increases the activity of ERK-MAPK and protein kinase A, which phosphorylate and increase the activity of PPARα, and/or (2) estrogen increases the intracellular concentration of both PUFA and eicosanoids, PPARα ligands, via elevated Ca^{2+}-PLA_2 and COX-2 activities, resulting in activation of PPARα. By increasing the activity of PPARα, estrogen likely causes elevated transcription of the enzymes involved in DHA synthesis, including desaturases, elongases, and peroxisomal β-oxidation enzymes. The increased activity of these enzymes results in the increased production of DHA, contributing to the elevated tissue DHA content observed in females.

A more complete understanding of these interactions is required to understand the health benefits of individual n-3 PUFA and to assist in the determination of dietary recommendations for ALA, EPA, DPAn-3 and DHA. The impact of hormonal status on n-3 PUFA metabolism may be important for DHA availability for maternal–fetal transport during pregnancy. In addition, subtle differences in DHA production may be contributing to observed sexual dimorphisms in sudden cardiac death. The interaction between estrogen and fatty acid metabolism requires further research including an examination of the effects of various gonadal hormones, the impact of fluctuations in hormones throughout the life cycle including physiological challenges such as pregnancy, and the impact of the dietary intake of specific fatty acids. In addition, an enhanced understanding of the regulation and control of DHA biosynthesis may assist in efforts to produce alternative food sources of DHA and aid in the challenge of meeting the potential global demand for EPA and DHA.

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