Synthesis of DHA (omega-3 fatty acid): FADS2 gene polymorphisms and regulation by PPARα

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Abstract – In humans, in several biological systems, in particular the nervous system, the FADS2 gene transcribes Δ6-desaturase, which is the rate-limiting enzyme for converting α-linolenic acid into docosahexaenoic acid (an n-3 fatty acid). The peroxisome proliferator-activated receptor α (PPARα) modulates the transcription of FADS2 gene by interacting with a second transcription factor: the retinoid X receptor α (RXRα). These transcription factors take the form of a PPARα-RXRα heterodimer and are modulated by the ligands that modify their respective structures and enable them to bind to the peroxisome proliferator response element (PPRE) located in the promoter region of the FADS2 gene. Free estradiol induces the activation of PPARα via two pathways (i) transcription through genomic action mediated by an estrogen receptor; (ii) a non-genomic effect that allows for phosphorylation and activates PPARα via the ERK1/2-MAPK pathway. Phosphorylation is an on/off switch for PPARα transcription activity. Since Δ6-desaturase expression is retro-inhibited by free intracellular DHA in a dose-dependent manner, this position paper proposes an original hypothesis: if DHA simultaneously binds to both phosphorylated PPARα and RXRα, the resulting DHA-PPARα-RXRα-DHA heterodimer represses FADS2 gene via PPRE. The retinoic acids-RARα-RXRα-DHA heterodimer would not dissociate from corepressors and would prevent coactivators from binding to FADS2. We speculate that SNPs, which are mostly located on PPRE, modulate the binding affinities of DHA-PPARα-P-RXRα-DHA heterodimer to PPRE. The DHA-PPARα-P-RXRα-DHA heterodimer’s greater affinity for PPRE results in a decreased production of D6D and DHA. FADS2 promoter polymorphism would increase the competition between DHA and other ligands, in accordance with their concentrations and affinities.

Keywords: FADS2 / Δ6-desaturase / DHA / PPARα / regulation

Résumé – Synthèse du DHA (acide gras oméga-3): polymorphismes du gène FADS2 et régulation par PPARα. Dans plusieurs systèmes biologiques humains, en particulier au niveau cérébral, le gène FADS2 produit la Δ6-desaturase, l’enzyme qui catalyse la conversion de l’acide α-linoléénique en acide docosahexaénoïque (DHA). Le récepteur activé par les proliférateurs de peroxyomes (PPARα) module la transcription de FADS2 en interagissant avec un second facteur de transcription, le récepteur des rétinoïdes X (RXRα). Sous forme d’hétérodimère PPARα-RXRα, ces facteurs de transcription sont modulés par leurs ligands respectifs qui modifient leurs structures, et leur permettent de se lier à l’élément de réponse au proliférateur de peroxyome (PPRE) situé dans la région promotorice de FADS2. L’œstradiol induit l’activation de PPARα selon deux voies : (i), par transcription grâce à son action génomique via un récepteur œstrogénique ; (ii) par son effet non génomique qui permet la phosphorylation et l’activation de PPARα par la voie ERK1/2-MAPK. L’expression de la Δ6-désaturase étant rétro-inhibée par le DHA intracellulaire de manière dose-dépendante, cet article présente l’hypothèse originale que la liaison simultanée de DHA sur PPARα (phosphorylé) et RXRα rendrait l’hétérodimère DHA-PPARα-P-RXRα-DHA répressif pour FADS2 via PPRE. L’hétérodimère acides rétinoïques-RARα-RXRα-DHA neromprait pas sa liaison avec les corépresseurs et empêcherait la liaison avec des coactivateurs sur FADS2. Et nous pensons que les SNP, principalement sur PPRE, modulent les affinités de liaison de DHA-PPARα-P-RXRα-DHA sur PPRE. Des productions plus faibles de D6D et de DHA résultaient d’une plus grande affinité de DHA-PPARα-P-RXRα-DHA. Le polymorphisme du promuteur de FADS2 augmenterait la compétition entre le DHA et d’autres ligands en fonction de leurs concentrations et de leurs affinités.

Mots clés : FADS2 / Δ6-désaturase / DHA / PPARα / régulation

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1 Introduction

In humans, the Δ6-desaturase gene, a FADS2 (fatty acid desaturase 2) gene is located on chromosome 11 (11q12–13.1) and is ubiquitously expressed, especially in the liver and brain (astrocytes) (Innis and Dyer, 2002; Nakamura and Nara, 2004), as well as in heart skeletal muscle, kidney, lung, prostate, testes, adipocytes, ovary, uterus and sebaceous glands (Ge et al., 2003; Nwankwo et al., 2003; Pedrón et al., 2010). The FADS2 gene transcribes Δ6-desaturase (D6D), which is a membrane-bound enzyme that desaturates and introduces a double bond at specific carbon positions along the chain of at least six substrates: four polyunsaturated fatty acids (PUFAs), one monounsaturated fatty acid (oleic acid) and one saturate (palmitic acid) (Guillou et al., 2004; Rioux et al., 2015). The same D6D acts on 18- and also on 24-carbon fatty acids in very-long-chain polyunsaturated fatty acid biosynthesis (d’Andrea et al., 2002) (Fig. 1). D6D is the rate-limiting enzyme for converting α-linolenic acid (ALA) into docosahexaenoic acid (DHA) (n-3 fatty acid) (Fig. 1). Moreover, this FASD2 enzyme displays a Δ8-desaturase activity on 20:2 n-6 and 20:3 n-3 which leads respectively to 20:3 n-6 and 20:4 n-3 (Park et al., 2009).

There is a local competition between the substrates. The reactivity of the enzyme depends on the concentration of available substrates and their affinity with D6D. This is usually expressed as the enzyme’s Km (Michaelis constant), an inverse measure of affinity (Ivanitch et al., 1996; Rodriguez et al., 1998). The conversion rate is also dependent on tissue type.

Several studies examining n-3 fatty acids have demonstrated the transcription of FADS2. Dietary n-3 fatty acid intake regulates D6D expression (Cho et al., 1999), but conversion to DHA is limited (Burdge, 2006). D6D expression is subject to feedback regulation by dietary polyunsaturated fatty acids (PUFAs) (Nara et al., 2002). Several studies in animals and in vitro models suggest that D6D expression and/or activity is regulated by steroid hormones (Childs et al., 2008), as well as by insulin in the liver (Cho et al., 1999). More 18:3n-3 is converted to 20:5n-3 and 22:6n-3 in women than in men (Burdge, 2006). However, the mechanisms regulating FADS2 transcription have not been well established to date. This position paper aims to highlight the possible mechanisms involved – active molecules and transcription factors – while also considering the impact of single-nucleotide FADS2 promoter polymorphisms.

2 Regulation of the FADS2 gene

Peroxisome proliferator-activated receptor α (PPARα) modulates transcription of the FADS2 gene (Guillou et al., 2002) by interacting with a second transcription factor, retinoid X receptor α (RXRα) (Tang et al., 2003). PPARα is stabilized through heterodimerization with RXRα, and there is a rapid turnover of excess protein unpaired with RXRα, unless it is bound by an appropriate ligand (Hirotani et al., 2001). 17β-estradiol treatment upregulates PPARα mRNA expression and PPARα protein content (Campbell et al., 2003).

Like most nuclear receptors, PPARα is organized into functional domains called A/B, C, D and E/F. The A/B domain supports an activation function that is independent of ligand binding (Activation Function-1: AF-1). The central region, which contains the DNA-binding domain (domain C), contains the best-conserved nuclear receptor: a highly conserved two-finger zinc structure that allows interaction with the DNA molecule at specific sites. The C-terminal region, which contains domain E, is the second-best conserved region and contains the E domain. This region is the repository of numerous functions, in particular, the ligand-binding-dependent activation of genes (activation function 2 (AF-2) (Bugge and Mandrup, 2010).

PPARα is a phosphoprotein. Phosphorylation is an on/off switch for transcriptional PPARα activity. The phosphorylation of serine residues (at Ser 6, 12 and 21) (Barger et al., 2001) takes place in the A/B domain. It affects intramolecular communication, since the phosphorylation status of the A/B domain affects the activity of the E/F region of the protein (Burns and Vandenhoevel, 2007).

These transcription factors take the form of a PPARα-RXRα heterodimer, located within the nucleus and are modulated by ligands that modify their tertiary structures, and enable them to bind to the peroxisome proliferator response element (PPRE) located in the promoter region of the FADS2 gene. The PPAR contains a direct repeat sequence 1 (DR1) that assists in the regulation of human FADS2 gene transcription (Tang et al., 2003). The heterodimer bond to the PPRE modulates the transcription of the gene under highly specific conditions.

Several fatty acids prevalent in vivo, including palmitoleic acid (de Souza et al., 2017; Bolsoni-Lopes et al., 2013); oleic acid; linoleic acid (LA); α-linolenic acid (ALA); arachidonic acid (ARA) (Popeijus et al., 2014; Kliewer et al., 1997); eicosapentaenoic acid (EPA); and docosahexaenoic acid (DHA) (Diep et al., 2002; Deckelbaum et al., 2006; Dziedzic et al., 2018) function as ligands for PPARα at concentrations consistent with those found in human serum. 8-HETE (8-hydroxyeicosatetraenoic acid) is also a natural agonist of PPARα (Yu et al., 1995). This eicosanoid metabolite is a lipoygenase product of arachidonic acid. It is believed to have tissue concentrations that are too low to be a ligand for PPARα.

In their free form, unlinked to binding proteins, these ligands compete with PPARα in accordance with their relative concentrations and dissociation constants. Competing free-form DHA ligands (de Urquiza et al., 2000); arachidonic acid (less efficiently) (Egea et al., 2002); and 9-cis-retinoic acid (9-cis-RA), an active metabolite of vitamin A (Lengqvist et al., 2004) activate RXR-mediated transcription. The crystal structure of DHA bound to the ligand-binding pocket of RXRα indicates that, despite its higher affinity, 9-cis-RA displays a significantly lower number of ligand-protein contacts than DHA, when positioned in the ligand-binding pocket (Egea et al., 2002). By contrast, EPA (another n-3 fatty acid) fails to activate RXR-mediated transcription even though both DHA and EPA activate PPARα (Deckelbaum et al., 2006).

Alternatively, transcription factor Elk-1, an ETS domain transcription factor encoded by the ELK1 gene, could be one of the proteins that binds to the promoter region of the FADS2 gene (Lattka et al., 2010). Elk-1 is known to play a role in transcription regulation by forming ternary complexes with serum response factor (SRF) on the serum response elements (SRE) of gene promoters (Hipskind et al., 1991). Elk-1
contains a MAPK-binding motif (Yang et al., 1998). Its activation and nuclear translocation require phosphorylation of serine 383–389, located in the C-terminal region of Elk-1, via the ERK-MAPK pathway, which results in a conformational change to Elk-1 (Lavaur et al., 2007). In silico, phosphorylated ELK-1 would act as a coactivator.

Does biological sex affect the regulation of the FADS2 gene? Observational evidence suggests that in populations that consume low levels of n-3 highly unsaturated fatty acids, women have higher blood DHA levels than men (Kitson et al., 2010). Women of reproductive age are known to convert more ALA into DHA than men (Burdge and Wootton, 2002). Estrogens cause higher DHA concentrations in plasma cholesteryl esters in women than in men, by upregulating synthesis from ALA. This difference is independent of dietary differences. It has also been suggested that estradiol may increase the activity of the desaturation pathway because DHA synthesis has been shown to be almost 3 times greater in women who take oral contraceptive pills that contain ethinylestradiol than in women who do not, while testosterone stimulus decreases DHA status (Giltay et al., 2004). This difference in conversion appears to be associated with estrogen and some evidence indicates that the expression of enzymes, including desaturases, involved in synthesizing DHA from ALA is higher in females. PPARα may mediate an estrogen-associated effect. However, because estrogens are weak ligands for PPARα, estrogen-mediated increases in PPARα activity probably occur through an indirect mechanism involving membrane-bound estrogen receptors (Kitson et al., 2010). What is the relationship between the regulation of the FADS2 gene by estradiol and by PPARα?

3 PPARα-RXRα regulation mechanisms governing the FADS2 gene

As we mentioned above, at least two key molecules are involved in the regulation of the FADS2 gene: free 17β-estradiol, when not bound to its binding protein SHBG (sex hormone-binding globulin) – when bound to SHBG it is not biologically active – and PPARα.

Free estradiol induces PPARα activation via two pathways. First, by transcription, through its genomic action on the PPARα gene, which is mediated by an estrogen receptor (ER) (Campbell et al., 2003). Then, via its non-genomic effect, mediated by ERα, which allows phosphorylation and activates PPARα via the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway (ERK1/2-MAPK) (Moreno et al., 2010), as we will show below. This is also true of ELK-1.

Free estradiol has a synergistic effect with insulin-like growth factor 1 (IGF-1) (Majou, 2015). For example, several studies have shown this interaction in different brain regions.
Garcia-Segura et al., 2006; Varea et al., 2010; Park et al., 2014) and in breast cancer cells (Song et al., 2010). The anabolic action of IGF-1 is mediated by the IGF-1 receptor (IGF-1R). When IGF-1 binds to the IGF-1R, it causes a conformational change to the receptor, inducing the auto-phosphorylation of tyrosine residues (Hubbard and Till, 2000). This leads to the recruitment of insulin receptor substrates (IRS-1–4), which in turn phosphorylate the tyrosine residues of the IRSs. Estradiol stimulates the rapid activation of the IGF-1R through phosphorylation via ERα, and induces the formation of a ternary protein complex comprising of phosphorylated Shc protein, ERα and IGF-1R (Song et al., 2004). Adapter protein Shc generally acts by activating MAPK and phosphoinositide-3-kinase/Akt signaling pathways (Gu et al., 2000; Vindis et al., 2003). The tyrosine phosphorylation of Shc is mediated by the tyrosine kinase Fyn (Src family kinase) associated with caveolin-1 (scaffolding protein). Upon integrin ligation, Fyn is activated and binds to Shc via its SH3 domain. Shc is subsequently phosphorylated at tyrosine 317 (Wary et al., 1998). IGF-1R activation stimulates MAPK kinases and consequently phosphorylation of ERK1/2. Activation of ERK1/2 may in turn lead to phosphorylation of ERα (Kato et al., 1995; Russo et al., 2002). This process leads to the activation of two main downstream signaling pathways: phosphoinositide-3-kinase (PI3K)/Akt and MAPK cascades (Zheng et al., 2000). Thus, free estradiol induces MAPK activation (Kahlert et al., 2000) and PPARα phosphorylation. This is also true of ELK-1 (Duan et al., 2001) (Fig. 2).

The activated PPARα function is then regulated by its ligands, initially by binding to RXRα. Each PPARα and RXRα ligand modifies the tertiary structure of each transcription factor, creating a specific PPARα-RXRα heterodimer conformation that modulates binding to the FADS2 gene. The change in ligand binding domain conformation (AF-2) following ligand binding induces the recruitment of coregulators. In the absence of a ligand or in the presence of an antagonist, PPARα interacts with nuclear receptor corepressors (NCoR1/2), which block coactivators from binding and prevent the formation of the transcriptional complex (Dowell et al., 1999). This repression is reversible. When ligands (all-trans retinoic acid, 9-cis-retinoic acid) (Allenby et al., 1993) are bound by RAR (the retinoic acid receptor), this promotes the dissociation of the PPRE-NCoR complex and the subsequent interaction of

![Diagram of Estradiol: PPARα transcription and phosphorylation.](image-url)
PPRE with one or more coactivator proteins including the cAMP response element-binding protein (CREB)/p300 (Dowell et al., 1997), and steroid receptor coactivator 1 (SRC-1) (Gocke et al., 2009).

Given that the expression of Δ6-desaturase is retro-inhibited by free intracellular DHA (Matzusaka et al., 2002; Bewicz-Binkowska et al., 2019), we propose the following original hypothesis: when DHA simultaneously binds to phosphorylated PPARα and RXRα, the DHA-PPARα-RXRα-DHA heterodimer represses the FADS2 gene via PPRE. Thus, depending on its concentration and binding affinities with PPARα and RXRα, DHA could block other competing ligands from accessing the two transcription factors. Both blockage and inhibition are reversible (Fig. 3). They can be overcome by competition between free DHA not bound to its binding protein called FABP (fatty acid binding protein), which is tissue-dependent (the FABP-bound fraction is not biologically active) and (i) free MUFA (palmitoleic acid, oleic acid) or PUFA (LA, ALA, EPA, ARA), not bound to FABP, on PPARα; (ii) free 9-cis-retinoic acid, not bound to its binding protein (retinoic acid-binding protein), on RXRα. Indeed, in the absence of 9-cis-retinoic acid, DHA diminishes FADS2 gene expression, and, in its presence, DHA upregulates its expression, especially in astrocytes (Dziedzic et al., 2018). At 75μM, DHA represses PPARα transactivation almost completely, in a dose-dependent manner, and acts as an antagonist (Lee and Hwang, 2014); conversely, DHA activates at lower doses (Popeijus et al., 2014).

Under basal conditions, the RARα-RXRα heterodimer represses the transcription of target genes by recruiting corepressor complexes on RARα, such as NCoR1/2 (Farboud et al., 2003). When a ligand binds to RARα, it causes a change to the receptor’s conformation. These changes cause a transition from transcriptional repressor activity to an activator state through the loss of corepressors and the recruitment of coactivator complexes, such as CBP/p300 and SRC-1. In the RARα-RXRα heterodimer, RXRα acts as a transcriptionally silent partner to RARα while it is active in the RXRα-RXRα homodimer. This phenomenon is known as the “subordination” of RXRα to RARα (Le Maire et al., 2019): (i) in the RARα-RXRα heterodimer, an RARα agonist alone allows for the transactivation of target genes; (ii) an RXRα agonist (9-cis-RA) alone is unable to dissociate corepressors from the complex, preventing RXRα/coactivator binding; (iii) the binding of retinoic ligands to RARα and RXRα induces a binding synergy with a coactivator. Above a certain concentration, we suggest that DHA would both bind to PPARα and replace 9-cis-RA in binding with RXRα. In such a case, RXRα-DHA would form one heterodimer with PPARα-DHA, and another with RXRα-retinoic acids. However, the retinoic acids-RARα-RXRα-DHA heterodimer would not dissociate in the presence of corepressors and would prevent binding with coactivators. Thus, above a certain concentration (Popeijus et al., 2014), DHA retro-inhibits the FADS2 gene, limits translation into D6D and the synthesis of DHA (Fig. 3).

It is interesting to note that the PPARα-RXRα regulation mechanisms governing the FADS2 gene are similar to those governing the human SCD gene. Stearoyl-CoA desaturase (SCD) is a lipid-metabolic enzyme that catalyses the insertion of a double bond between carbon atoms 9 and 10 in the
saturated fatty acids palmitic acid and stearic acid to generate the mono-unsaturated fatty acids palmitoleic acid and oleic acid respectively (Fig. 1). SCD is a key, rate-limiting enzyme in the cellular synthesis of MUFA from saturated fatty acids. A functional PPRE has been identified in the promoter of the SCD gene (Rakhshandehroo et al., 2010) and its gene expression is regulated by PPARα (Miller and Ntambi, 1996; Hebbachi et al., 2008).

DHA lowers the SCD gene’s protein expression and mRNA expression levels (Bellenger et al., 2004; On et al., 2019). All-trans-retinoic acid and 9-cis-retinoic acid increase SCD mRNA expression in a dose-dependent manner (Miller et al., 1997; Samuel et al., 2001; Mahesh et al., 2016). All these elements suggest that SCD regulation appears to be mediated by PPARα-RXRα and RARα-RXRα heterodimers with inhibition by DHA and activation by retinoic acids.

Thus, DHA seems to retro-regulate the FADS2 gene in two ways: (i) directly, by inhibiting the expression of the FADS2 gene, (ii) indirectly, by inhibiting the SCD gene. This repression downregulates SCD and decreases palmitoleic acid and oleic levels; these two MUFAs are PPARα ligands in competition with DHA.

Some studies suggest that D6D expression is dually regulated by SREBP-1c and PPARα (Matsuzaka et al., 2002). However, SREBP-1c’s mechanism of action remains under debate. The transcription factor, sterol regulatory element-binding protein (SREBP)-1c, controls lipogenesis in the liver (Shimano et al., 1999), but also in astrocytes (Tabernero et al., 2002) among other tissues. It regulates the expression of several genes that encode enzymes involved in fatty acid synthesis, in particular stearoyl-CoA desaturase (SCD)-1. SCD-1 catalyzes the rate-limiting step in biosynthesis (Kim et al., 2002). SCD-1 has a specific affinity for palmitic and stearic acid. Palmitoleic acid is synthesized from palmitic acid (Fig. 1) directly by desaturation (SCD-1) (likewise, oleic acid is synthesized from stearic acid). Palmitoleic acid is regarded as a lipid-controlling hormone, or "lipokine", because it modulates lipid metabolism. This characteristic (regulatory signal) distinguishes palmitoleic acid from oleic acid (Cao et al., 2008). Otherwise, palmitoleic acid functions as a ligand for PPARα and increases its transactivation (Popeijus et al., 2014).

Thus, we can assume that the regulation of D6D expression by SREBP-1c is carried out indirectly via SCD-1 and the synthesis of palmitoleic acid. This regulation would be upstream of PPARα and complementary to this transcription factor (Fig. 4).

SREBP-1c is bound to two other proteins: SCAP (SREBP-cleavage-activating protein), a polytopic membrane protein that serves as an escort for both SREBPs (Sakai et al., 1997) and Insig-1 (insulin-induced gene 1). The dissociation of Insig-1 from the SREBP-SCAP complex allows the complex to migrate from the endoplasmic reticulum to the Golgi apparatus, where SREBP is cleaved by S1P and S2P (site-1 and –2 proteases) – two enzymes that are activated by SCAP. The cleaved SREBP-1c then migrates to the nucleus and acts as a transcription factor to bind to the sterol regulatory element (SRE). The retention of the SREBP-SCAP complex in the endoplasmic reticulum depends on whether it binds to the endoplasmic reticulum resident proteins, Insig-1. By binding with SREBP-1c, Insig-1 inhibits these proteins depending on the level of Insig-1. Insig-1 is a critical regulator of SREBPs (Yang et al., 2002; Yabe et al., 2002) (Fig. 4).

It should be noted that DHA has been known to negatively regulate SREBP-1c (Song et al., 2014). Otherwise, the PPARγ-RXRα heterodimer, activated by DHA (Song et al., 2017), transactivates the Insig-1 promoter (Kast-Woelbern et al., 2004). Proteolytic processing of SREBP-1c is inhibited (Hannah et al., 2001). Thus, by intervening on the synthesis of Insig-1, DHA slows the maturation of SREBP-1c and reduces its lipogenic action through the synthesis of D6D (Fig. 4).

4 Promoter polymorphisms of the FADS2 gene and their consequences

Several significant associations between FADS genotypes and long-chain polyunsaturated fatty acids have been confirmed in diverse types of human tissue (erythrocytes, plasma, skin, breast milk, etc.), demonstrating that FADS2 gene cluster polymorphisms are major regulators of the synthesis of this type of fatty acid. One study (Ameur et al., 2012) examined the genotyping of the FADS cluster in five European cohorts, as well as the genomic data available from human populations, archaic hominids and more distant primates. The results show that modern humans have two haplotypes (groups of alleles at different loci on the same chromosome) for the FADS cluster: A and D, defined by 28 SNPs. These two haplotypes differ considerably in their capacity to synthesize long-chain fatty acids. In the two families of fatty acids – omega-3 and omega-6 – haplotype D is strongly associated with lower levels of precursors to the synthesis of fatty acids (α-linolenic acid, linoleic acid) and higher levels of EPA, DHA and arachidonic acid. This indicates that this haplotype is more effective at converting precursors. People who are homozygous for haplotype D have 24% more DHA and 43% higher levels of arachidonic acid than homozygotes for haplotype A. Several studies have shown a close correlation between several single nucleotide polymorphisms (SNPs) in the FADS1 and FADS2 genes and the concentrations of omega-3 and -6 fatty acids (Schaeffer et al., 2006; Xie and Innis, 2008; Rzehak et al., 2009; Glaser et al., 2011). Homozygous carriers of different minor alleles have higher levels of desaturase substrates (α-linolenic acid, linoleic acid) and lower levels of desaturation products (DHA, EPA, arachidonic acid) (Glaser et al., 2011). This suggests the reduced expression of desaturases in these polymorphisms (Molto-Puigmarti et al., 2010). Conversely, several studies suggest that some alleles of several SNPs in the FADS2 gene cluster are associated with higher D6D activity (homozygous carriers of the minor allele of rs1535) (Harsløf et al., 2013).

Nwankwo et al. (2003) demonstrated that the insertion of a nucleotide in the transcriptional regulatory region of the human FADS2 gene resulted in D6D deficiency and decreased FADS2 transcription (minor allele rs968567). Promoter-reporter assays demonstrated a 6-fold decrease in promoter activity in the regulatory region of the polymorphic FADS2 variant compared with the normal gene, confirming the functional relevance of the insertion mutation to diminished expression of the gene. Lattka et al. (2010) showed that the FADS2 promoter region surrounding SNP rs968567 exhibits promoter activity, which increases when the major C allele of SNP rs968567 is replaced by the minor T allele. This effect could be caused by the allele-specific differential binding affinity of transcription factors.
Given the hypothesis that the DHA-PPARα-RXRα-DHA heterodimer represses the FADS2 gene via its PPRE and the results of these studies, we speculate that SNPs, especially those on PPRE, modulate the binding affinity of DHA-PPARα-RXRα-DHA heterodimer on PPRE. If this affinity is increased, it will take less DHA to bind to PPARα and inhibit the transcription of FADS2 gene, and vice versa with decreased affinity. Lower production of D6D and DHA result from greater DHA-PPARα-RXRα-DHA heterodimer affinity for PPRE (Lattka et al., 2010). FADS2 promoter polymorphism would enhance competition between DHA and other ligands, depending on their concentrations and affinities (dissociation constants Kd).

5 Conclusion

The DHA concentration level is highly dependent on the catalytic capacity of D6D, a key enzyme in DHA synthesis. The regulation of the FADS2 gene by PPARα-RXRα and their ligands is compatible with the recruitment of coactivators and corepressors. DHA causes dose-dependent genetic feedback inhibition of the FADS2 promoter.

Mutations in the FADS2 gene can have positive or negative effects on D6D levels. The polymorphism of this gene appears to have been essential in allowing the Homo genus to adapt to its diet and evolve (Majou, 2018). The geographic distribution of FADS2 genetic variants now differs dramatically between continents. These differences in the capacity to synthesize DHA might contribute to health disparities. For example, over the past 10 years or so, studies have increasingly highlighted the relationship between DHA deficiency and certain pediatric neuropathologies, such as hyperactivity, learning difficulties (Milte et al., 2012), mental retardation (Negers et al., 2009), epilepsy (Emory University Health Sciences Center, 2004) and autism (Bent et al., 2009). In the elderly, DHA depletion is an aggravating factor in the etiology of Alzheimer’s disease (Majou, 2015).

It is essential to understand the regulation modalities of this gene, particularly in relation to its various alleles. Some SNPs in the FADS2 gene are considered an aggravating factor in DHA deficiency. The dose-dependent feedback inhibition caused by DHA in the DHA-PPARα-RXRα-DHA heterodimer could be a key factor for people with a genetic DHA deficiency. DHA depletion caused by FADS gene polymorphisms should be compensated entirely or partially by dietary DHA intake.
Conflicts of interest. The author declares that he has no conflicts of interest in relation to this article.

References

Allenby G, Bocquel MT, Saunders M, et al. 1993. Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. 

Ameur A, Enroth S, Johansson A, et al. 2012. Genetic adaptation of fatty-acid metabolism: A human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. 

Balfour PM, Browning AC, Garner AN, Kelly DP. 2001. P38 mitogen-activated protein kinase activates peroxisome proliferator-activated receptor alpha: a potential role in the cardiac metabolic stress response. 

Bolger J, Bellenger S, Clément L, et al. 2004. A new hypotensive polysaturated fatty acid diet combination regulates oleic acid accumulation by suppression of stearoyl CoA desaturase 1 gene expression in the SHR model of genetic hypertension. 

Barger PM, Browning AC, Garner AN, Kelly DP. 2001. P38 mitogen-activated protein kinase activates peroxisome proliferator-activated receptor alpha: a potential role in the cardiac metabolic stress response. 

Childs CE, Romeu-Nadal M, Burdge GC, Calder PC. 2008. Gender differences in the n-3 fatty acid content of tissues. 

Diep QN, Amiri F, Touyz RM, et al. 2002. PPARalpha activator effects on Ang II-induced vascular oxidative stress and inflammation. 

Dowell P, Ishmael JE, Avram D, Peterson VJ, Nevrivy DJ, Leid M. 1997. p300 functions as a coactivator for the peroxisome proliferator-activated receptor alpha. 

Dowell P, Ishmael JE, Avram D, Peterson VJ, Nevrivy DJ, Leid M. 1999. Identification of nuclear receptor coressor as a peroxisome proliferator-activated receptor alpha interacting protein. 

Duan R, Xie W, Safe S. 2001. Estrogen receptor-mediated activation of theserum response element in MCF-7 cells through MAPK-dependent phosphorylation of Elk-1. 

Dziedzic B, Biewicz-Binkowska D, Zgorzynska E, et al. 2018. DHA upregulates FADS2 expression in primary cortical astrocytes exposed to vitamin A. 

Egea P, Mirtschler A, Mosas D. 2002. Molecular recognition of agonist ligands by RXRs. 

Emory University Health Sciences Center. 2004. Patients with uncontrolled epilepsy have low levels of fatty acids. 

Farboud B, Hauksdottir H, Wu Y, Privalks ML. 2003. Isoytype-restricted corepressor recruitment: a constitutively closed helix 12 conformation in retinoic acid receptors beta and gamma interferes with corepressor recruitment and prevents transcriptional repression. 

Garcia-Segura LM, Sanz A, Mendez P. 2006. Cross-Talk between GF-I and Estradiol in the Brain: Focus on Neuroprotection. 

Ge L, Gordon JS, Hsuan C, Stenn K, Prouty SM. 2003. Identification of the delta-6 desaturase of human sebaceous glands: expression and enzyme activity. 

Gilgaj EJ, Gooren LJG, Toorians AWFT, Katan MB, Zook PL. 2004. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. 

Glaser C, Lattka E, Rzezak P, Steer C, Koletzko C. 2011. Genetic variation in polysaturated fatty acid metabolism and its potential relevance for human development and health. 

Gocke AR, Hussain RZ, Yang Y, et al. 2009. Transcriptional modulation of the immune response by peroxisome proliferator-activated receptor-alpha agonists in autoimmune disease. 

Gu H, Maeda H, Moon JJ, et al. 2000. New role for Shc in activation of the phosphatidylinositol 3-kinase/Akt pathway. 

Harsløf LB, Larsen LH, Ritz C, et al. 2012. Genetic adaptation of a haplotype increasing the very-long-chain polyunsaturated fatty acid biosynthesis. 

Harms r LF, Larsen LH, Ritz C, et al. 2013. FADS genotype and diet are important determinants of DHA status: a cross-sectional study in Danish infants. 

Hebbachi AM, Knight BL, Wiggins D, Patel DD, Gibbons GF. 2008. Peroxisome proliferator-activated receptor alpha deficiency
abolishes the response of lipogenic gene expression to re-feeding: restoration of the normal response by activation of liver X receptor alpha. J Biol Chem 283(8): 4866–4876.

Hipskind RA, Rao VN, Mueller CG, Reddy ES, Nordheim A. 1991. Ets-related protein Elk-1 is homologous to the c-fos regulatory factor p62TCF. Nature 354(6354): 531–534.

Hirotani M, Tsukamoto T, Bourdeaux J, Sadano H, Osumi T. 2001. Stabilization of peroxisome proliferator-activated receptor alpha by the ligand. Biochim Biophys Acta 1485(1): 106–110.

Hubbard SR, Till JH. 2000. Protein tyrosine kinase structure and function. Annu Rev Biochem 69: 373–398.

Innis SM, Dyer RA. 2002. Brain astrocyte synthesis of docosahexaenoic acid from n-3 fatty acids is limited at the elongation of docosapentaenoic acid. J Lipid Res 43(9): 1529–1536.

Ivanetich KM, Bradshaw JJ, Ziman MR. 1996. Delta 6-desaturase: improved methodology and analysis of the kinetics in a multi-enzyme system. Biochim Biophys Acta 1292(1): 120–132.

Kahlert S, Nuedling S, van Eickels M, Vetter H, Meyer R, Grohe C. 2000. Estrogen receptor alpha rapidly activates the IGF-I receptor pathway. J Biol Chem 275: 18447–18453.

Kast-Woelbern HR, Dana SL, Cesario RM, et al. 2004. Rosiglitazone induction of Insig-1 in white adipose tissue reveals a novel interplay of peroxisome proliferator-activated receptor gamma and sterol regulatory element-binding protein in the regulation of adipogenesis. J Biol Chem 279(23): 23908–23915.

Kato S, Endoh H, Masuhiro Y, et al. 1995. Activation of the estrogen receptor by phosphorylation by mitogen-activated protein kinase. Science 270(5241): 1491–1494.

Kim HJ, Miyazaki M, Nambi JM. 2002. Dietary cholesterol opposes PUFA-mediated repression of the stearoyl-CoA desaturase-1 gene by SREBP-1 independent mechanism. J Lipid Res 43(10): 1750–1757.

Kitson AP, Stroud CK, Stark KD. 2010. Elevated production of docosahexaenoic acid in females: potential molecular mechanisms. Lipids 45(3): 209–224.

Kliewer SA, Sundseth SS, Jones SA, et al. 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci U S A 94(9): 4318–4323.

Lattka E, Eggers S, Moeller G, et al. 2010. A common FADS2 promoter polymorphism increases promoter activity and facilitates binding of transcription factor ELK1. J Lipid Res 51(1): 182–191.

Lavau J, Bernard F, Trifillieff P, et al. 2007. TAT-DEF-Elk-1 peptide regulates the cytonuclear trafficking of Elk-1 and controls cytoskeleton dynamics. J Neurosci 27(52): 14448–14458.

Le Maire A, Teysssier C, Balaguer P, Bourguet W, Germain P. 2019. Regulation of RXR-RAR Heterodimers by RXR- and RAR-Specific Ligands and Their Combinations. Cells 8(11): 1392.

Lee KY, Hwang DH. 2002. Docosahexaenoic acid suppresses the activity of peroxisome proliferator-activated receptors in a colon tumor cell line. Biochem Biophys Res Commun 298(5): 667–674.

Lengqvist J, de Urquiza A, Bergman AC, et al. 2004. Polynsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain. Mol Cell Proteomics 3(7): 692–703.

Mahesh M, Bharath M, Reddy MR, et al. 2016. Carrot juice administration decreases liver stearyl-CoA desaturase 1 and improves docosahexaenoic acid levels, but not steatosis in high fructose diet-fed weanling wistar rats. Prev Nutr Food Sci 21(3): 171–180.

Majou D. 2015. Alzheimer’s disease: origins, mechanisms, people at risk and prevention by DHA (omega-3 fatty acid). Paris (France): Actia Editions.

Majou D. 2018. Evolution of the Human Brain: the key roles of DHA (omega-3 fatty acid) and Δ6-desaturase gene. OCL 25(4): A401.

Matsuzaka T, Shimano H, Yahagi N, et al. 2002. Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPARα. J Lipid Res 43(1): 107–114.

Miller CW, Nambi JM. 1996. Peroxisome proliferators induce mouse liver stearoyl-CoA desaturase 1 gene expression. Proc Natl Acad Sci U S A 93(18): 9443–9448.

Miller CW, Waters KM, Nambi JM. 1997. Regulation of hepatic stearoyl-CoA desaturase gene 1 by vitamin A. Biochem Biophys Res Commun 231(1): 206–210.

Milet CM, Parletta N, Buckley JD, Coates AM, Young RM, Howe PR. 2012. Eicosapentaenoic and docosahexaenoic acids, cognition, and behavior in children with attention-deficit/hyperactivity disorder: a randomized controlled trial. Nutrition 28(6): 670–677.

Moltó-Puigmarti C, Plat J, Mensink RP et al. 2010. FADS1 FADS2 gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk. Am J Clin Nutr 91: 1368–1376.

Moreno M, Lombardi A, Silvestri E, et al. 2010. PPARs: Nuclear receptors controlled by, and controlling, nutrient handling through nuclear and cytosolic signaling. PPAR Res 2010: 435689.

Nakamura MT, Nara TY. 2004. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Ann Rev Nutr 24: 345–376.

Nara TY, He WS, Tang C, Clarke SD, Nakamura MT. 2002. The E-b ox like sterol regulatory element mediates the suppression of human Delta-6 desaturase gene by highly unsaturated fatty acids. Biochim Biophys Acta 1485(1): 111–117.

Neggers YH, Kim EK, Song JM, Chung EJ, Um YS, Park T. 2009. Mental retardation is associated with plasma omega-3 fatty acid levels and the omega-3/omega-6 ratio in children. Asia Pac J Clin Nutr 18(1): 22–28.

Nwankwo JO, Spector AA, Domann FE. 2003. A nucleotide insertion in the transcriptional regulatory region of FADS2 gives rise to human fatty acid delta6-desaturase deficiency. J Lipid Res 44 (12): 2311–2319.

On S, Kim HY, Kim HS, Park J, Kang KW. 2019. Involvement of G-Protein-Coupled Receptor 40 in the Inhibitory Effects of Docosahexaenoic Acid on SREBP1-Mediated Lipogenic Enzyme Expression in Primary Hepatocytes. Int J Mol Sci 20(11): 2625.

Park S, Nozaki K, Smith JA, Krause JS, Banik NL. 2014. Cross-talk between IGF-1 and estrogen receptors attenuates intracellular changes in ventral spinal cord 41.1 motoneuron cells because of interferon-gamma exposure. J Neurochem 128(6): 904–918.

Park WJ, Kothapalli KS, Lawrence P, Tyburczy C, Brenna JT. 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(6): 1195–1202.

Pedróno F, Blanchard H, Kloareg M, et al. 2010. The fatty acid desaturase 3 gene encodes for different FADS3 protein isoforms in mammalian tissues. J Lipid Res 51(3): 472–479.

Popeijus HE, van Otterdijk SD, van der Krieken SE, et al. 2014. Fatty acid chain length and saturation influences PPARα transcriptional activation and repression in HepG2 cells. Mol Nutr Food Res 58 (12): 2342–2349.

Rakhshandehroo M, Knoch B, Müller M, Kersten S. 2010. Peroxisome proliferator-activated receptor alpha target genes. PPAR Res 2010: 612089.

Rioux V, Choque B, Ezanno H, Duby C, Catheline D, Legrand P. 2015. Influence of the cis-9, cis-12 and cis-15 double bond position in octadecenoic acid (18:1) isomers on the rat FADS2-catalyzed Δ6-desaturation. Chem Phys Lipids 187: 10–19.
Rodriguez A, Sarda P, Nessmann C, Boulot P, Leger CL, Descomps B. 1998. Delta6- and delta5-desaturase activities in the human fetal liver: kinetic aspects. *J Lipid Res* 39(9): 1825–1832.

Russo C, Dolcini V, Salis S, et al. 2002. Signal transduction through tyrosine-phosphorylated carboxy-terminal fragments of APP via an enhanced interaction with Shc/Grb2 adaptor proteins in reactive astrocytes of Alzheimer’s disease brain. *Ann NY Acad Sci* 973: 322–333.

Rzhak P, Heinrich J, Klopp N, et al. 2009. Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. *Br J Nutr* 101(1): 20–26.

Sakai J, Nohturfft A, Cheng D, Ho YK, Brown MS, Goldstein JL. 1999. Identification of complexes between the COOH-terminal domains of sterol regulatory element-binding proteins (SREBPs) and SREBP cleavage-activating protein. *J Biol Chem* 272(32): 20213–20221.

Samuel W, Kuty RK, Nagniemen S, et al. 2001. Regulation of stearoyl coenzyme A desaturase expression in human retinal pigment epithelial cells by retinoic acid. *J Biol Chem* 276(31): 28744–28750.

Schaeffer L, Gohke H, Müller 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. *Hum Mol Genet* 15(11): 1745–1756.

Shimano H, Yahagi N, Amemiya-Kudo M, et al. 1999. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J Biol Chem* 274(50): 35832–35839.

Song J, Li C, Lv Y, Zhang Y, Amakye WK, Mao L. 2017. DHA increases adiponectin expression more effectively than EPA at relative low concentrations by regulating PPARα and its phosphorylation at Ser273 in 3T3-L1 adipocytes. *Natu Metab* 14: 52.

Song NY, Na HK, Back JH, Surh YJ. 2014. Docosahexaenoic acid inhibits insulin-induced activation of sterol regulatory-element binding protein 1 and cyclooxygenase-2 expression through upregulation of SIRT1 in human colon epithelial cells. *Biochem Pharmacol* 92(1): 142–148.

Song RX, Barnes CJ, Zhang Z, Bao Y, Kumar R, Santen RJ. 2004. The role of Shc and insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor alpha to the plasma membrane. *Proc Natl Acad Sci USA* 101(7): 2076–2081.

Song RX, Chen Y, Zhang Z, et al. 2010. Estrogen utilization of IGF-1-R and EGF-R to signal in breast cancer cells. *J Steroid Biochem Mol. Biol* 118(4–5): 219–230.

de Souza CO, Teixeira AAS, Biondo LA, Lima Junior EA, Batatinha HAP, Rosa Neto JC. 2017. Palmitoleic acid improves metabolic functions in fatty liver by PPARα-dependent AMPK activation. *J Cell Physiol* 232(8): 2168–2177.

Tabernerio A, Velasco A, Granda B, Lavado EM, Medina JM. 2002. Transcytosis of albumin in astrocytes activates the sterol regulatory element-binding protein-1, which promotes the synthesis of the neurotrophic factor oleic acid. *J Biol Chem* 277(6): 4240–4246.

Tang C, Cho HP, Nakamura MT, Clarke SD. 2003. Regulation of human delta-6 desaturase gene transcription: identification of a functional direct repeat-1 element. *J Biol Res* 44(4): 686–695.

de Urquiza AM, Liu S, Sjöberg M, et al. 2000. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science* 290 (5499): 2140–2144.

Varea O, Arevalo MA, Garrido JJ, Garcia-Segura LM, Wandosell Mendez P. 2010. Interaction of estrogen receptors with insulin-like growth factor-1 and Wnt signaling in the nervous system. *Steroids* 75(8–9): 565–569.

Vindis C, Cerretti DP, Daniel TO, Huynh-Do U. 2003. EphB1 recruits c-Src and p52Shc to activate MAPK/ERK and promote chemotaxis. *J Cell Biol* 62(4): 661–671.

Wary KK, Mariotti A, Zurrzolo C, Giancotti FG. 1998. A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell* 94(5): 625–634.

Xie L, Inmis 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 (11): 2222–2228.

Yabe D, Brown MS, Goldstein JL. 2002. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc Natl Acad Sci USA* 99(20): 12753–12758.

Yang SH, Whitmarsh AJ, Davis RJ, Sharrocks AD. 1998. Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1. *EMBO J* 17(6): 1740–1749.

Yang T, Espenshade PJ, Wright ME, et al. 2002. Crucial step in cholesterol homeostasis: steroids promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110(4): 489–500.

Yu K, Bayona W, Kallen CB, et al. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J Biol Chem* 270(41): 23975–23983.

Zheng WH, Kar S, Dore S, Quirion R. 2000. Insulin-like growth factor-1 (IGF-1): a neuroprotective trophic factor acting via the Akt kinase pathway. *J Neural Transm Suppl* 60: 261–272.

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