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Endogenous Production of Long-Chain Polyunsaturated Fatty Acids and Metabolic Disease Risk

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Abstract

Long chain polyunsaturated fatty acids (PUFAs) are important structural components of cellular membranes and are converted into eicosanoids which serve various biological roles. The most common dietary n-6 and n-3 PUFAs are linoleic acid and α -linoleic acid, respectively. These 18-carbon chain fatty acids undergo a series of desaturation and elongation steps to become the 20-carbon fatty acids arachidonic acid and eicosapentaenoic acid, respectively. Evidence from genome wide association studies has consistently demonstrated that plasma and tissue levels of the n-6 long-chain PUFA arachidonic acid and to a lesser extent the n-3 long-chain PUFA eicosapentaenoic acid, are strongly influenced by variation in *fatty acid desaturase-1,-2*, and *elongation of very long chain fatty acid* genes. Studies of functional variants in these genes, as well as studies in which desaturase activity has been indirectly estimated by fatty acid product-to-precursor ratios, have suggested that endogenous capacity to synthesize long-chain PUFAs may be associated with metabolic diseases such as diabetes mellitus. Interventional studies are starting to tease out the complicated relationship between dietary intakes of specific fatty acids, variation in desaturase and elongase genes and tissue levels of long chain PUFAs. Thus future studies of dietary PUFA interventions designed to reduce inflammatory and metabolic diseases will need to carefully consider how an individual's genetically-determined endogenous long-chain PUFA synthesis capacity might modify therapeutic response.

Keywords

Acids; polyunsaturated; fatty acid; delta 5 desaturase; fatty acid delta 6 desaturase; eicosanoids; diabetes mellitus

Introduction

Long-chain polyunsaturated fatty acids (PUFA) are chains of 18 to 22 carbon atoms that contain two or more sequential double bonds. The two predominant classes of PUFAs are

n-6 and n-3, categorized based on the position of the first double bond from the methyl end of the carbon chain (n- × nomenclature). In the Western diet, the n-6 PUFA linoleic acid (LA) (18:2n-6) accounts for 84-89% of total PUFA dietary intake [1]. LA is metabolized to arachidonic acid (ARA) (20:4n-6) through a series of desaturation and elongation reactions. The rate limiting step in this metabolic pathway is Δ^6 -desaturase (D6D, *FADS2*) which converts LA to γ -linolenic acid (GLA) (18:3n-6) [2]. GLA is then elongated to di-homo- γ -linolenic acid (DGLA) (20:3n-6) and then desaturated by Δ^5 -desaturase (D5D, *FADS1*) to ARA. Both the genes for D6D and D5D (*Fatty Acid Desaturase (FADS)-2* and *FADS1* respectively) exist in a cluster on chromosome 11 (11q12-q13.1) [3]. The elongase step involves *Elongation of Very Long Chain Fatty Acid (ELOVL) 5* which is located on chromosome 6 (6p21.1) [4].

In the Western diet the most commonly consumed n-3 PUFA is α -linolenic acid (ALA) (18:3n3) which accounts for 9-11% of total PUFA intake. [1] ALA can be converted to eicosapentaenoic acid (EPA) (20:5n-3) through the same series of reactions. ALA is desaturated by D6D to stearidonic acid (SDA) (18:4n-3) then subsequently elongated to eicosatetraenoic acid (20:4n-3) and finally desaturated by D5D to EPA. Through an additional elongation (*ELOVL2*) and desaturation (D6D) EPA can be converted to docosahexaenoic acid (DHA) (22:6 n-3). While EPA can be produced *in vivo* from ALA, in humans, this process is extremely inefficient and most tissue EPA is derived from dietary consumption of fatty fish [5-7]. This inefficiency may result from the much higher levels of LA typically consumed in the Western diet. Stable isotope studies of ALA suggest that consuming 6.9% of dietary energy from ALA can result in increased production of endogenous EPA although DHA production is less influenced [8]. D6D has a greater affinity for ALA and is inhibited by EPA [9].

Endogenous and exogenous sources of long chain PUFA

Humans cannot synthesize 18-carbon chain n-6 or n-3 PUFAs so all sources of LA and ALA are exogenous. Sources of LA include sunflower, corn, cottonseed, and safflower oils. ALA is commonly found in seed oils such as flax, rapeseed, and chia oils. Long-chain PUFAs such as ARA and EPA can come from both exogenous and endogenous sources. ARA is directly consumed through meat and egg products while EPA and DHA are the primary PUFAs in fatty fish.

Endogenous synthesis of ARA from LA may comprise a significant proportion of total body stores of ARA. Interestingly, in the largest study to date in which red blood cell (RBC) membrane phospholipid fatty acids were measured in 160,000 patients, Harris *et al.* reported that RBC membrane ARA changed very little with age despite finding age-related changes in LA, EPA, and DHA [4]. These very consistent concentrations of ARA over multiple age groups suggest a homeostatic mechanism. Compared with other PUFAs, older individuals tended to have lower LA and higher EPA and DHA RBC membrane content when compared to younger adults. A recent systematic review of interventional studies concluded that typical dietary intakes of LA, as well as marked increases or decrease of dietary LA, did not seem to affect tissue ARA levels [10]. Tracer studies have reported very low levels of synthesis of ARA from LA ranging from 0.2% to 0.6% [8, 11].

Despite studies suggesting a low rate of conversion of ARA from LA, multiple genetic studies have suggested variants in *FADS1*, and -2 make major contributions to tissue ARA levels. In a study conducted in kibbutz settlements in Israel of 80 families, the heritability estimated for RBC membrane concentrations were 0.60 ± 0.11 for ARA, 0.52 ± 0.11 for EPA, and 0.65 ± 0.09 for DHA, suggesting a major heritable component of variance in tissue levels of long chain PUFAs [12]. Correlations of RBC membrane ARA levels between spouses were estimated at 0.08 compared to 0.18 between parent-child and 0.41 between siblings. Similarly, genomic studies have established that variants in *FADS1* strongly influence RBC membrane and plasma levels of ARA [13]. To date nine genome-wide association studies (GWAS) found a strong association between single nucleotide polymorphisms (SNPs) at the *FADS* gene cluster and PUFA levels [14-22] with 18 to 28% of the additive variance in tissue ARA levels explained by *FADS* genotypes [23, 24]. In a GWAS of the InCHIANTI study, Tanaka et al. investigated genetic factors associated with plasma PUFA levels and found a dose-response relationship between alleles at the rs174537 SNP in *FADS1* and ARA levels (8.76% for GG, 7.39% for GT, 6.35% for TT, *P*-value <0.0001) [14]. This finding was replicated in the GOLDN cohort with a similar statistically significant association with ARA concentration [14]. This SNP tags haplotype blocks described by two prior studies that found strong associations between *FADS1* gene variation and tissue levels of ARA [23, 24]. Additional GWAS studies have also associated variants in *ELOVL2* with n-3 PUFA levels [17, 19-21] although these results have not been consistently demonstrated in other studies [25].

Although the *FADS* gene cluster has a well-described role in PUFA metabolism, recent studies have demonstrated that *FADS* may also have a role in saturated and monounsaturated synthesis via *de novo* lipogenesis. In a large GWAS including 8,961 European ancestry individuals from 5 population-based cohorts, variants in *FADS1* and -2 were associated with higher plasma palmitoleic acid (16:1n-7) and oleic acid (18:1n-9) levels and lower stearic acid (18:0) levels[26]. Mechanisms behind these intriguing findings are unknown but suggest there may be an association between PUFA synthesis and *de novo* lipogenesis.

In order to determine whether variation in desaturase genes might influence desaturase activity several studies have investigated polymorphisms in *FADS1* and 2, and indirectly calculated fatty acid desaturase product to precursor ratios. D6D activity is usually estimated by dividing the RBC membrane concentration of GLA by the concentration of LA and D5D activity is estimated by dividing the concentration of DGLA by ARA [27]. This method has been utilized in several observational studies, although in some cases the ratio is calculated by dividing ARA by LA, which considers desaturase activity and elongase activity. In a random sample of 2,066 participants from the European Prospective Investigation into Cancer and Nutrition-Potsdam Study, Zietemann et al. found that variation at rs174546 was related to D6D and D5D activity and that the dietary ratio of n-6 to n-3 PUFA might modify the relationship between this variant and D5D activity [28]. An additional study also demonstrated that genetic variants in *FADS* are associated with indirectly calculated *FADS* activity [29].

Estimated desaturase activity can be influenced by diet, particularly PUFA intake [27]. Vessby et al. conducted a randomized controlled, three-month dietary intervention

comparing a diet containing butter fat (n = 17) with a diet containing monounsaturated fats (n = 17) [30]. Both diets contained 37% total energy from fats, with 17% energy from saturated fats (butter fat) versus 23% energy from MUFA in the comparison diet. In each diet participants were also randomized to receive fish oil capsules (2.4 g EPA and DHA) or placebo. There was no change in estimated D6D and D5D ratios in either serum cholesterol esters or phospholipids over the course of the study in either arm; however, participants randomized to fish oil had a decreased estimated D6D activity and an increased estimated D5D activity. These results are somewhat different from an 8-week crossover trial in 17 women comparing a high-fat diet (40% fat), a low-fat diet (20% fat) and a low-fat diet with the addition of 3% energy from n-3 PUFA (ALA, EPA, and DHA). The ratio of saturated to MUFA to PUFA was 1:1:1 in the high fat and the low fat diets [31]. In this study, the high-fat diet reduced D6D and increased D5D activity from baseline while the low-fat diet only increased D5D activity [31]. The low-fat with n-3 fatty acids diet had no effect on indirectly calculated desaturase activity which is intriguing as animal studies suggest that n-3 LC PUFAs inhibit D6D [32]. In this study, the total daily amount of EPA and DHA was 1.45 g and somewhat lower than the Vessby trial.

In addition, variants in desaturase and elongase genes might interact with dietary fats to influence PUFA tissue levels. In a secondary analysis of the Modulation of Atherosclerosis Risk by increasing dose of N-3 fatty Acids (MARINA) study, variants in *ELOVL2* significantly influenced participant responses to n-3 supplementation [33]. This study found that minor alleles in three SNPs in *ELOVL2* were associated with lower baseline levels of DHA but higher post-treatment levels of EPA and DHA compared to homozygotes for the major allele. This interaction was only seen in the high dose group (1.8 g EPA/DHA per day). Gillingham et al. compared a diet enriched with flaxseed oil or high-oleic acid canola oil to a Western diet in a randomized crossover clinical trial [34]. Although carriers of *FADS* variant minor alleles had lower EPA levels, they also had a marked increase in EPA levels in response to increasing ALA intake. Thus, a complicated relationship exists between intake of dietary PUFAs, genetic variation in desaturase and elongase enzymes, and tissue levels of long chain PUFAs, which has not been fully elucidated.

Lifestyle factors might also influence estimated desaturase activities [35]. In a cross-sectional analysis of 1,782 participants of the EPIC-Potsdam study estimated desaturase activities were calculated from RBC membrane phospholipids to discover dietary and lifestyle factors which might be associated with estimated activities [36]. Body mass index and waist-to-hip ratio were only weakly correlated with desaturase activity (positively correlated for D6D and negatively correlated for D5D). Alcohol use was positively correlated to D6D activity but only explained a small percentage of the variance (1.52%).

Arachidonic Acid Derived Eicosanoids

Understanding the contributions of endogenous long chain PUFA synthesis to tissue ARA stores could have important clinical implications given the biological function of ARA-derived eicosanoids and their strong role in many common diseases [37, 38]. ARA is released from the cellular membranes through the action of phospholipase A₂. Free ARA can be converted into various series of 2, 4 prostaglandins (PG), leukotrienes (LT) and

thromboxanes (TXs). In the cyclooxygenase (COX) pathway, ARA is enzymatically converted to PGG₂ which is further reduced to PGH₂. PGH₂ is then metabolized via specific PG synthases into various bioactive lipid molecules including PGE₂, PGD₂, PGF_{2α}, PGI₂ and TXA₂. These molecules then exert their cellular functions through binding to cell surface receptors of the 7 transmembrane G-coupled rhodopsin-type receptors. Some of the cellular functions of these ARA-derived eicosanoids include: inducing inflammation and promoting tumor angiogenesis (PGE₂) inhibiting platelet aggregation and vasodilation (PGI₂), and promoting platelet aggregation and vasoconstriction (TXA₂).

EPA is converted to eicosanoids through the same enzymatic pathways as ARA but produces series-3 prostanoids that have less inflammatory actions due to lower receptor affinities when compared to ARA derived series-2 prostanoids [39, 40]. Several studies have demonstrated that increased consumption of marine fatty fish results in an increased phospholipid membrane proportion of EPA and a concomitant decreased in ARA [41-43].

Data on whether dietary changes in PUFA might impact the production of eicosanoids-derived from ARA has been inconsistent. In a randomized crossover trial including 17 postmenopausal women who consumed an 8-week high-fat diet, low-fat diet and a low-fat diet enriched with n-3 PUFAs, urinary prostaglandin E metabolite and 11-dehydro-thromboxane B₂ concentrations were greater in the high-fat group compared to the low-fat group [44]. Interestingly plasma ARA increased with the low fat diet and decreased with the high fat diet. Clinical trials have demonstrated increased eicosanoid production with increased dietary n-6 PUFAs [45], decreased eicosanoid production with n-3 supplementation [46] or even increased concentrations of prostaglandin-F_{2α} and thromboxane B₂ with n-3 supplementation [47].

Although the effect of COX-2 (*PTGS2*) inhibitors on eicosanoid production, particularly PGE₂ is well described [48], several studies have suggested that inhibition of D6D might also impact PGE₂ production. He et al. found that reducing D6D activity through either RNAi knockdown or a selective D6D inhibitor reduced production of PGD₂, PGE₂, 12-HETE and 15-HETE by up to 80-95% in B16 melanoma cell lines [49]. They found a similar but lower reduction in these ARA-derived eicosanoids in LLC lung cancer cell lines.

Desaturase Activity and Intermediate Endpoints

Both genetic variation in *FADS* and alterations in indirectly calculated desaturase activity have been found to be associated with intermediate endpoints of disease. Recently published GWAS studies have found *FADS* variants associated with total cholesterol [50, 51], LDL cholesterol [50-52], HDL-cholesterol [50, 51, 53, 54], and triglyceride levels [50, 51, 53, 54]. *FADS* polymorphisms may not only impact absolute concentrations of certain blood lipids but also have other effects. Solakivi et al. genotyped 58 healthy volunteers for the *FADS2* deletion variant rs3834458 located in the promoter region of D6D and measured oxidation of LDL and HDL₂ cholesterol in response to CU²⁺ induced oxidation [55]. In homozygote carriers of the deletion, plasma total EPA and ARA were reduced. In addition the peroxidizability index of LDL cholesterol was markedly reduced. Stancakova et al. found that the *FADS1* variant rs174550 was associated with increased concentrations of very large and large HDL particles [56]. Additional variants in this gene cluster have also been

found to be associated with HDL particle size, although which variants are determinants of particle size and which are associated by linkage disequilibrium is unresolved [57].

The complex interaction between diet and genetic variation in endogenous long chain PUFA synthesis has also been investigated. Several studies have suggested that genetic variation in *FADS* appears to interact with dietary PUFA and possibly statin medications to influence serum lipids [58-60]. Interactions have been reported between variants within the *FADS* gene cluster, diet, and blood lipids. Within the Doetinchem Cohort Study, the *C* allele in rs174546 (*FADS1*) was associated with higher HDL cholesterol only in individuals with high intake of n-6 PUFAs (*P* for interaction = 0.02) [60]. Hellstrand et al. found no evidence of an interaction between the *C* allele in rs174547, HDL cholesterol, and intake of n-6 PUFAs however only carriers of the *C* allele had higher HDL cholesterol levels with increasing ALA to LA ratio. [61] These two studies suggest that diet and desaturase genotype may influence HDL levels but the direction of such an effect is still uncertain. To date no studies have investigated interactions between variants within the *FADS* gene cluster, dietary PUFAs, and glycemic traits, an area requiring future study. Fewer studies have investigated variants in elongase genes but noteworthy a recent population-based myocardial infarction case-control study investigated the association of seven *ELOVL* polymorphisms located in *ELOVL2*, *ELOVL4* and *ELOVL5* was unable to find any replicating association between any variants and serum lipids [25].

Although limited investigations utilizing genetic markers have been conducted, the effect of indirectly assessed desaturase activity has been investigated for inflammatory markers. In a study of Japanese school children, increased D6D and decreased D5D activity were correlated with CRP level [62]. This association was only seen for boys and not girls, which might suggest hormone interactions. In adults, Martinelli et al. found an increasing ratio of ARA to LA was associated with increased high-sensitivity C-reactive protein (hsCRP) [63]. Additionally, hsCRP concentrations increased with *FADS* alleles associated with the AA/LA ratio. Hong followed 122 non-obese healthy men for 3 years to determine the impact of variants in *FADS* on markers of oxidative stress. After 3 years, men with the rs17457 T allele had lower serum ARA, lower indirectly assessed D5D activity, and lower urinary 8-epi-prostaglandin F_{2α}, a marker of oxidative stress [64]. In general these studies suggest that decreasing D5D activity and increasing D6D activity might be associated with increased inflammation, although the evidence is still limited. In addition, lower D5D appeared to be associated with reduced oxidative stress.

Desaturase Activity and Metabolic Risk Factors

The observation that diabetes and insulin resistance may be associated with defects in n-6 PUFA metabolism has been reported in several studies using varied approaches [35, 65-88]. To date, three GWAS studies have reported associations between *FADS* variants and fasting glucose or metabolic syndrome [89-91]. In cross-sectional studies, insulin resistance, as determined by euglycemic clamp studies or the homeostatic model assessment for insulin resistance (HOMA-IR) have consistently demonstrated that D6D activity is positively correlated with insulin resistance while D5D activity is negatively correlated [66, 68, 69, 73,

74, 88]. In longitudinal studies, indirectly calculated D6D activity is associated with worsening hyperglycemia based on oral-glucose tolerance testing [92].

In the Uppsala Longitudinal Study of Adult Men, high activity of D6D was associated with an increased risk of developing metabolic syndrome (OR=1.35, 95% CI 1.10-1.65) while higher activity of D5D was associated with a decreased risk (OR = 0.71, 95% CI 0.57-0.87). [72] Another prospective study found the risk of developing diabetes to be increased with greater D6D activity (OR = 1.31, 95% CI 0.92-1.88) and reduced with increasing D5D activity (OR = 0.83; 95% CI 0.60-1.14) [67]. These results were not statistically significant as the study included 450 participants and was limited in power. To date, five prospective cohort studies have associated decreased D5D and increased D6D activity with development of insulin resistance [72, 92-95].

In the European Prospective Investigation into Cancer and Nutrition study, high activity of D6D was associated with an increased risk of type 2 diabetes (T2D)(OR=2.46, 95% CI 1.67-3.63) while higher activity of D5D was associated with decreased risk (OR = 0.46, 95% CI 0.31-0.70) [94]. This study was notable in that the investigators confirmed these results using a Mendelian randomization approach using the SNP rs174546 as the instrumental variable, strongly supporting a causal relationship. In this study, genetically determined low D6D activity was associated with a reduced risk for diabetes while lower D5D activity was associated with increased risk. Additional evidence supporting a causal association between polymorphisms in *FADS* and D6D/D5D activity comes from expression quantitative trait locus (eQTL) mapping studies. Westra et al. conducted an eQTL meta-analysis which included a discovery set of 5,311 and a replication set of 2,775 non-transformed peripheral blood samples pooled from 7 studies [96]. These investigators found a SNP in the 3' UTR region of *FADS1* (rs174546) to effect expression of *FADS1*, *FADS2* and *TMEM258*. This SNP has been found to be associated with LDL cholesterol levels [50-52] and interestingly this variant was also associated with *LDLR* gene expression levels suggesting additional downstream pathway effects of fatty acid desaturase polymorphisms.

Genetic variation in *FADS* genes may also modify the effects of n-3 PUFAs on fasting glucose. In a 6-week study of fish oil supplementation, a significant interaction was seen between supplement and *FADS* genotype on fasting glucose levels and HOMA-IR [97]. Participants took 5 g/d of fish oil and were genotyped for 18 SNPs in *FADS1*, -2. These investigators found a significant interaction between n-3 PUFA and fasting glucose based on rs482548 genotype.

While most prior studies of diabetes have focused on T2D in adults there is also evidence to suggest genetic variation in *FADS* might be associated with pancreatic beta cell dysfunction and type I diabetes. In the Diabetes Autoimmunity Study in the Young (DAISY) study 2,547 children at increased risk for type I diabetes, determine either through the detection of diabetes susceptibility alleles measured in cord blood or having a first-degree relative with type I diabetes were enrolled and followed with serial measurements for serum autoantibodies to insulin, glutamic acid decarboxylase, and insulinoma antigen 2. In this case-cohort study SNPs in *FADS1*, 2 (rs174556, rs174570, rs174583) significantly interacted with ALA intake, reducing the risk of islet autoimmunity [98].

Fatty Acid Desaturase Genetic Variants in CAD

The relationship of genetic variants of the *FADS* gene cluster to cardiovascular disease has been less clear. Baylin et al. found no association between *FADS* promoter variant rs3834458 which was related to EPA, ALA, and ARA levels in adipose tissue and plasma and myocardial infarction risk in a population-based case-control study conducted in Costa Rica [99]. In the Verona Heart Study, *FADS* haplotypes were associated with a higher desaturase activity and cardiovascular risk [63]. In a case-control study of coronary artery disease (CAD) in a Chinese Han population, rs174460 was found to be associated with CAD risk and increased D6D activity [100], and Qin et al. found rs174556 to be associated with CAD in Chinese Han [101]. In a case-cohort design from the prospective CAREMA study, estimated D5D activity, derived from measures of plasma cholesterol esters, was inversely associated with CAD risk [102]. In sub-analyses, participants with high baseline D5D activity estimates along with a high D5D activity genotype had a marked reduction in CAD risk compared to individuals with the lowest D5D activity and low activity genotype (HR = 0.35, 95% CI 0.15-0.81).

Conclusions

It is becoming increasingly clear that capacity to endogenously synthesize long-chain PUFAs, whether measured through genetic markers or fatty acid product-to-precursor ratios, is associated with metabolic diseases, with consistent effects noted for diabetes mellitus risk. It is possible that this association is mediated through the production of inflammatory eicosanoids; however additional studies are needed. Prior studies of PUFA-based dietary interventions on inflammatory mediators and disease outcomes have produced mixed results and this may stem from a failure to account for factors which might influence endogenous long-chain PUFA synthesis. A deeper understanding of the complicated relationship between dietary PUFAs and desaturase and elongase activity *in vivo* could help lead to better designed clinical studies and more patient-specific PUFA-based dietary interventions.

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Figure 1. Endogenous Production of Polyunsaturated Fatty Acids