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Gamma-linolenic acid, Dihommo-gamma linolenic, Eicosanoids and Inflammatory Processes

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Abstract

Gamma-linolenic acid (GLA, 18:3n-6) is an omega-6 (n-6), 18 carbon (18C-) polyunsaturated fatty acid (PUFA) found in human milk and several botanical seed oils and is typically consumed as part of a dietary supplement. While there have been numerous *in vitro* and *in vivo* animal models which illustrate that GLA-supplemented diets attenuate inflammatory responses, clinical studies utilizing GLA or GLA in combination with omega-3 (n-3) PUFAs have been much less conclusive. A central premise of this review is that there are critical metabolic and genetic factors that affect the conversion of GLA to dihommo-gamma linolenic acid (DGLA, 20:3n-6) and arachidonic acid (AA, 20:4n-6), which consequently affects the balance of DGLA- and AA-derived metabolites. As a result, these factors impact the clinical effectiveness of GLA or GLA/n-3 PUFA supplementations in treating inflammatory conditions. Specifically, these factors include: 1) the capacity for different human cells and tissues to convert GLA to DGLA and AA and to metabolize DGLA and AA to bioactive metabolites; 2) the opposing effects of DGLA and AA metabolites on inflammatory processes and diseases; and 3) the impact of genetic variations within the fatty acid desaturase (*FADS*) gene cluster, in particular, on AA/DGLA ratios and bioactive metabolites. We postulate that these factors influence the heterogeneity of results observed in GLA supplement-based clinical trials and suggest that “one-size fits all” approaches utilizing PUFA-based supplements may no longer be appropriate for the prevention and treatment of complex human diseases.

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Conflict of Interest

Floyd H. Chilton is a paid consultant for Eagle Wellness, LLC. This information has been revealed to Wake Forest Baptist Medical Center and is institutionally managed. Other authors have no conflict of interest.

Keywords

gamma-linolenic acid; dihommo gamma-linolenic acid; arachidonic acid; eicosanoid; inflammation

1. Introduction

Gamma-linolenic acid (GLA, 18:3n-6) is an omega-6 (n-6), 18 carbon (18C) polyunsaturated fatty acid (PUFA) found in human milk and several botanical seed oils (borage [~21% GLA], blackcurrant [~17%GLA] and evening primrose [~9%GLA]), and is typically consumed as a part of a dietary supplement. The scientific literature examining the clinical effects of GLA-containing supplements is both complex and confusing. While there have been numerous *in vitro* and *in vivo* animal models illustrating that GLA-supplemented diets attenuate various inflammatory responses, the clinical literature has been less conclusive (for a review, see (Fan and Chapkin, 1998)). The introduction of GLA supplementation strategies to achieve symptomatic relief of atopic dermatitis/eczema was historically preceded by the use of relatively large daily doses of oral linoleic acid (LA, 18:2n-6) containing oils. This was based on the premise that patients with atopic dermatitis/eczema had hallmark cutaneous signs of essential fatty acid deficiency and an impairment in PUFA biosynthesis at an early desaturase step (*FADS2;* Δ-6 desaturase) (Burr and Burr, 1929; Burr et al., 1932; Horrobin, 2000). It was hypothesized that GLA supplements could restore needed PUFAs and mitigate the disease.

Numerous studies primarily carried out in the 1980s and 1990s demonstrated that GLA-enriched botanical oils (evening primrose, borage, blackcurrant seed, and fungal-derived) had the capacity to relieve the signs and symptoms of several chronic inflammatory diseases, including rheumatoid arthritis (RA) and atopic dermatitis (Andreassi et al., 1997; Foolad et al., 2013; Kunkel et al., 1981; Leventhal et al., 1994; Leventhal et al., 1993; Lovell et al., 1981; Morse et al., 1989; Tate et al., 1989; Zurier et al., 1996). However, several more recent reviews and meta-analyses have questioned these earlier studies and raised doubts about the clinical effectiveness of GLA-enriched supplements particularly in the context of atopic dermatitis and RA (Bamford et al., 2013; Belch and Hill, 2000; Kitz et al., 2006; Macfarlane et al., 2011; Van Gool et al., 2004) (Table 1). A variety of issues complicate these studies including the fact that many of the trials have: 1) relatively low subject numbers; 2) less than ideal study designs (e.g. the absence of washout period in cross-over design trials); 3) variations in the types of GLA supplements and how they are administered (e.g. dose, duration); and 4) differences in selection/inclusion criteria (e.g. population demographics and disease states)(Foster et al., 2010; Van Gool et al., 2004).

Several studies have also investigated the effects of GLA when given in combination with botanical or marine omega-3 (n-3) enriched PUFA supplements. Enteral diets enriched with marine oils containing n-3 LC-PUFAs (i.e. eicosapentaenoic acid [EPA, 20:5n-3] and docosahexaenoic acid [DHA, 22:6n-3]) and GLA have been shown to reduce cytokine production and neutrophil recruitment into the lung resulting in fewer days on ventilation and shorter stays in the intensive care unit in patients with acute lung injury or acute

respiratory distress syndrome (Gadek et al., 1999; Pontes-Arruda et al., 2006; Singer et al., 2006). Importantly, these dietary combinations of GLA and n-3 LC-PUFAs were also shown to reduce both morbidity and mortality of critically ill patients (Gadek et al., 1999; Li et al., 2015; Pontes-Arruda et al., 2006; Singer et al., 2006). However, as with the studies of GLA alone, the results combining GLA and n-3 LC-PUFAs have not been reproducible. Other clinical studies, such as the OMEGA trial, did not show a benefit of these GLA/n-3 LC-PUFA combinations on patient outcomes (Rice et al., 2011).

Supplementation strategies providing GLA together with n-3 LC-PUFAs (i.e. EPA and DHA) have also been utilized in patients with atopic asthma (Surette et al., 2003a; Surette et al., 2003b; Surette et al., 2008) and have been shown to block *ex vivo* synthesis of leukotrienes from whole blood and isolated neutrophils. Importantly when provided as an emulsion, daily consumption of these combinations was associated with an improved quality of life in asthma patients and a decreased reliance on rescue medication (Surette et al., 2008). These results compared favorably with quality of life scores obtained in mild asthmatics treated with montelukast or zafirlukast (Riccioni et al., 2004). However, to our knowledge, no randomized, placebo-controlled trials have been conducted to investigate the effect of these combinations on the improved quality of life or other relevant clinical outcomes in asthma patients.

Alternatively, botanical oil combinations (e.g. borage and echium oils) containing GLA, the n-3 18C-PUFAs, alpha-linolenic acid (ALA, 18:3n-3) and stearidonic acid (SDA, 18:4n-3), have been shown to reduce leukotriene generation and forced expiratory volume in mild asthmatics (Arm et al., 2013; Kazani et al., 2014), improve glucose tolerance in insulin-resistant monkeys (Kavanagh et al., 2013) and reduce total and LDL cholesterol levels in patients with diabetes and metabolic syndrome (Lee et al., 2014). These botanical oil studies, however, have yet to be replicated in larger human clinical trials.

Together, these data indicate that the outcomes of clinical studies utilizing GLA supplementation, alone or in combination with other fatty acid-based supplements, while promising are highly inconsistent. These observations raise serious questions about our current understanding of the highly complex and dynamic nature of PUFA metabolism. More recent studies suggest that there are important metabolic and genetic factors within the human host that significantly impact the study of GLA or GLA/n-3 PUFAs combinations and reveal that a “one size fits all” model of supplementation may not be appropriate. Further, these studies suggest that it may be necessary to better understand key metabolic and genetic issues regarding GLA metabolism before GLA-enriched supplements can be effectively used to address human disease. This review will focus on potential key metabolic and genetic factors that may impact the use and clinical effectiveness of GLA or GLA/n-3 PUFA combinations.

2.0 Polyunsaturated Fatty Acid Metabolism

2.1 Long Chain Polyunsaturated Fatty Acid Biosynthesis

In mammals, n-6 and n-3, long chain (>20 carbons, LC) PUFAs such as arachidonic acid (AA, 20:4n-6), dihommo-gamma linolenic acid (DGLA, 20:3n-6), EPA (20:5n-3) and DHA

(22:6n-3) can be synthesized from their respective precursors, n-6 and n-3 18C-PUFAs such as LA, GLA, ALA, and SDA. The PUFA pathways and attendant enzymes are illustrated in Fig. 1. Biologically important n-6 LC-PUFAs, DGLA and AA can be synthesized from LA using either two (one desaturation and one elongation step) or three (two desaturation and one elongation step) enzymatic steps, respectively (Sprecher, 1981). The desaturation reactions have long been recognized as the rate-limiting steps in this pathway (Bernert and Sprecher, 1975) and the enzymes that catalyze these reactions are encoded by the fatty acid desaturase 1 and 2 (i.e. *FADS1* and *FADS2*) genes located on chromosome 11. This region is commonly referred to as the *FADS* cluster (11Q12.2-q13.1) (Glaser et al., 2011). These same enzymes are responsible for the rate-limiting steps in the conversion n-3 18C-PUFAs (ALA and SDA) to n-3 long chain-PUFAs including EPA. Recent studies suggest that the efficiency of several steps in the pathway, in particular the desaturase steps, is highly impacted by genetic variations within the *FADS* cluster (Chilton et al., 2014; Eaton, 1992). The potential impact of these genetic variations on GLA, DGLA and AA levels and their respective ratios will be discussed in greater detail in section 4. In addition, small quantities of LC-PUFAs can be obtained directly from the diet (Fig. 1). Dietary AA is obtained primarily from animal products such as, organ meats, eggs, poultry, and fish, whereas dietary EPA and DHA are found primarily in seafood, particularly cold-water fish (Hibbeln et al., 2006).

GLA enters the n-6 pathway distal to the *FADS2* enzymatic step and is efficiently converted to DGLA by an enzymatic activity, encoded for by a gene known as *ELOVL5*, in a wide range of cells (including several inflammatory cells) and tissues. Because of its rapid conversion, GLA is found in low levels in circulating lipids, cells or tissues. In contrast to GLA, the ELOVL5 product, DGLA is readily measured in circulating lipids and most cells, and levels of DGLA are consistently elevated after GLA supplementation (Chilton-Lopez et al., 1996; Johnson et al., 1997; Lee et al., 2014; Simon et al., 2014) (Table 2). Once formed, DGLA can be incorporated into cellular glycerolipids (primarily phospholipids). Upon cell activation, DGLA can be released as a free fatty acid by phospholipase A_2(s) and enzymatically converted to several metabolites with predominantly anti-inflammatory properties. These pathways are described in further detail in section 3.3 below.

2.2 Metabolism of PUFAs to Lipid Meditators that Impact the Immune Response

DGLA and its metabolites have long been recognized to have potent inhibitory effects on platelet aggregation and inflammation. The impact of DGLA on platelet aggregation was first recognized in the early 1970s (Willis et al., 1974a), and Lagarde and colleagues showed that ten times more collagen and twice as much thrombin were necessary to obtain aggregation when platelets and endothelial cells were pretreated with DGLA as compared to untreated platelets and endothelial cells (Lagarde et al., 1981). Interestingly, DGLA was much more potent than EPA in inhibiting platelet aggregation.

The anti-inflammatory effects of DGLA have been attributed to both i) the anti-inflammatory properties of DGLA-derived metabolites and ii) the ability of DGLA to compete with AA in the synthesis of pro-inflammatory AA products (Billah et al., 1985; Chilton-Lopez et al., 1996; Iversen et al., 1991; Iversen et al., 1992; Vanderhoek et al., 1980)

(Table 3). The synthesis of DGLA oxygenated metabolites and their impact is discussed in detail below in section 3.3.

Somewhat paradoxically from an inflammation perspective, AA can also be synthesized from DGLA utilizing an enzymatic activity originally known as the Δ-5 desaturase. As shown in Fig. 1, this activity is encoded for by *FADS1* within the *FADS* cluster. AA and its metabolic products have long been known to play important roles in immunity and inflammation (Boyce, 2008; Calder, 2013; Schmitz and Ecker, 2008; Simopoulos, 2008) via their ability to impact normal and pathophysiologic responses through the conversion of AA to potent eicosanoid products (including prostaglandins [PG], thromboxanes [TX], leukotrienes [LT] and lipoxins). Additionally, AA and its oxidized products can regulate transcription and consequently a wide range of cellular activities via cellular and nuclear receptors (such NF-κB, PPAR and SREBP-1c (Berger et al., 2006; Caputo et al., 2011; Chinetti et al., 2000; Deckelbaum et al., 2006; Jump and Clarke, 1999; Jung et al., 2012; Schmitz and Ecker, 2008; Soberman and Christmas, 2003; Vanden Heuvel, 2012), thereby modulating the expression of numerous genes that impact immune responses. Therefore, dietary supplementation with GLA has the capacity to both increase levels of both DGLA, which can lead to several anti-inflammatory metabolites, and AA, whose metabolic products generally promote inflammation.

3. Factors that Determine the Balance of Pro- and Anti-inflammatory PUFAs and PUFA Metabolites after GLA Supplementation

3.1 Differential Metabolism of GLA to DGLA and AA in Human Cells and Tissues

Since metabolites of DGLA have predominantly anti-inflammatory effects and AA products generally enhance inflammation, it stands to reason that the balance of AA to DGLA (i.e. the ratio of AA/DGLA) in circulation, cells and tissues is a critical factor that impacts inflammatory processes. Several factors determine the levels of AA and DGLA and thus the ratio of AA metabolites and DGLA metabolites within cells and tissues. One is the differential capacities of cells or tissues to elongate GLA to DGLA and then to further desaturate it to AA. Differential expression of enzymatic activities is observed when comparing GLA metabolism within an inflammatory cell, such as the human neutrophil, and within a tissue bed or organ, such as the human liver. Both *in vitro* and *in vivo* studies demonstrate that human neutrophils contain the elongase (*ELOVL5*) but not the Δ-5 desaturase (i.e. *FADS1*) activity. In addition to human neutrophils, skin, murine peritoneal macrophages and platelets also appear to have high *ELOVL5* elongase activity relative to *FADS1* (Δ-5) desaturase activity (Chapkin and Coble, 1991; Chapkin et al., 1988b; Chapkin and Ziboh, 1984; de Bravo et al., 1985; Navarette et al., 1992; Ziboh et al., 2000). In contrast, several other tissues including liver, kidney, testes, brain and intestine appear to contain both activities (Bernert and Sprecher, 1975; Blond and Bézard, 1991; Hurtado de Catalfo et al., 1992; Irazu et al., 1993; Luthria and Sprecher, 1994; Pawlosky et al., 1994).

The PUFA pathway enzymatic portfolio of human neutrophils results in the accumulation of cellular DGLA upon, dietary GLA supplementation. DGLA formed within the human neutrophil generally resides in the same phospholipid pools as AA. For example, the largest

pools of AA and DGLA within the neutrophil lipids are found in phosphatidylethanoamine (PE) molecular species. Additionally, there are significant increases in the amount of DGLA associated with PE after supplementation with GLA (Chilton-Lopez et al., 1996; Johnson et al., 1997). Thus, the AA/DGLA ratio in PE species markedly decreases after supplementation with GLA. Perhaps more importantly, both DGLA and AA are released from membrane phospholipids, particularly PE, after neutrophil stimulation indicating that DGLA is located in membrane phospholipids that are readily accessible to hydrolysis by phospholipase(s).An altered AA/DGLA ratio has functional implications for immune cells (Table 3).

In contrast to these observations made in several inflammatory cell types, dietary GLA supplementation markedly increases circulating levels of both DGLA and AA in humans, suggesting that ingested GLA is both elongated to DGLA and subsequently desaturated to AA *in vivo* by tissues such as the liver (Barham et al., 2000; Johnson et al., 1997; Surette et al., 2003a). Examination of circulating lipoproteins reveals that DGLA and AA are almost exclusively localized in phospholipid pools, and GLA supplementation further enriches phospholipid pools with both DGLA and AA, but does not cause any appreciable change in other serum lipid classes (Johnson et al., 1997). *In vitro* studies utilizing Hep-G2 liver cells confirm that these transformed liver cells contain the enzymatic capacities to both elongate GLA to DGLA and then to further desaturate it to form AA (Barham et al., 2000).

Thus, *in vivo* GLA metabolism in humans is extremely complex since all cellular compartments do not metabolize it in a uniform manner due to the differential expression of PUFA metabolizing enzymes. GLA supplementation leads to elevated DGLA and has no effect on AA levels in certain inflammatory cells (e.g. neutrophils), but also increases both DGLA and AA levels in circulating lipids. The biological ramification of AA accumulation in circulation of humans is controversial. Some studies suggest that dietary AA has no influence on immune responses, blood lipids, lipoproteins or health in general (Kelley et al., 1997; Nelson et al., 1997). However, other studies show a strong association between elevated levels of AA and the formation of platelet-aggregating endoperoxides and thromboxanes (Hamberg and Samuelsson, 1974; Hamberg et al., 1975; Smith and Lands, 1972; Willis et al., 1974b). Additionally, high levels of AA in humans have also been shown to result in an increased tendency for the secondary irreversible phase of platelet aggregation (Seyberth et al., 1975).

3.2 Impact of n-3 PUFAs on GLA Metabolism

As mentioned in the Introduction, GLA-enriched supplements have also been given in combination with marine n-3 long chain-PUFA supplements. These supplementation strategies often provide GLA together with the n-3 long chain-PUFAs, EPA and DHA. There are three primary rationales for using these combinations. First, addition of n-3 long chain-PUFAs inhibits the conversion of GLA-derived DGLA to AA. *In vitro* experiments show that EPA blocks *FADS1* activity in cultured HEP-G2 cells (Barham et al., 2000). Additionally, *in vivo* studies that demonstrate that the addition of fish oil (with EPA and DHA) to GLA-enriched diets prevents the accumulation of serum AA in response to GLA without inhibiting accumulation of DGLA in neutrophils (Barham et al., 2000). Other

studies show that inclusion of as little as 0.25 g/d EPA + DHA can block GLA-induced elevations in plasma AA levels (Surette et al., 2003a; Surette et al., 2003b).

Secondly, like GLA alone, supplementation with borage + fish oil combinations inhibit leukotriene generation (Barham et al., 2000; Surette et al., 2003a; Surette et al., 2003b) and attenuate the expression of early steps in signal transduction, as well as the expression of genes for pro-inflammatory cytokines (Weaver et al., 2009). Finally, addition of fish oil to GLA supplemented diets enriches cells and tissues with EPA, DPA and DHA and their metabolites. Many of these metabolites have potent anti-inflammatory effects (Ariel and Serhan, 2007; Serhan et al., 2004; Serhan et al., 2002). Consequently, GLA/n-3 long chain-PUFA combinations theoretically would induce a powerful combination of anti-inflammatory metabolites from DGLA, EPA and DHA.

Botanical oil combinations that contain borage oil, enriched in GLA, and echium oil, (from *Echium plantagineum* L.) enriched in n-3 PUFAs (ALA and SDA), also markedly increase circulating levels of DGLA and have little impact on circulating AA levels (Arm et al., 2013; Lee et al., 2014). These studies suggest that botanical n-3 18C-PUFAs not only enhance the conversion of dietary GLA to DGLA but also inhibit further conversion of that DGLA to AA.

3.3 Differential Function and Impact of DGLA- and AA-Derived Eicosanoids

Free (unesterified) DGLA and AA released by phospholipases A_2(s) are substrates for cyclooxygenases (COX) and lipoxygenases (LOX), leading to the synthesis of a variety of eicosanoid products including PGs, TXs, LTs and hydroxyl epoxides. As mentioned above (section 2.2), there are many eicosanoid derivatives of AA. In particular, the 2-series PGs and TXs, and the 4-series LTs, are by far the most commonly studied and are very well characterized. These lipid mediators tend to exhibit pro-inflammatory activities in numerous cell types and disease states. Additionally, there is emerging scientific literature revealing that free AA and oxidized products of AA can regulate gene expression, and consequently a wide range of cellular activities via cellular and nuclear receptors (Berger et al., 2006; Caputo et al., 2011; Chinetti et al., 2000; Deckelbaum et al., 2006; Jump and Clarke, 1999; Jung et al., 2012; Schmitz and Ecker, 2008; Soberman and Christmas, 2003; Vanden Heuvel, 2012).

Depending on the cell type, DGLA can be metabolized by COX 1/2 (prostaglandin endoperoxide H synthase-1 and −2, *PGHS1/2*) to 1-series PGs, particularly PGE_1, and by 15-lipoxygenase into 15-(S)-hydroxy-8,11,13-eicosatrienoic acid (15-HETrE) (Borgeat et al., 1976). These two metabolites of DGLA have been shown to suppress inflammation, promote vasodilation, lower blood pressure, inhibit smooth muscle cell proliferation, and exert anti-neoplastic activities (Fan et al., 1995; Tabolacci et al., 2010; Zurier, 1991). DGLA supplementation has also been observed to regulate PGD_1 and PGD_2 levels (Amagai et al., 2015) (Table 3).

To better understand the capacity of *PGHS1/2* to synthesize PGE_1 and PGE_2, it is important to understand 1) the cells that contain *PGHS1/2* activities, 2) conditions in which *PGHS1* and *PGHS2* genes are expressed, 3) whether *PGHS1* and *PGHS2* can equally utilize DGLA

and AA as substrates for prostaglandin biosynthesis, and 4) the capacity of PGE_1 and PGE_2 to bind E-type prostanoid receptors and induce biological responses. A great deal remains unknown in all of these areas. Levin and colleagues compared the DGLA and AA affinities and reaction rates for PGHS1 and PGHS2. DGLA and AA had similar affinities (Km) and maximal reaction rates (Vmax) for PGHS2 (Levin et al., 2002). In contrast, AA was metabolized preferentially by PGHS1. PGHS2 (Levin et al., 2002) is thought to be the dominant source for prostaglandin formation during inflammation (Ricciotti and FitzGerald, 2011). Together, these data suggest that both DGLA and AA can be efficiently converted to PGE_1 and PGE_2 during inflammatory responses.

Prostaglandins, including PGE_1 and PGE_2, exert their effects by binding to rhodopsin-like seven transmembrane spanning G protein-coupled receptors (Ricciotti and FitzGerald, 2011). The prostanoid receptor family has several members including EP1 (E prostanoid receptor 1), EP2, EP3, and EP4 subtypes and while it has been suggested that certain biological properties of PGE_1 are ~20 times stronger than PGE_2 (Fan and Chapkin, 1998), much remains to be learned about the affinity of PGE_1 compared to PGE_2 for PGE receptor subtypes and their subsequent biological activities.

Both PGE_1 and 15-HETrE have been shown to antagonize the synthesis of AA-derived eicosanoids. When exogenously provided, 15-HETrE reduces A23187-stimulated LT generation in a dose-dependent manner in human neutrophils (Chilton-Lopez et al., 1996), peripheral blood mononuclear cells (Iversen et al., 1992) and murine peritoneal macrophages (Chapkin et al., 1988a) by 75–90% (Table 3). The inhibitory effect of 15-HETrE on neutrophil LTB_4 generation is reversible and required only short (5min) exposure time (Chilton-Lopez et al., 1996). Interestingly, 15-HETrE is a much more potent inhibitor (90% inhibition at 20μM) of 5-lipoxygenase than 15-lipoxygenase derivatives of AA (15-HETE; 25% at 20μM), EPA (15-HEPE; 33% at 20μM) and DHA (17-HODHE; 33% at 20μM) (Iversen et al., 1992; Ziboh, 1996). PGE_1 appears to also contribute to the inhibitory impact of GLA supplementation on LT synthesis. In mouse dendritic cells, both PGE_1 and PGE_2 suppress LTB_4 production, the latter by an IL-10 dependent mechanism that interferes with the 5-LO activating protein (FLAP) expression (Harizi et al., 2003). Taken together, the isomeric series of the lipid mediators synthesized from AA and DGLA are functionally distinct and typically have opposing actions.

While all putative mechanisms have not been elucidated, the functional consequence of elevated DGLA content in neutrophils is a dramatic reduction in LTB_4 generation in response to stimulation (Chilton-Lopez et al., 1996; Johnson et al., 1997; Ziboh and Fletcher, 1992). Cysteinyl LTs are important in the pathology of asthma and utilize receptors ($CysLT_1T$ and $CysLT_2R$) localized in bronchial smooth muscle, vascular endothelium and secretory cells (Heise et al., 2000; Lynch et al., 1999). Basophil cysteinyl LT generation is reduced up to 50% in mild asthmatics by supplementation with botanical oil (borage + echium) combinations that contain both GLA and SDA (Arm et al., 2013). The generation of other potent lipid mediators including platelet activating factor is also inhibited as a result of dietary supplementation with GLA (Johnson et al., 1997).

4. The Impact of Genetic Variation in the Fatty Acid Desaturase (*FADS*) Gene Cluster on AA/DGLA Ratios and Eicosanoid Production

Until recently, the conversion of LA and ALA to AA and DHA, respectively, via the pathway shown in Fig. 1 was thought to be inefficient and uniform for all populations. However, mounting evidence indicates that common genetic and epigenetic variations in close proximity to and within the *FADS* cluster markedly affect the rate of conversion of 18C-PUFAs, including GLA, to LC-PUFAs and thus affecting the amount of circulating and tissue LC-PUFA levels. Specifically, single nucleotide polymorphisms (SNPs) and the methylation status of CpG sites in the *FADS* gene cluster are strongly associated with DGLA, AA and DHA levels in plasma and liver tissues (Chilton et al., 2014; Hoile et al., 2014; Howard et al., 2014; Mathias et al., 2014). As discussed in section 2, the human *FADS* gene cluster is located on chromosome 11q12-q13.1, comprised of 91.9kb and has nearly 500 SNPs annotated to this region with exon/intron organization (Fig. 2) (Glaser et al., 2010).

Recent studies from our lab have revealed dramatic population-based differences in the frequency of genetic variations that impact long chain (LC; >20 carbons) PUFAs and their metabolites (e.g. eicosanoids) (Hester et al., 2014; Mathias et al., 2011; Sergeant et al., 2012). These studies clearly support that associations between genetic variants and PUFA levels are strongly related to ethnicity. For example ~80% of African Americans carry two copies of the alleles associated with increased levels of AA and DHA and reduced levels of DGLA, compared to only ~45% of European Americans. Together these studies raise important questions of whether gene-PUFA interactions induced by a modern western diet are differentially driving the risk of diseases of inflammation in diverse populations, and are these interactions leading to health disparities. They also suggest that “one size fits all” dietary recommendations and supplementation strategies may not be appropriate for all populations or even individuals within specific populations.

Our laboratory has focused on numerous SNPs and epigenetic sites within the *FADS* cluster but particularly the SNP, rs174537. Rs 174537, located 13–15kb downstream of *FADS1*, was originally chosen because it was shown in a large GWAS to be the strongest genetic determinant associated circulating plasma AA levels ($p = 5.95 \times 10^{-46}$) (Tanaka et al., 2009) Additionally, there are marked frequency differences in genotypes at rs174537 between African-Americans and European-Americans. Our studies, across several cohorts, show that this SNP in particular, is robustly associated with ratios of AA to DGLA and thus the enzymatic efficiency of *FADS1*. Fig. 2 shows the relationships between genotypes at rs174537 and serum AA/DGLA ratios in African American and European American populations (Mathias et al., 2011). These data indicate that there is a greater than 3-fold difference (e.g. African American GG versus European American TT) in the AA/DGLA ratio between all genotypes in both populations and a greater than 2-fold difference between genotypes within either population. African Americans with the GG genotype have a mean ratio of AA/DGLA approaching 7.5 to 1, with some individuals well over 10 to 1. Studies have not been performed to determine how genetic variation within the *FADS* cluster impacts AA/DGLA in tissue or inflammatory cell lipids. These observations indicate the

critical need for studies that are focused on the impact of gene variations, such as rs174537, on AA/DGLA ratios after supplementation with GLA-enriched oils. However, findings to date suggest that variation within the *FADS* cluster is likely to have significant impact on how individuals respond to GLA supplementation.

5. Discussion and Future Directions

This review emphasizes that the study of GLA and DGLA metabolism and its relationship to eicosanoid biosynthesis and inflammatory processes is a complex area of research. On the one hand, there are promising studies that suggest that supplementation with GLA and particularly combinations of GLA with n-3 long chain-PUFAs have great potential to dampen inflammatory processes and improve signs and symptoms of several inflammatory diseases. However, as a whole, this field of study is currently riddled with confusion. Much of the perplexity arises from many of the issues raised in this review including a limited knowledge about how genetic variation affects PUFA supplementation and subsequent metabolism.

A critical question is where does the field go from here? First, even in its current state, we feel the clinical studies indicate that this is an important area of research that should continue to be emphasized. Currently, the clinical effectiveness of a wide variety of supplementation strategies (with fish, flaxseed and GLA-containing oils) is being questioned. For example, recent clinical trials and meta-analyses have challenged the efficacy of supplementation with fish oils containing n-3 long chain-PUFA (Chen et al., 2011; Filion et al., 2010; Rizos et al., 2012; Zhao et al., 2009). Similarly, a meta-analysis of 27 studies (Pan et al., 2012) showed higher ALA exposure was associated with a moderately lower risk of CVD, but found “high unexplained heterogeneity” that warranted further studies. A central issue in all of these studies is the fact that large, diverse clinical trials inevitably have sizeable subsets of individuals with markedly different circulating and tissue levels of 18C- and LC-PUFAs, and great variability in how individuals respond to PUFA-based supplements. It seems clear that in light of such host-related complexities, study approaches that provide complex n-6 or n-3 dietary supplements to diverse groups of individuals using a “one size fits all” model, are unlikely to yield conclusive results.

In contrast, when genetic diversity is taken into consideration and the resultant marked differences in n-6 to n-3 LC-PUFA levels and ratios are recognized, then complex n-6 or n-3 dietary supplementation strategies can be used to correct critical diet-gene interactions in a targeted manner for individuals that need them. Moreover, *in vitro*, animal and human studies have demonstrated the benefits of balancing n-6 and n-3 metabolic pathways to reduce inflammatory processes, prevent disease and improve human health. It seems that understanding and recognizing individual and population differences provides this field with a great opportunity to optimize the use of PUFA-based supplements (including GLA-enriched supplements) as we move into the era of *individualized medicine*.

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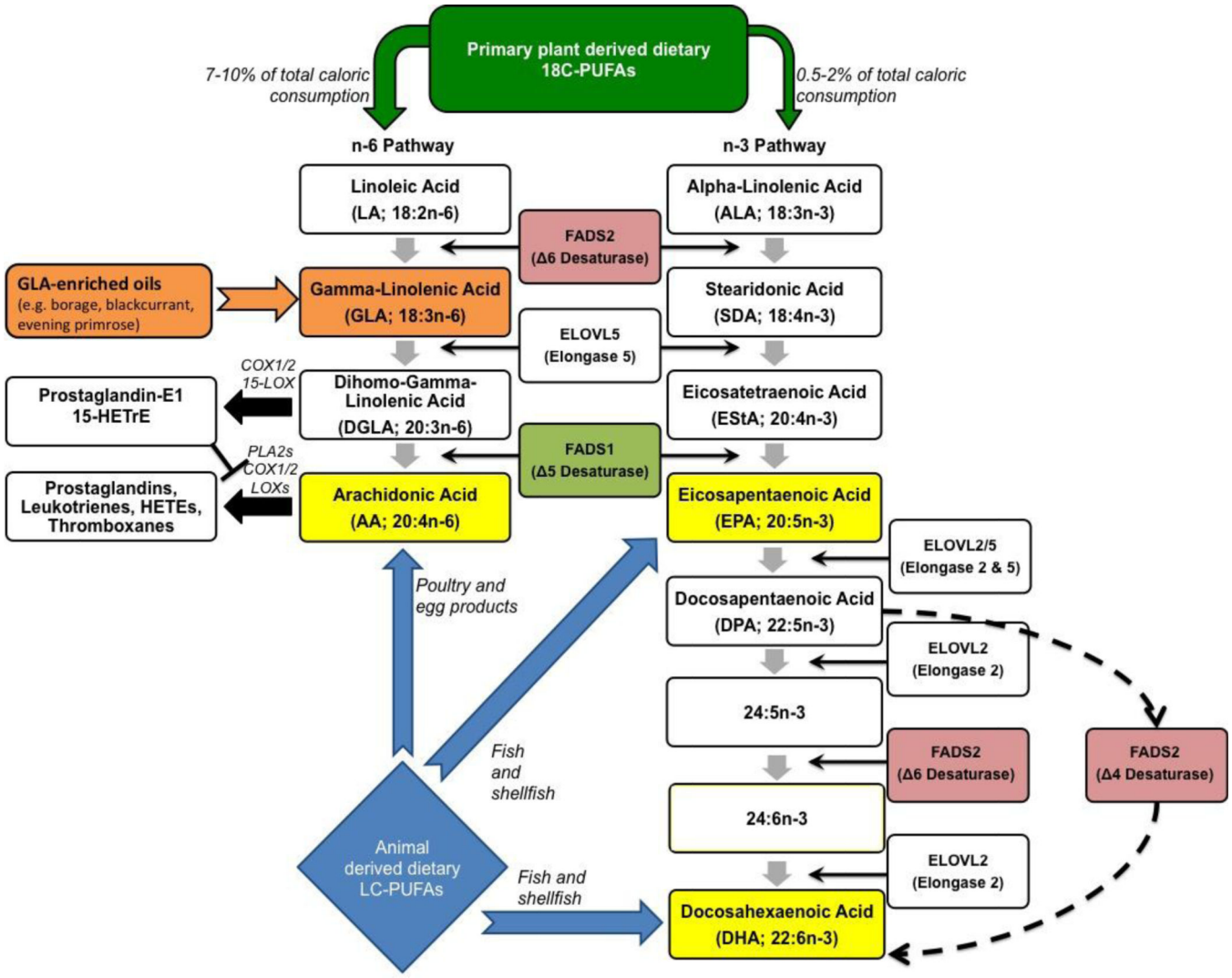

Fig. 1.
Illustration of PUFA pathway

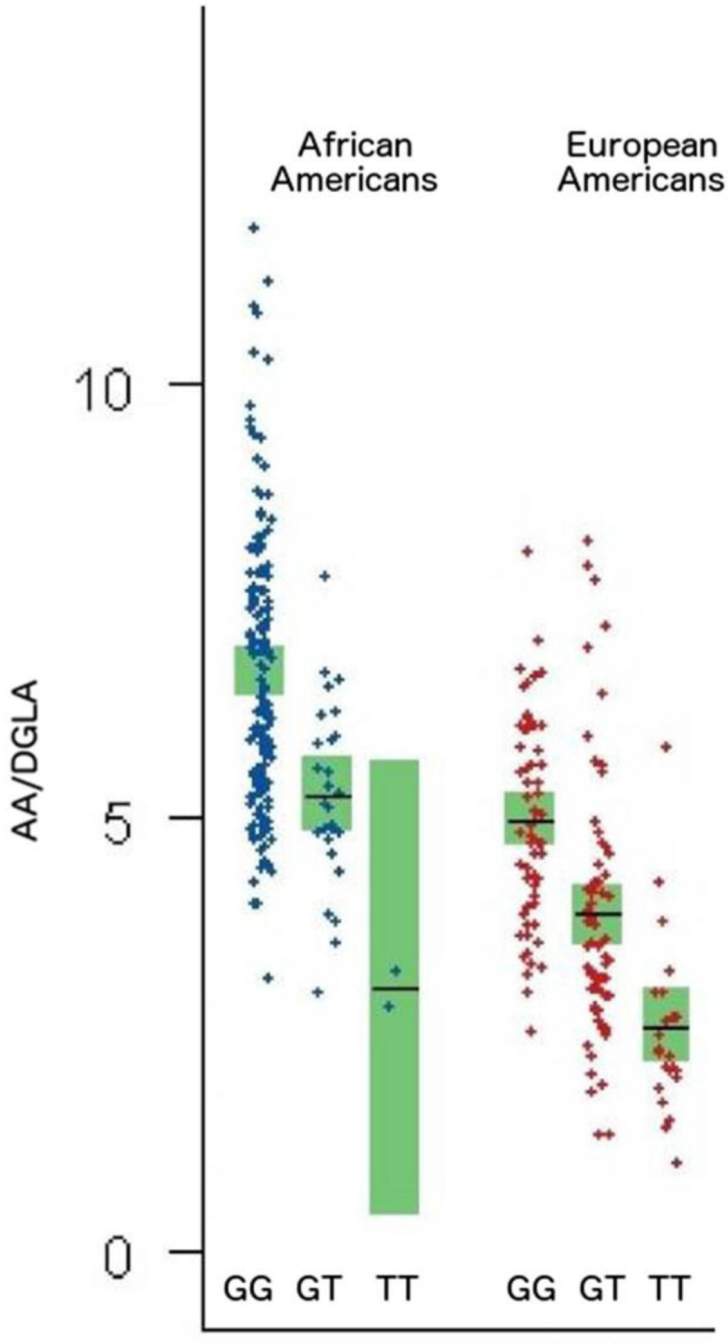

Fig. 2.

Plasma AA/DGLA ratios vary by genotype at rs174537 in both African American and European American populations (Adapted from (Mathias et al., 2011))

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Table 1

Effect of GLA-enriched oil supplements on various human disease from meta-analyses and recent studies

Study	Disease[1] and Study Type[2]	Supplement[3]	location	# subjects	# studies	duration	outcome	effect
Skin								
(Morse et al., 1989)	Atoptic dermatitis (CO, parallel)	EPO (Epogam)	UK, Italy, Finland	311	9 (EPO)	4, 8, or 12 wk	Severity of symptoms	reduced severity of symptoms
(Van Gool et al., 2004)	Atoptic dermatitis (RCT, CO, CCT)	EPO, BO, BCO; 90–480mg GLA/d (children); 132–720mg GLA/d (adult)	Germany, Italy, UK, Canada, USA, Finland, Sweden, Switzerland,	1071	22 (total) BO (6) EPO (12) BCO (1)	3–24wk	Severity of symptoms	no effect
(Bamford et al., 2013)	Eczema (AE, AD, AEDS) adult, children (RCTs)	EPO, BO	UK, Italy, Germany, India, NZ, Finland, Sweden, USA, Switzerland	1596	27 (total) 19 (EPO) 8 (BO)	3–24wk	Severity of symptoms	no effect
(Morse and Clough, 2006)	Atopic eczema	EPO (Efamol®)		1207	26	4–8wks	Severity of symptoms	reduced severity of symptoms
(Fiocchi et al., 1994)	Atoptic dermatitis, infants	EPO, 3g oil/d	Italy	10	na	4wk	Lesion number; Severity of Symptoms	decrease number (trend); reduced severity of symptoms
(van Gool et al., 2003)	Atoptic dermatitis, infants (RCT)	BO, 100mg/d	Netherlands	118	na	6mo	Incidence in 1st yr; Severity of symptoms	no prevention benefit; reduced severity of symptoms (trend)
(Kitz et al., 2006)	Atoptic dermatitis, infants	GLA, 40mg/d	Germany	131	na	6 mo	Prevention	no effect
(Kawamura et al., 2011)	Atoptic dermatitis, adult	GLA, 200mg/d, oil of *Mucor circinelloides* in food	Japan	130	na	16wk	Trans-water loss; Nocturnal itching	no effect; decreased
(Simon et al., 2014)	Atoptic dermatitis, children and adult (open study, non-controlled)	EPA, 4–6g GLA/d	Switzerland	21	na	12wk	SCORAD[4] index	plasma GLA content correlates with SCORAD
Arthritis								

Study	Disease[1] and Study Type[2]	Supplement[3]	location	# subjects	# studies	duration	outcome	effect
(Cameron et al., 2011) (Macfarlane et al., 2011)	Rheumatoid arthritis (RCT, parallel, placebo controlled)	Herbal intervention 525–540mg GLA/d	UK, USA	286 (total) >90 (in 3 studies)	22 (total) EPO (2) BCO (1)	6mo	Morning stiffness; Pain	decreased (2 of 3); no effect
(Cameron et al., 2011) (Macfarlane et al., 2011)	Rheumatoid arthritis	1400-2800mg GLA/d	USA, Finland	>111	EPO (1) BO (2) BCO (1)	6mo	Pain; Morning stiffness; Joint tenderness; Joint swelling;	decreased; decreased; improvement; decreased;
Asthma								
(Arm et al., 2013)	Mild asthma, adults (randomized)	BO+EO (GLA, 1.67g/d+ SDA, 0.88g/d)	USA	37	na	3wk	Basophil, Neutrophil leukotriene production (*ex vivo*)	>50% decrease (basophil response); >35% decrease (neutrophil response)
(Ziboh et al., 2004)	Mild asthma, adults (randomized)	BO (2g GLA/d)	USA	24	na	12mo	Neutrophil leukotriene production (*ex vivo*); Peak flow	>20% decrease ($p<0.05$); no effect

[1] AD, atopic dermatitis; AE, atopic eczema; AEDS, atopic eczema/dermatitis syndrome;

[2] RCT, randomized clinical trial; CO crossover; CCT, controlled clinical trial

[3] BO, borage oil: BCO. Blackcurrant oil; EPO, evening primrose oil; EO, echium oil; GLA, gamma-linolenic acid; SDA, stearidonic acid

[4] SCORAD, SCOing Atopic Dermatitis

Table 2

Effect of GLA supplementation on the DGLA content of immune cells

						Biochemical Outcomes			
Reference	**design**	**supplement**	**dose**	**duration**	**cell type tested**	**[DGLA]-pre**	**[DGLA]-post**	**p value**	**unit**
(Fletcher and Ziboh, 1990)	*In vivo* fatty acid supplementation	borage oil	3% oil (by weight) in diet	12wk	guinea pig neutrophil	0.27	2.41	p[1]	mg /100mg total phospholipid
(Ziboh and Fletcher, 1992)	*In vivo* fatty acid supplementation	borage oil	0.48g GLA/d	6wk	human neutrophil	1.35	1.96	<0.05	mg /100mg total phospholipid
(Ziboh and Fletcher, 1992)	*In vivo* fatty acid supplementation	blackcurrant oil	0.48g GLA/d	6wk	human neutrophil	1.35	1.95	<0.05	mg /100mg total phospholipid
(Chilton-Lopez et al., 1996)	*In vitro* fatty acid incorporation	albumin-conjugated GLA	200nmol	24 hr	human neutrophil	0.4	1.0	p[1]	nmol
(Chilton- Lopez et al., 1996)	*In vivo* fatty acid supplementation	borage oil	3g GLA/d	21d	human neutrophil	0.45	0.80	<0.05	nmol/million cells
(Johnson et al., 1997)	*In vivo* fatty acid supplementation	borage oil + controlled diet	1.5g GLA/d 3g GLA/d 6g GLA/d	21d 21d 21d	human neutrophil human neutrophil human neutrophil	0.20 0.15 0.13	0.20 0.27 0.35	ns[2] <0.05 <0.05	nmol/5 million cells nmol/5 million cells nmol/5 million cells
(Chapkin and Coble, 1991)	*In vitro* fatty acid incorporation	albumin-conjugated radiolabeled GLA	tracer amounts	3hr	murine peritoneal macrophage	0	84	p[1]	% conversion to radiolabel product
(Ziboh et al., 2004)	*In vivo* fatty acid supplementation	borage oil	2g GLA/d	12mo	human neutrophil	0.7	1.4	<0.05	mg /100mg total phospholipid

[1] p value not specified

[2] ns, not significant

Table 3

Effect of GLA supplementation on functional endpoints of immune cells

Reference	design	supplement	dose	duration	cells	*Functional Outcomes*
(Fletcher and Ziboh, 1990)	*In vivo* fatty acid supplementation	borage oil	3% oil (by weight) in diet	12wk	guinea pig neutrophil	~25% inhibition of fMLP-stimulated superoxide production (vs control diet; $p<0.005$) No effect on PMA-stimulated superoxide production (vs control diet)
(Iversen et al., 1991)	*In vitro* fatty acid Competition	LA and DGLA conc. Curve	0–100μM	10m	human neutrophil	50μM DGLA, 75% ↓ in LTB_4 generation 50μM LA, 60% ↓ in LTB_4 generation
(Iversen et al., 1992)	*In vitro* fatty acid treatment	DGLA conc. curve	0–100μM	10m	human PBMC[1]	50μM DGLA, 60% ↓ in LTB_4 generation 50μM DGLA, >7-fold ↑ in PGE1 generation 50μm DGLA, significant ↑ in 15-HETrE generation (>250ng/10mil cells)
(Ziboh and Fletcher, 1992)	*In vivo* fatty acid supplementation	borage oil	0.48g GLA/d	6wk	human neutrophil	50% ↓ LTB_4 generation (vs olive oil control), *ex vivo*
(Ziboh and Fletcher, 1992)	*In vivo* fatty acid supplementation	black currant oil	0.48g GLA/d	6wk	human neutrophil	50% ↓ LTB_4 generation (vs olive oil control), *ex vivo*
(Chapkin et al., 1988a)	*In vitro* treatment	15-HETrE	0–30μM	1hr	murine peritoneal macrophage	10μM 15-HETrE, 90% ↓ in LTB_4 generation
(Chilton- Lopez et al., 1996)	*In vitro* treatment	15-HETrE	0–20μM		human neutrophil	10μM 15-HETrE, 75% ↓ in LTB_4 generation
(Johnson et al., 1997)	*In vivo* fatty acid Supplementation	borage oil + controlled diet	3g GLA/day	21d	human neutrophil	58% ↓ LTB_4 generation (vs baseline), *ex vivo*
(Barham et al., 2000)	*In vivo* fatty acid supplementation	borage + fish oils + controlled diet	3g GLA + 3g EPA/day	21d	human neutrophil	30% ↓ LTB_4 generation (vs baseline), *ex vivo*
(Amagai et al., 2015)	*In vitro* fatty acid treatment	DGLA conc. curve	0–30μM	48hr	RBL-2H3 cells	30μM, significant (>30ng/ml) PGD_1 formation
(Amagai et al., 2015)	*In vivo* fatty acid supplementation	DGLA	11% of dietary fatty	5wk	NC/Tnd mouse skin	significant ↑ in PDG_1, PDE_1, PGD_2, 8-HETrE, 15-HETrE (vs control diet)

[1] PBMC, peripheral blood mononuclear cells