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Variation in the *FADS1/2* gene cluster alters plasma *n*−6 PUFA and is weakly associated with hsCRP levels in healthy young adults

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ABSTRACT

Introduction: Past research has reported that single nucleotide polymorphisms (SNPs) in fatty acid desaturase 1 and 2 (*FADS1/2*) can influence plasma fatty acid (FA) profiles. Changes in FA profiles are known to influence inflammatory processes; therefore both FA and SNPs in *FADS1/2* may affect inflammation. The goals of this study were to (i) examine the relationships between individual *n*−6 FA and estimates of FA desaturation with circulating high sensitivity C-reactive protein (hsCRP) levels, and (ii) determine whether SNPs in *FADS1/2* are associated with changes in hsCRP.
Methods: FA and hsCRP were measured in fasted plasma samples from 878 healthy young adults (20–29 yrs). Circulating levels of plasma linoleic (LA), γ-linolenic (GLA), dihomo-γ-linolenic (DGLA) and arachidonic (AA) acids were measured by gas chromatography and used to calculate desaturase indices for *FADS1/2*. Nineteen SNPs in *FADS1/2* were genotyped in all subjects and six (rs174579, rs174593, rs174626, rs526126, rs968567 and rs17831757) were further analyzed.
Results: Significant inverse associations were found between LA and hsCRP ($p=8.55\times10^{-9}$) and the *FADS1* desaturase index and hsCRP ($p=4.41\times10^{-6}$). A significant positive association was found between DGLA and hsCRP ($p=9.10\times10^{-11}$). Several SNPs were associated with circulating levels of individual FA and desaturase indices, with minor allele carriers having lower AA levels and reduced desaturase indices. A single SNP in *FADS2* (rs526126) was weakly associated with hsCRP ($p=0.05$).
Conclusions: This study highlights the relationships between FA and hsCRP, and confirms that FA are strongly influenced by SNPs in *FADS1/2*. Furthermore, we found weak evidence that SNPs in *FADS1/2* may influence hsCRP levels in young adults.

1. Introduction

Low-grade chronic inflammation is a hallmark of many common metabolic disorders, such as obesity, type-2 diabetes, and cardiovascular disease [1]. One of the major contributors to the increased prevalence of these disorders is the Western-style diet, which is characterized by high fat and energy-dense foods [2,3]. Dietary fats are capable of influencing a myriad of physiological parameters, including inflammation, metabolism, and insulin sensitivity; however, the influence is related to the type of fat consumed [4,5]. Of particular relevance to the current study is the recognized link between polyunsaturated fatty acids (PUFA) and inflammation.

In general, *n*−3 PUFA are widely considered to inhibit and/or resolve inflammation, while *n*−6 PUFA are thought to be pro-inflammatory [6]. Current research questions the link between *n*−6 PUFA and inflammation [7,8]. Linoleic acid (LA; 18:2*n*−6) has long been thought to be pro-inflammatory, but a recent review by Johnson and Fritsche indicated that such a role is not supported by existing literature [9]. This questions whether LA itself is pro-inflammatory, or rather if it's a downstream product of LA metabolism (e.g. γ-linolenic acid (GLA; 18:3*n*−6), dihomo-γ-linolenic acid (DGLA; 20:3*n*−6) or arachidonic acid (AA; 20:4*n*−6)) that promotes inflammation. However, this is not without controversy itself. For

Abbreviations: FA, fatty acids; SNPs, single nucleotide polymorphisms; FADS1/2, fatty acid desaturase gene cluster; FADS1, FADS2, fatty acid desaturase 1 and 2; hsCRP, high sensitivity C-reactive protein; LA, linoleic acid; GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; PUFA, polyunsaturated fatty acids; TNH, Toronto Nutrigenomics and Health Study; FFQ, food frequency questionnaire; tSNPs, tag SNPs; BMI, body mass index.

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example, GLA is a downstream product of LA metabolism and has previously received attention as a dietary supplement because of its anti-inflammatory and anti-proliferative effects [10,11]. Moreover, Johnson et al. suggested that GLA is rapidly converted into DGLA, which then serves as a precursor for the production of anti-inflammatory eicosanoids [11]. The final product of LA metabolism is AA; however, a previous cross-sectional study by Ferrucci et al. showed no evidence of a relationship between plasma AA and markers of inflammation [12]. Calder also reported that results from AA supplementation studies are inconsistent [13]. Together, this research proposes that neither LA nor its downstream FA metabolites are directly pro-inflammatory [12,13], but rather that their association with inflammation may be primarily related to their roles as precursors for AA-derived pro-inflammatory eicosanoids [6,14]. In light of the continued uncertainty regarding the link between fatty acids and inflammation, further investigation is warranted.

While it is important to study the relationships between individual *n*−6 PUFA and inflammation, it is also necessary to better understand how the enzymes regulating FA metabolism may influence this relationship. Although many FA are consumed in the diet, GLA, DGLA and AA can also be produced endogenously from LA via a pathway comprising several desaturation and elongation steps [15]. Of relevance to the current project, a previous genome-wide association study found that several genes in this pathway contributed to variability in plasma PUFA concentrations [16]. The strongest associations were found with single nucleotide polymorphisms (SNPs) in the fatty acid desaturase 1 and 2 (*FADS1/2*) gene cluster (11). *FADS1* and *FADS2* code for fatty acid desaturases, which are enzymes responsible for the formation of double bonds at the Δ−5 and Δ−6 positions in PUFA, respectfully [7,15]. The association between SNPs in the *FADS1/2* gene cluster and plasma PUFA levels have been independently replicated in a number of studies, and these SNPs have also been shown to influence an individual's risk for chronic diseases such as type 2 diabetes and cardiovascular disease [15,17–20]. While the role of *FADS1/2* in regulating fatty acid metabolism is well documented, limited evidence exists to support a relationship between *FADS1/2* and inflammation [21,22]. To the best of our knowledge only Martinelli et al. have previously reported an association between a haplotype in the *FADS1/2* gene cluster and high sensitivity C-reactive protein (hsCRP) levels, which was also related to a greater risk for coronary artery disease [22]; however, this link has not been previously examined in healthy young adults. As such, the link between *FADS1/2* and inflammation merits continued examination.

The goal of this work was to examine the relationships between individual *n*−6 FA and estimates of FA desaturation with circulating hsCRP levels in a population of healthy young adults. Further, we subsequently examined to what extent SNPs in the *FADS1/2* gene cluster could also influence these relationships.

2. Materials and methods

2.1. Study participants

Subjects participating in the Toronto Nutrigenomics and Health (TNH) study were used for the present investigation [23]. All participants were between the ages of 20–29 yrs and were recruited from the University of Toronto campus between October 2004 and June 2009. Subjects were excluded from the analysis if they met one of the following criteria: (1) a body mass index > 30 kg/m^2, (2) plasma hsCRP > 10 mg/L, (3) plasma hsCRP > 3 mg/L and a recorded inflammatory event (e.g. a piercing or a fever) 2 weeks prior to providing a blood sample, or (4) had taken an analgesic in the month prior to providing a blood sample (n=91 excluded). The final number of participants used for the current study was 878, consisting of both men (n=250) and women (n=628). Individuals in the study population corresponded to three self-reported ethnic backgrounds: European (n=439), East Asian (n=339) and South Asian (n=100). The study protocol was approved by the Research Ethics Boards at the University of Toronto and the University of Guelph. Written informed consent was obtained from all participants.

2.2. Anthropometric and clinical measurements

Anthropometric measurements were recorded and subjects completed general health and lifestyle questionnaires, including a food frequency questionnaire (FFQ) that has been used in previous studies examining diet-gene interactions [23]. The FFQ was used to calculate total calories consumed and the amount of energy obtained from fat. A physical activity score, which is representative of the number of hours a week one spends doing light, moderate, and vigorous activities and sleeping and/or lying down, was determined from questionnaire data, as described by Lee and Paffenbarger Jr [24]. Following a 12hr overnight fast, plasma samples were collected by a LifeLabs Medical laboratory (Toronto, ON, Canada) and analyzed for markers of glucose (glucose, insulin), lipid (triglycerides, total- and HDL-cholesterol) and inflammation (hsCRP).

2.3. Analysis of plasma FA using gas chromatography

Plasma FA content was determined from fasted blood samples using gas chromatography, as previously described [18]. Fatty acids of interest (LA, GLA, DGLA and AA) were expressed as a percentage of total FA composition. The product-to-precursor ratios of AA/DGLA, GLA/LA and AA/LA were used to estimate the *FADS1*, the *FADS2*, and the aggregate *FADS1/2* desaturase indices, respectively; an approach that is routinely used in the literature to estimate desaturase activity [18,22]. The aggregate *FADS1/2* desaturase index was calculated in order to obtain a global overview of the entire desaturation/elongation pathway.

2.4. SNP selection and genotyping

SNPs in *FADS1* and *FADS2*, including 10 kb up-and downstream flanking regions, were identified with the International HapMap Project SNP database using the NCBI B36 assembly—HapMap Genome Browser Data release 24, phase II, build 126. Tag SNPs (tSNPs) were selected with the Tagger feature in Haploview V4.2 using a minor allele frequency (MAF) > 5% and pairwise tagging (r^2≥0.8), leading to the identification of 19 tSNPs in total (3 in *FADS1* and 16 in *FADS2*). The selection of tSNPs for *FADS1* and *FADS2* was performed independently for each gene; however, it is important to note that these genes appear side-by-side on chromosome 11 and therefore a degree of overlap in our tSNP selection may have existed. To determine the extent of this overlap, we examined linkage disequilibrium (LD) between all of our tSNPs (i.e. those identified for *FADS1* and *FADS2*) using the SNP Annotation and Proxy Search (SNAP) database [25]. Two tSNPs were in high LD (r^2≥0.8): rs174547 in *FADS1* and rs174576 in *FADS2*. Genotyping was performed using the Sequenom MassARRAY platform, which is based on detection through MALDI-TOF MS (Mass Array, Sequenom, San Diego, CA). Twenty-nine DNA samples were randomly selected for replication for all 19 tSNPs and 100% concordance was achieved.