The Effect of Genetic Variations in the FADS1 Gene on Fatty Acid Metabolism

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Abstract

FADS1 is a key regulator of fatty acid metabolism with important implications for athlete health and performance. Mutations in the FADS1 gene have been linked to a variety of adverse health conditions, in addition to alterations in biochemical levels of fatty acids. Polyunsaturated fatty acids, including omega-3 and omega-6 fatty acids, are associated with rates of inflammation, immune function, and brain health in athletes. The purpose of this study was to explore the relationship between genetic variability, specifically in the FADS1 gene, and fatty acid metabolism in an athletic population. Methods: 20 collegiate football players were recruited for this study. Saliva samples were collected for the purpose of obtaining genetic information. Serum samples for ALA, AA, EPA, and DHA were collected to measure omega-3 and omega-6 fatty acid levels. Genotypes and serum measurements were compared using 3x3 Fisher Exact tests, and the online software PROVEAN was used to identify potential novel variants in the FADS1 gene. Results of this exploratory study suggest the minor allele for 9 variants in the FADS1 gene decrease baseline serum levels of EPA. These results suggest that genetic data may be useful in assessing individual athlete risk for n-3 PUFA deficiency and associated health consequences. Subsequently, this may allow athletes and sports practitioners to make better informed decisions about individual intake and supplementation of n-3 PUFAs, specifically, EPA and DHA.

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Introduction and Purpose Statement

Fatty acids are an important energy source. They are necessary for intracellular signaling pathways, transcription factor activity, and gene expression, making them important for human health (Calder, 2015). Fatty acids can also influence a variety of conditions, including cardiovascular disease (CVD), metabolic syndrome, diabetes, inflammatory diseases, and cancer (Calder, 2015). Essential fatty acids (EFAs) are also important for the performance and well-being of athletes. In addition to serving as an energy source, EFAs serve as structural components of cell membranes and are necessary for immune function and wound repair. Potential benefits of Omega-3 fatty acids (n-3) for athletic performance include enhanced muscle recovery, reduced inflammation, and improved brain health and function (Gatorade Sport Science Institute, n.d.).

There are two major categories of fatty acids; saturated fatty acids, characterized by single bonds, and unsaturated fatty acids, characterized by double bonds. Unsaturated fatty acids can be characterized further into monounsaturated fatty acids (MUFAs), containing 1 double bond, and polyunsaturated fatty acids (PUFAs), containing multiple double bonds. Omega-6 (n-6) and omega-3 (n-3) fatty acids are two types of PUFAs with important functions for human health. N-3 PUFAs include eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and alpha-linolenic acid (ALA). N-6 PUFAs include arachidonic acid (AA) and linoleic acid (LA) (U.S. Department of Health and Human Services [DHHS], 2021).

Biochemical levels of n-3 and n-6 fatty acids are influenced by endogenous metabolism and dietary intake (Simopoulos, 2010). Foods such as fish, fish oil, nuts, seeds, vegetable oils, and

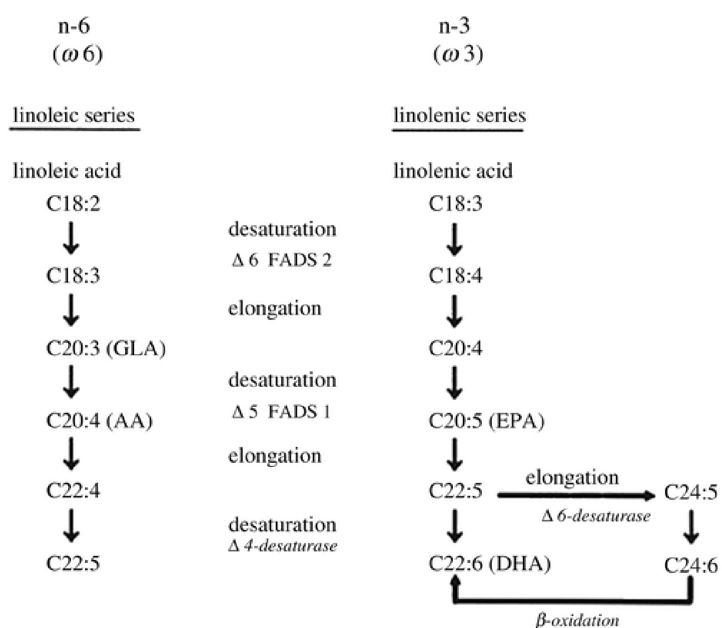
some fruits are quality sources of dietary PUFAs (White, 2009). Biomarkers which can be used to measure fatty acid levels include serum, erythrocytes, triglycerides, phospholipids or cholesterol esters, and adipose tissue (Arab, 2003). Adipose tissue is recognized as the preferred medium for measuring fatty acid levels because it most accurately reflects long-term intake of dietary fatty acids. Erythrocytes also reflect long-term fatty acid intake and have the advantage of being more accessible compared to adipose tissue. Additionally, compared to serum measurements, erythrocyte fatty acids are more strongly correlated with dietary intake, especially for dietary n-3 fatty acids (Sun, Q., et al, 2007). This is likely due to a longer half-life (120 days), which results in slower turnover and reduced sensitivity to dietary intake. Research on PUFAs indicates that genetics strongly regulate endogenous fatty acid metabolism (Lankinen, et al., 2018). PUFAs have been a popular area of research in recent years, and, as summarized in Lankinen, et al., 2018, many genes are associated with alterations in fatty acid metabolism and individual fatty acid status. Specifically, variants of the Fatty Acid Desaturase 1 (FADS1) gene, an important enzyme in fatty acid metabolism, may influence erythrocyte and serum fatty acid concentrations.

According to Genecards, the human gene database, the FADS1 gene is responsible for producing the protein for regulating inflammatory responses throughout the body. As shown in Figure 1, FADS1 encodes the Δ5 desaturase enzyme, which catalyzes a double bond at carbon 5, converting dihomo-γ-linolenic acid (DGLA) to AA and eicosatetraenoic acid (ETA) to EPA. It is a rate-limiting enzyme required for the synthesis of AA and EPA from essential fatty acids (EFAs) LA and ALA, respectively (Plunde, et al., 2020). EFAs are fatty acids required by the body for biological processes but cannot be synthesized endogenously, therefore must be

obtained through diet. EPA and DHA can be synthesized from ALA but have very low conversion rates. For this reason, it is recommended that DHA and EPA are consumed through diet (Libretexts, 2022.; Linus Pauling Institute, 2003). Mutations in the FADS1 gene have been linked to cardiovascular disease, cancer, obesity, metabolic syndrome, and dyslipidemias (Corella, D., & Ordovás, J. M., 2012).

Figure 1

Omega-6 and Omega-3 Polyunsaturated Fatty Acid Pathway



FADS1 encodes the Δ5 desaturase enzyme, which catalyzes a double bond at carbon 5, converting dihomo-γ-linolenic acid (DGLA) to arachidonic acid (AA) and eicosatetraenoic acid (ETA) to EPA.

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What then is the effect of inactivating or altering the function of the FADS1 gene, and could this influence circulating levels of fatty acids? In a mouse model, inactivation of the FADS1 gene promotes M1 activation, increasing an inflammatory response (Figure 2). Macrophages are specialized white blood cells involved in immunity, homeostasis, and tissue development and repair. They can be characterized into two groups, M1 and M2, both of which are critical for maintaining homeostasis within the body. M1 macrophages function to destroy foreign pathogens and bacteria through phagocytosis, resulting in a proinflammatory effect. Alternatively, M2 macrophages function to regenerate tissues during wound repair by secreting growth factors, yielding an anti-inflammatory effect (Guo, n.d.; Watanabe, et al., 2019). Gromovsky et al., (2017) suggests that loss of function in FADS1 results in diminished levels of anti-inflammatory mediators derived from AA, EPA, and DHA. SNPs in the FADS1 and FADS2 gene cluster have been suggested to influence serum levels of LA and ALA. These genetic variants are suggested to impact cholesterol levels, triglycerides, and potentially risk for prostate cancer (Dumont, et al., 2011; Simopolous, A. P., 2010). However, the FADS1 gene has been researched extensively in recent years, many of the SNPs linked to it are not in the protein-coding region of the gene. Protein coding regions of a gene are the portion of a gene's DNA that is translated and transcribed into a protein. Therefore, many studies on FADS1 can suggest links between certain SNPs and their influence on health conditions, however the true cause of these health conditions cannot be confirmed.

In athletic populations, the function of FADS1 is extremely important for regulating inflammatory responses in the body. Athletes experience acute inflammation in response to exercise, which promotes repair processes for damaged tissues. However, athletes are also at an

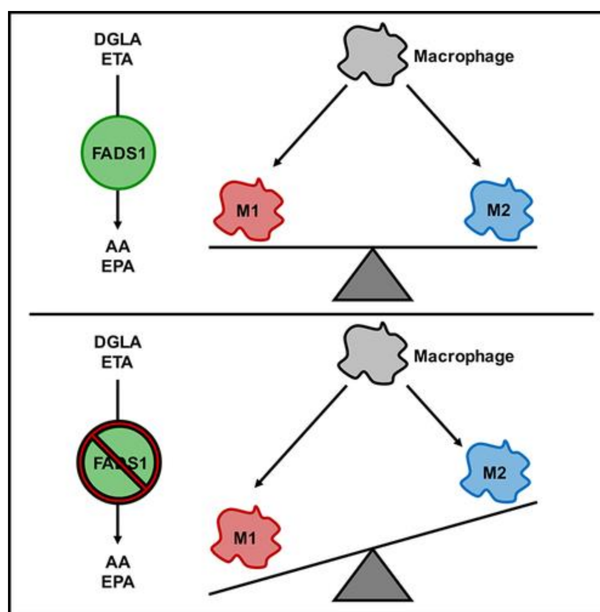
increased risk for chronic inflammation, especially when training at higher volumes with reduced recovery times (Cerqueira, et al, 2019). Prolonged inflammation can cause fatigue, muscle damage or soreness, limit muscle growth and training progression, and loss of muscle mass (Wentz, n.d.). N-3 fatty acids have anti-inflammatory properties which may benefit human health and athletic performance (Harvard Health, 2020). In athletes, dietary EPA and DHA have been suggested to enhance endurance, promote muscle protein synthesis (MPS), improve markers of functional response to exercise, and offer neuroprotective benefits (Thielecke and Blannin, 2020). The benefits of dietary EPA and DHA for brain health in athletes has received special attention due to the risk of traumatic brain injuries (TBI) in sport. EPA and DHA have the unique ability to cross the blood brain barrier (BBB) by attaching to erythrocytes. It is believed that both n-3 FAs reduce inflammation in the brain post-TBI by reducing apoptosis, oxidative stress, and mitochondrial dysfunction, in addition to increasing antioxidant enzyme activity (Bailes, et al., 2020). DHA makes up most of the n-3 FAs found in the brain. It has been shown that changes in DHA composition of central nervous system membranes impacts axonal and dendritic stability, neuronal plasticity, glucose uptake, neuroinflammation, and hypothalamic function (Gupta, 2019). As mentioned before, n-3 fatty acids, particularly EPA and DHA, must be acquired through food. However, metabolism of beneficial fatty acids may be hindered due to genetic variations in the FADS1 gene.

The purpose of this study was to characterize SNPs located within the protein coding region of the FADS1 gene and compare athlete genotypes for these SNPs and SNPs used in other studies from non-coding regions of the FADS1 gene with levels of fatty acids in serum. This study tests the hypothesis that genetic differences in FADS1 may influence fatty acid

metabolism. Specifically, and based on previous literature, it is hypothesized that variants in the FADS1 gene will alter baseline n-3 and n-6 fatty acid levels.

Figure 2

Impact of FADS1 Knockout in a Mouse Model



This image depicts the impact of FADS1 gene knockout in a mouse model. Inactivation of FADS1 prevents the conversion of dihomo-γ-linolenic acid (DGLA) to arachidonic acid (AA) and eicosatetraenoic acid (ETA) to eicosapentaenoic acid (EPA). This increases M1 macrophage activity and decreases M2 macrophage activity, resulting in a proinflammatory effect. Macrophages (M1 and M2) are specialized types of white blood cells. M1 macrophages detect and destroy pathogens, increasing inflammation in the cells. M2 macrophages secrete growth factors which repair damaged tissues, decreasing inflammation in the cells.

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Literature Review

Role of PUFAs in Human Health and Performance

Research studies suggest that genetic variability plays a significant role in individual levels of fatty acids. It is well known that PUFAs play an important role in health. As stated by Harvard University's School of Public Health (2019), n-3 PUFAS (EPA, DHA, and ALA), have protective effects against heart disease and can lower blood pressure, heart rate, triglycerides, and decrease inflammation. The U.S. Department of Health and Human Services (2021) confirms that EPA and DHA have also been shown to protect against rheumatoid arthritis, promote healthy fetal growth, and prevent certain cancers. The last 5 years have seen extensive research exploring the connection between n-3 fatty acids and cognitive function. There is strong evidence suggesting increased dietary EPA and DHA promote brain health and have important roles in the prevention of neurodegenerative disease (Dyall, S. C., 2015). N-6 PUFA's include linolenic acid (LA) and arachidonic acid (AA) and are essential to health. However, research suggests that increased intake of n-6 PUFA's may contribute to chronic inflammation and subsequent diseases such as CVD, metabolic syndrome, irritable bowel disease, rheumatoid arthritis, cancer, and neurodegenerative disease (Patterson, E. et al, 2012). Dietary recommendations for the ratio of n-6:n-3 PUFA's are approximately 1:4. However, most Americans fall into a much higher ratio ranging from 10:1-20:1 (Patterson, E. et al, 2012).

The balance of n-6/n-3 fatty acids is an important consideration when assessing dietary intake of fatty acids. In 2004, the Omega-3 Index (O3i) was validated as a method for assessing dietary intake of EPA and DHA and subsequent risk for coronary heart disease (CHD) (Harris and von Shacky, 2004). Since then, the O3i has become a common criterion used to assess cardiovascular health (von Shacky, 2014). The greatest cardioprotective effects are observed at a O3i of >8%. It has also been suggested that the EPA:AA ratio, mediated by dietary intake of EPA, may be a useful marker for chronic inflammation and associated health conditions (Nelson and Raskin, 2019). A lower ratio is associated with increased quantities of AA-derived metabolites, including prostaglandins, leukotrienes, thromboxanes, hydroxy fatty acids, and lipoxins (Nelson and Raskin, 2019; Simopoulos, 2008). In large amounts, these metabolic products can contribute to the formation of thrombus and atheroma, in addition to increasing blood viscosity, vasospasm and vasoconstriction. It is possible that consuming higher quantities of n-3 fatty acids can decrease these metabolites, because n-3 fatty acids displace AA within the cell membrane (White, 2009).

Potential benefits of n-3 PUFAs for athlete health include reduced bodily inflammation, enhanced immune function, prevention of muscle breakdown, and promotion of brain health (Stupin, et al., 2019; Thielecke and Blannin, 2020; Witard and Davis, 2021). This is an important consideration for athletes who are at an increased risk for TBI. According to Ledreux and colleagues (2020), male football players are at the highest risk for suffering mild TBI, followed by female soccer players and ice hockey players of both genders. In general, athletes participating in contact sports are at higher risk for head injury compared to those participating in non-contact sports. Furthermore, it is estimated that 50% of concussions sustained in athletes go

undetected or unreported (UPMC Sports Medicine, n.d.). The beneficial role of n-3 FAs in TBI has been primarily explored in animal models. Desai, et al. (2014) concluded that mice with deficient levels of DHA experienced decreased recovery following incidents of TBI. A 2011 study found that DHA offered neuroprotective effects in rodents who were supplemented with DHA prior to sustaining a TBI (Mills, et al.) Salberg, et al., (2017) found that mice supplemented with a combination of DHA, prebiotic fiber, and resveratrol had improved post-TBI outcomes compared to controls. Preliminary research on n-3 fatty acids and brain health in athletes is promising, however at this time no definitive recommendations can be made for n-3 fatty acids in the treatment of TBI (Klein, 2022). However, considering the important implications of PUFAs on athlete health and performance, it is necessary to examine the influence of genetic variability on fatty acid status.

Impact of Genetic Variability on PUFA Status

Several studies provide evidence to support the theory that individuals may respond differently when provided the same dose of PUFAs. A genome-wide association study (GWAS) from March 2021 identified four genetic loci which had modified impacts on blood lipids following fish oil supplementation. Interestingly, participants carrying the minor allele of rs112803755, a SNP in the GJB6-GJB2-GJA3 gene cluster, had decreased triglyceride (TG) levels after consuming fish oils, and an increase in TG levels without fish oil (Francis, M. et al, 2021). Another GWAS identified thirteen SNPs that were influenced by n-3 PUFA supplementation. SNPs located in or near the IQCJ-SCHIP1, MYB, NELL1, NXPH1, PHF17, and SLIT2 genes were linked to PPAR signaling pathway, tight junction signaling pathways, and ceramide signaling (Rudkowska, et al., 2014). A 2015 meta-analysis examined the relationship

between genetics, diet, and risk for various disease states known to be impacted by blood lipid levels. This review revealed that SNPs located in the PPARA, APOA5, ADIPOQ, PPARG2, FADS cluster, PARG2, MTHFR, and PLIN4 were all influenced by dietary n-3 PUFAs. The FADS1/FADS2 cluster had some of the strongest associations with dietary fatty acid intake. Disease states affected by genetic variations in these genes include cardiovascular disease, cancer, obesity, metabolic syndrome, and dyslipidemias (Corella, D., & Ordovás, J. M., 2012). Polymorphisms in the FADS1 and FADS2 gene cluster have also been suggested to influence serum levels of LA and ALA. These genetic variants are suggested to impact cholesterol levels, triglycerides, and potentially risk for prostate cancer (Dumont, et al., 2011; Simopolous, A. P,. 2010).

The influence of variants in the FADS1 gene on fatty acid metabolism may also be attributed to differences in genetic ancestry. Coltell (2020) found that 6 SNPs in the FADS gene cluster (rs2727270, rs174547, rs174550, rs174546, rs1535, and rs174570) were statistically associated with serum PUFA status in a population of Mediterranean subjects. Three of these polymorphisms, rs174547, rs174550, rs174546, are found in the FADS1 gene. The minor allele (C) of rs174547, a variant in the intron of FADS1, was associated with lower serum n-3 levels. Matthias and colleagues analyzed data from GeneSTAR, the Diabetes Heart Study (DHS), and HapMap to compare seven FADS variants with levels of long-chain polyunsaturated fatty acids (LC-PUFAs) in African American and European American populations. These data suggest that the allele associated with higher levels of LC-PUFAs is more frequent in African American populations. In the same study, Matthias determined that African American populations have a 97.5% prevalence rate of the homozygous GG genotype (rs174537), compared to 50.6% in

European Americans. This genotype is associated with enhanced levels of AA, suggesting that African Americans may be genetically predisposed to convert medium-chain polyunsaturated fatty acids (MC-PUFAs) to LC-PUFAs more efficiently. Similar findings were seen in an island population of European descent. Individuals with the homozygous GG genotype had significantly higher concentrations of AA and lower levels of DGLA compared to heterozygous GT or wildtype (WT) homozygous TT genotypes. Han and colleagues found that single nucleotide variants (SNVs) rs174548 and rs174549 had bivariate associations with compressive strength index (CSI) and appendicular lean mass (ALM) in male populations, suggesting that FADS1 may be involved in the regulation of bone metabolism. Smith (2015) found that two FADS1 variants, rs174538 (minor allele A) and rs174548 (minor allele G) interacted with dietary ALA to modulate plasma levels of DPA and DHA. Vazquez-Vidal suggests that in Mexican populations, the minor allele (C) for rs174546 contributes to plasma levels of triglycerides (TG) and very-low-density lipoprotein (VLDL).

In respect to athletic populations, research on genetic variability of blood fatty acid status and diet is minimal. A recent study published by sports dietitians at Virginia Tech showed that athletes from multiple sports and institutions do not meet recommended levels of n-3 fatty acids. In addition to shedding light on low n-3 fatty acid status among athletes, this study also determined that male athletes, on average, consume higher quantities of EPA and DHA, but do not have higher O3i compared to female athletes (Ritz, P., et al, 2021). Although body mass may be a factor in this finding, it is also plausible that genetic differences between genders contributed to PUFA levels. Another study published in August 2021 investigated the effects of n-3 supplementation on the PPAR and UCP2 pathways in elite male athletes (Moradi, S., et al.

2021). The results of this study suggested that supplementation of n-3 PUFAs influenced both pathways and contributed to changes in body composition, metabolic rate, appetite, blood pressure, UCP2 protein level, blood lipid profile, and dietary intake. This provides further support that dietary n-3 FAs have the potential to alter gene expression and impact athletic performance.

Research on nutritional genomics is still in its infancy. However, more studies are being published every year to explore the relationship between genetics, dietary intake, and health status. The purpose of this study is to explore genetic differences in the FADS1 gene and to evaluate the relationship between genotype and PUFA status in collegiate football players.

Methods

Design

This was a two-part study which aimed to explore the relationship between genetics and fatty acid metabolism. Part 1 examines the influence of genetic variations in the FADS1 gene on baseline circulating PUFAs. Part 2 explores the influence of genetic variation in the FADS1 gene on PUFA status following omega-3 supplementation. Serum blood samples were used to measure ALA, AA, EPA, and DHA levels. Genetic testing was administered utilizing 23andMe™ kits. Further information on 23andMe is provided in Appendix A. Participants also completed pre- and post-surveys which provided additional health information. Both parts of the study are described in further detail below. Prior to starting the study, all participants signed a written informed consent form.

Part 1

Part 1 of this study compared genotypes from 9 SNPs in the FADS1 gene (rs174545, rs174546, rs174547, rs174548, rs174549, rs174550, rs174555, rs174556, and rs174561) to baseline serum levels of ALA, AA, EPA, and DHA. No additional fatty acids were included in this portion of the study. 23andMe genetic testing kits were used to collect DNA samples. Subjects provided one saliva sample each, which were sent back to 23andMe for processing. Prior to providing saliva samples, all subjects were asked to complete a pre-survey questionnaire to provide further health and dietary information (Appendix B). Subjects were also asked to complete a post-survey questionnaire after receiving their individual 23andMe results.

Part 2

Part 2 of this study compares the previously mentioned SNPs to serum measurements of ALA, AA, EPA, and DHA following omega-3 supplementation. 12 subjects from Part 1 continued into the second portion of this study. Subjects were blindly and randomly assigned to the supplementation group (Group A), or placebo group (Group B). Group A received a DHA supplement which they took daily for a total of 16 weeks. Group B received a placebo pill which they also took daily for the entire 16-week period. Serum blood fatty acid levels were measured pre-supplementation at baseline (T1), 8-weeks post supplementation (T2) and 16-weeks post supplementation (T3). T1 serum PUFA measurements from all 12 subjects were included in the analysis of Part 1, alongside the rest of the Part 1 subjects.

Participants

Participants for this study were recruited on a first come first serve volunteer basis from a National Collegiate Athletic Association (NCAA) Division 1 football program. To ensure confidentiality, each player was assigned a unique ID code which was used throughout the study for data collection. Each participant signed a consent form prior to data collection and was compensated with $25 in cash for their participation. Height and weight measurements for participants were assessed in duplicate, using standard protocols and a wall-mounted stadiometer and scale. Duplicate values were averages.

Setting

This research project took place at an institution within the NCAA Power 5 Conference. Blood and saliva samples were collected following team practice in the athletic training room located directly next to the team locker room and practice area. This was the preferred setting for data collection because subjects could easily be recruited at the same time. Additionally, most of the team was present immediately post-practice. Recruiting subjects at this time increased the chances of having a diverse study population and helped create equal opportunity for participation in the study. The athletic training room is also a sanitary location equipped with necessary supplies for handling blood and saliva.

Data Collection

Blood Samples

Serum fatty acid levels were measured at three separate time intervals, T1, T2, and T3. T1 represents week 0, before any participants received n-3 supplementation. T2 was after 8 weeks of supplementation, and T3 was after 16 weeks of supplementation. Part 1 subjects provided serum blood samples for ALA, AA, EPA, and DHA only at T1. Part 2 subjects provided additional blood samples at T2 and T3 following administration of treatment. In total, the following 20 fatty acids were measured for Part 2 subjects: Myristic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, vaccenic acid, linoleic acid, y-linolenic acid, alpha-linolenic acid, arachidic acid, gondoic acid, eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, EPA, behenic acid, docosahexaenoic acid, lignoceric acid, and nervonic acid. To stay consistent with data from Part 1, only blood measurements for ALA, AA, EPA, and DHA were analyzed at T2. Blood samples were centrifuged and processed using standard techniques and stored at -40 degrees until analysis. Fatty acids were analyzed using gas chromatography.

Saliva Samples

Saliva samples were collected using 23andMe kits for the purpose of obtaining DNA information. Following directions included in the 23andMe kit, participants were instructed not to eat, drink, smoke, chew gum, brush their teeth, or rinse their mouth in the 30 minutes prior to providing saliva samples. Each subject provided one saliva sample under the supervision of

researchers. Immediately after the recommended amount of saliva was collected, samples were sealed and re-packaged according to kit instructions. Each subject's kit was registered in 23andMe under separate email addresses corresponding with their ID code. Kits were mailed back to 23andMe for processing, and results were available within 2-3 months. Subjects were provided with physical copies of their 23andMe results, including reports for ancestry composition and traits. Subjects were also provided with the opportunity to review results with researchers, to allow them an opportunity to ask questions about their data. Following dissemination of 23andMe results, each participant was also asked to complete a post-survey based on their results.

23andMe testing provides raw genome data consisting of thousands of different genes and SNPs. It is an easy and fast way to collect DNA, and only requires a couple minutes of time to complete. Results are available after 3-4 weeks and are easily accessible through the 23andMe website. Additionally, 23andMe kits are less expensive than other genetic testing available. Three of the 23andMe kits returned with results titled "inconclusive data" meaning they did not include any genetic data due to errors in saliva genotyping. It is believed that the results from these three samples were inconclusive due to saliva samples that were diluted with water, or subjects did not provide enough saliva for testing. All three subjects with inconclusive data were asked to come back and provide second saliva samples, which were sent for processing a second time. All three samples resulted in conclusive data on the second attempt. These results are included in the results section of this paper.

Statistical Analysis

Fisher Exact

3x3 Fisher Exact tests (Lowry, n.d.) were applied to determine the probability between circulating serum fatty acid and genotype. This test was chosen due to its ability to determine probability in populations under 90. Genotypes were compared to the mean and standard deviation (+/- 1 SD) of each PUFA blood measurement at T1 and T2.

PROVEAN

PROVEAN (Protein Variation Effect Analyzer) (Choi et al., 2012) is an online software tool which predicts whether an amino acid mutation has an impact on the function of a protein. Compared to other amino acid prediction software tools, PROVEAN has a higher performance rating for human protein variants. The average prediction accuracy of PROVEAN (for amino acid substitutions, deletions, insertions, and replacements) is 79.50% in human proteins. Additionally, PROVEAN is an easily accessible and free tool which generates results within minutes. Variants with a score of -2.5 or greater are classified as significant as having a deleterious effect. Scores above -2.5 are considered neutral, having no effect. Variants of both the NP and XP sequences with deleterious scores have the potential to have an impact on the protein-coding region of the FADS1 gene.

Results

In total, 20 collegiate football players from the same institution participated in this study. All participants were male and of college age, ranging from 19-24 years old. Demographic data for participants is displayed in Table 1.

Table 1

Demographic, Ancestral, and Clinical Characteristics of Study Participants at Baseline

Variable	Totals (n=20)
Age (years)	20 +/- 1.28
Height (in)	73.78 +/- 2.52
Weight (lbs)	263.21 +/- 52.68
Sub-Saharan African	60.12 +/-36.93
European	33.18 +/- 35.29
Indigenous American	0.36 +/- 0.50
East Asian	0.66 +/- 2.43
Central and South Asian	0.12 +/- 0.35
Western Asian and North African	0.17 +/- 0.63
Melanesian	4.44 +/- 19.86
ALA (gram per 100/g fatty acid)	0.41 +/-0.45
AA (gram per 100/g fatty acid)	11.76 +/-3.28
EPA (gram per 100/g fatty acid)	0.37 +/-0.13
DHA (gram per 100/g fatty acid)	2.24 +/- 1.04

All data is displayed in averages +/- 1 standard deviation

FADS1 Variants Identified in 23andMe

Raw genome data was used to search the FADS1 gene and identify genotypes of various SNPs within the gene. As previously discussed, the FADS1 gene contains thousands of variants, the majority of which lack substantial research and are not included in the 23andMe database. 10 variants of the FADS1 gene are included in the 23andMe raw genome database. Genotype data was unavailable for one of these variants, rs174544. The remaining 9 variants are displayed in

Table 2. Figure 3 shows the location for each of the 9 variants within chromosome 11 of the FADS1 gene.

Table 2

FADS1 Variants Identified Through 23andMe with Corresponding Position in the Gene, Minor Allele, and Minor Allele Frequency

SNP	Position	Minor Allele	MAF (TOPMED)
RS174545	3-prime UTR	G	0.288443
RS174546	3-prime UTR	T	0.288402
RS174547	Intron	C	0.288424
RS174548	Intron	G	0.315172
RS174549	Intron	A	0.263950
RS174550	Intron	C	0.288413
RS174555	Intron	C	0.263278
RS174556	Intron	T	0.262787
RS174561	Intron	C	0.263361

UTR, untranslated region; MAF, minor allele frequency; TOPMED, Trans-Omics for Precision Medicine.

Figure 3

Location of Variants Identified Through 23andMe in the FADS1 Gene

This image shows single nucleotide polymorphisms identified in 23andMe located in the intronic region of the FADS1 gene. Variants listed in order from left to right: rs174545, rs174546, rs174547, rs174548, rs174549, rs174550, rs174555, rs174556, rs174561.

This picture was taken from the US National Library of Medicine gene database (2021).

FADS1 Variants Identified in PROVEAN

The FADS1 gene, located in chromosome 11 (chr11:61,799,627-61,829,318), is a large gene consisting of 29,692 nucleotides and 444 amino acids (Genecards, n.d.). To narrow down the search, only missense and deleterious SNPs were analyzed in PROVEAN. Missense variants are a type of amino acid substitution in which one amino acid is switched with another in the protein from that same gene. Missense variants may result in a change in protein function. Deletions are a type of mutation where 1 or more nucleotides are removed from a DNA sequence. This can alter the function of a single protein, and possibly several proteins (U.S. National Library of Medicine, 2021). A total of 349 variants were identified as missense or deletions. Variants belonged to the natural protein (NP) sequence, transcript protein (XP) sequence, and in some cases both. Each variant was analyzed in PROVEAN according to their respective sequence. Table 3 displays SNPs within the FADS1 gene with a significant PROVEAN score. Figure 4 shows the location of each significant missense SNP, in addition to variants from 23andMe, within the FADS1 gene. None of the missense SNPs were included in the 23andMe reports.

Table 3

Missense Variants in the FADS1 Gene Predicted Through PROVEAN Analysis to Have Significant Impact on Protein Functionality

SNP	Classification	PROVEAN score
rs1565320852	Inframe deletion	-11.37
rs567224080	missense	-8.387
rs751206996	missense	-7.725
rs752093425	missense	-7.686
rs759817584	missense	-7.455
rs763381968	missense	-12.027
rs770410678	missense	-8.35
rs772061257	missense	-8.656
rs777508094	missense	-7.591
rs1013439914	missense	-9.232
rs1223680364	missense	-7.454
rs1241231078	missense	-7.421
rs1380250440	missense	-7.468
rs1591149236	missense	-8.562
rs2066874300	missense	-11.384
rs2066945061	missense	-7.269
rs2066986983	missense	-10.887
rs2066987485	missense	-10.975
rs11548149	missense	-2.989

SNP: Single nucleotide polymorphism; Classification: Variants are classified based on the consequence a mutation in the variant has on protein function; PROVEAN: Protein Variation Effect Analyzer, scores >-2.5 are considered significant and included in this table.

Figure 4

Location of Significant Missense Variants Identified Through PROVEAN Analysis and Variants Identified in 23andMe in the FADS1 Gene.

This image shows missense single nucleotide polymorphisms identified as significant through PROVEAN analysis (score >-2.5) in addition to the 9 single nucleotide polymorphisms identified in 23andMe within the FADS1 gene. Variants are listed in order from left to right: rs174545, rs174546, rs751206996, rs1591149236, rs777508094, rs174547, rs567224080, rs1380250440, rs2066874300, rs174548, rs174549, rs174550, rs770410678, rs1241231078, rs11548149, rs759817584, rs752093425, rs174555, rs1565320852, rs174556, rs772061257, rs2066945061, rs174561, rs1013439914, rs2066986983, rs2066987485.

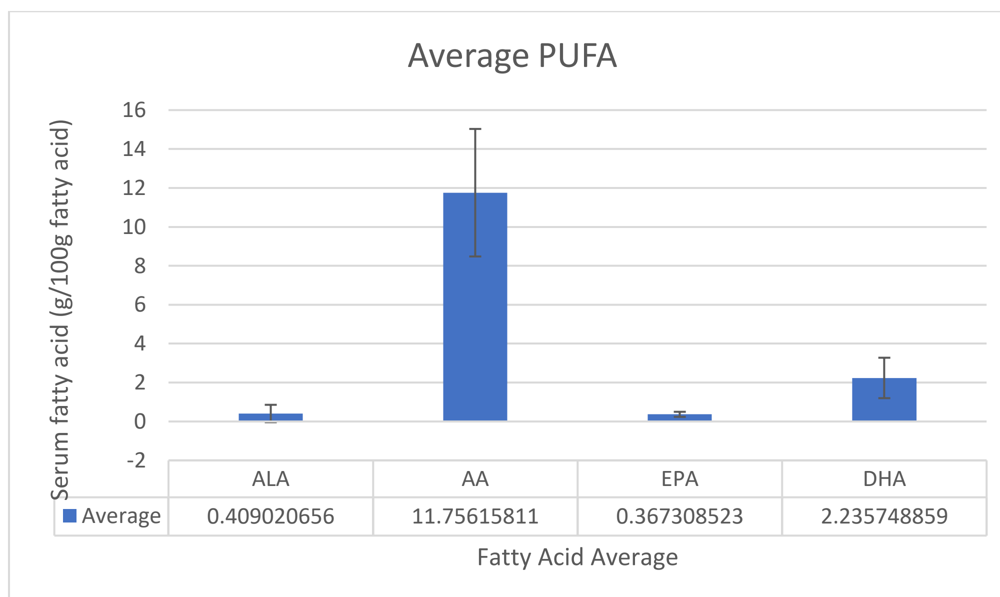
This image was taken from the US National Library of Medicine gene database (2021).

Serum PUFA Measurements

One participant did not return for T2 measurements, and three subjects did not return for T3 measurements. Low participation resulted in insufficient data beyond T2, therefore T3 data was not used. Figure 5 displays mean baseline serum measurement (g/100g of fatty acid) taken at T1 for ALA, AA, EPA, and DHA. The average ALA was 0.409 (+/- 0.448) g/100g of fatty acid. Average AA was 11.756 (+/- 3.277) g/100g of fatty acid. Average EPA was 0.367 (+/- 0.130) g/100g of fatty acid. Average DHA was 2.236 (+/- 1.039) g/100g of fatty acid. Individual serum measurements for each fatty acid at T1 are included in Appendix C.

Figure 5

Fatty Acid Averages at Baseline (T1) for All Subjects (n=20)



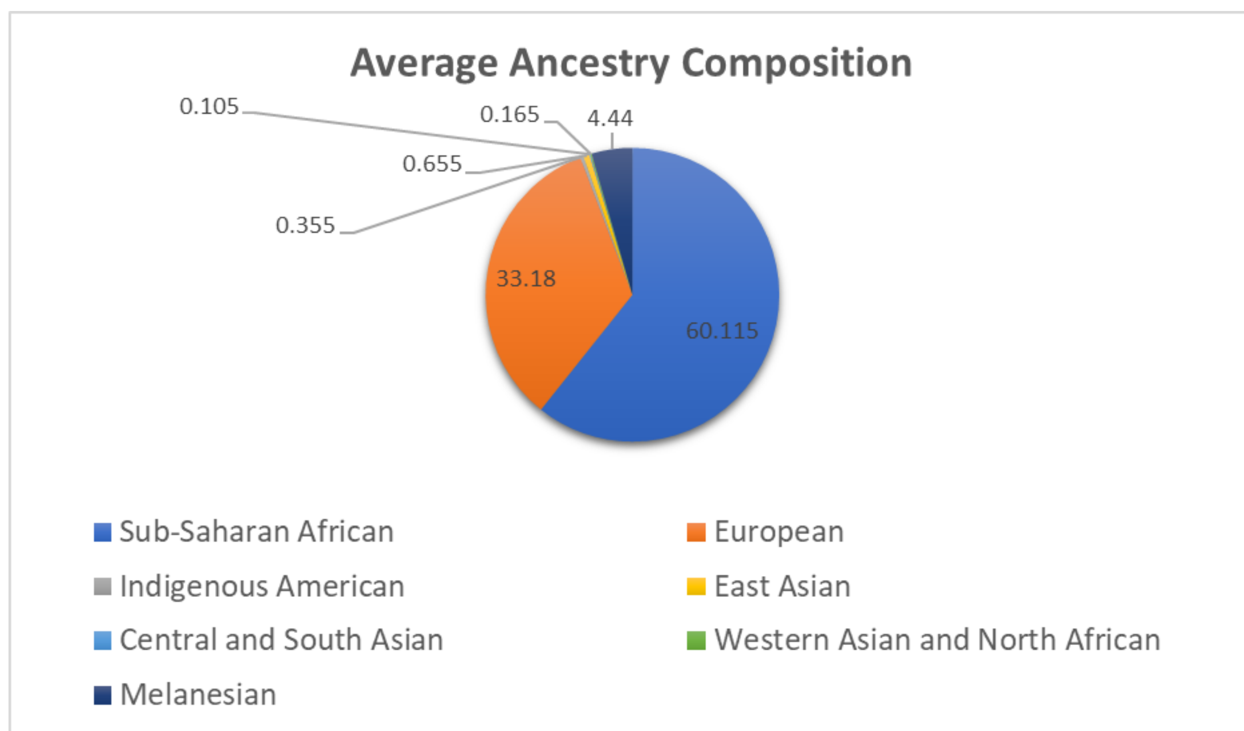
ALA: Alpha-linolenic acid; AA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

Ancestry Composition

Average ancestry composition data is shown in Figure 6. In total, seven regions were represented in this study. In descending order, regions included Europe (n=19), Sub-Saharan Africa (n=15), Indigenous American (n=8), East Asian (n=4), Western Asian and North African (n=2), Central and South Asian (n=2), and Melanesian (n=1). For most subjects, ancestry composition also included a small percentage of “trace” ancestry which did not identify any particular region. This data was insignificant and therefore not included in the analysis or discussion of this study.

Figure 6

Percent Average Ancestry Composition for All Subjects (n=20)



Average ancestry percentages for subjects are listed in descending order: Sub-Saharan African (60.115%); European (33.18%); Melanesian (4.44%); East Asian (0.655%); Indigenous American (0.355%); Western Asian and North African (0.165%); Central and South Asian (0.105%).

Genotypes

Table 4 shows the number of individuals carrying at least 1 minor allele for each of the 9 FADS1 variants available in the 23andMe database. With the exception of rs174548, all variants had 2 individuals carrying a minor allele. The two individuals with minor alleles were the same across all variants. RS174548 had an additional 5 individuals with the minor allele, for a total of 7 minor allele carriers.

Table 4

Number of Subjects Carrying At Least 1 Minor Allele per FADS1 Variant Identified in 23andMe

SNP	Minor Allele	Minor Allele Carriers
RS174545	G	2
RS174546	T	2
RS174547	C	2
RS174548	G	7
RS174549	A	2
RS174550	C	2
RS174555	C	2
RS174556	T	2
RS174561	C	2

SNP: Single-nucleotide polymorphism. Minor allele carriers include subjects with 1 or 2 copies of the minor allele for each respective SNP.

Genotypes vs. Fatty Acid Measurements

Table 5 shows the T1 averages of the four fatty acids across all variants for each genotype. There were no statistically significant findings for ALA, AA, or DHA among any of the variants at baseline (T1). Additionally, there were no statistically significant findings for Part 2 subjects at T2. For all 9 variants, the minor allele was found to significantly decrease baseline levels of circulating EPA. On average, carriers with 1 minor allele had a 38.08% (-0.147 g/100g EPA) decrease in EPA levels while carriers with two minor alleles had a 41.79% (-0.162 g/100g EPA) decrease in EPA levels. Table 6 displays Fisher Exact test results for EPA across all nine variants.

Table 5

Average Level of Serum Polyunsaturated Fatty Acid per Genotype at Baseline (T1)

	Genotype		
Fatty Acid	**WT Homozygous**	**Homozygous**	**Heterozygous**
ALA (g/100 g FA)	0.385	0.391	0.807
AA (g/100 g FA)	12.135	8.994	8.206
EPA (g/100 g FA)	0.386518833	0.225	0.239314953
DHA (g/100 g FA)	2.332	1.681	1.298

WT: Wildtype; ALA: Alpha-linolenic acid; AA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

Table 6

P-value for 3x3 Fisher Exact Tests Comparing Genotype to Average EPA at Baseline (T1) for Variants Identified in 23andMe

SNP	Minor Allele Carriers	P-value (EPA)
RS174545	2	0.021
RS174546	2	0.021
RS174547	2	0.021
RS174548	7	0.031
RS174549	2	0.021
RS174550	2	0.021
RS174555	2	0.021
RS174556	2	0.021
RS174561	2	0.021

SNP: Single-nucleotide polymorphism; EPA: Eicosapentaenoic Acid

Summary of Outcomes, Discussion, and Recommendations

Summary

This project was conducted to better understand the complex role of genetics in fatty acid metabolism, specifically in athletes. Recommended ranges for biochemical levels of ALA, AA, EPA, and DHA have not yet been established for general or athlete populations. However, based on recommendations for dietary intake of PUFAs and the O3i, and it has been shown that n-3 fatty acid levels are deficient in athletic populations (Ritz, P., et al, 2021). It has also been suggested that there may be a need for personalized fatty acid recommendations for athletic populations (OmegaQuant, 2019). This two-part study compared FADS1 genotypes to PUFA levels at baseline (T1) and post-supplementation of n-3 fatty acids (T2). It was hypothesized that variants in the FADS1 gene would alter baseline and post-supplementation n-3 and n-6 fatty acid levels. Saliva and serum blood samples were collected to obtain genotype and fatty acid data. Part 1 compared FADS1 variants only to baseline serum fatty acid levels. Part 2 went a step further and compared the same FADS1 variants to serum fatty acid levels following n-3 supplementation. Results indicate that the minor allele for all SNPs (rs174545, rs174546, rs174547, rs174548, rs174549, rs174550, rs174555, rs174556, rs174561) decreases serum levels of EPA at baseline. Interestingly, rs174548 was the only SNP to have a higher frequency of the minor allele among subjects.

This study also attempted to identify new SNPs within the protein-coding region of the FADS1 gene which may influence gene expression. 349 variants of the FADS1 gene, obtained from the NCBI database, were analyzed in PROVEAN to identify SNVs/SNPs of interest. From

this pool, 19 missense variants had significant scores (>-2.5), indicating they are predicted to impact protein functionality. Unfortunately, none of the missense variants were included in the 23andMe database, therefore no genetic comparisons could be made. Additionally, there was no existing literature available for the variants, making it difficult to determine if they have the potential to be causative for PUFA status or other genetic disorders.

Discussion

Results from this study support previous findings on the interaction between FADS1 genotypes and EPA status. Takkunen et al. found that rs174550 significantly impacted EPA status in minor allele carriers who did not supplement with omega-3 fish oils. Gillingham (2012) found that minor allele carriers for rs174545, rs174583, rs174561, and rs174537 had lower levels of plasma EPA and AA 24 and 48 hours after consuming high quantities of flaxseed oil. Juan (2018) suggests that minor allele carriers of rs174546 have lower circulating levels of EPA, therefore suggesting the need to supplement with higher doses of EPA.

Previous research has identified influential SNPs within the FADS cluster and, specifically, the FADS1 gene which impact PUFA status. However, the majority of previously identified SNPs are not located within the protein-coding region of the FADS1 gene. This study identified variants in the protein-coding region that are predicted to have deleterious impacts on protein functionality. Interestingly, no evidence currently exists to support the theory that these variants have causal influence on biological conditions. In fact, there is a lack of research exploring the influence of these protein-coding variants entirely. This observation is not unique to the FADS1 gene. Most trait-associated SNPs/SNVs identified through GWAS have been

located in non-coding regions of their respective gene (Jo, 2015; Zhang, 2015). It is certainly possible that these SNPs/SNVs may be linked to nearby coding variants which influence gene expression. In fact, as shown earlier, many of the missense variants identified as potentially deleterious in this study are in close proximity to the 9 intronic variants associated with PUFA status. However, it is also plausible that non-coding regions of genes contain functionally relevant variants which regulate gene expression.

The extent to which mutations in non-coding regions impact gene expression is not entirely clear. Intron variants make up approximately 40% of the total length of genes and are involved in splicing, mRNA transport, nonsense mediated decay (NMD) and regulation of gene expression. Untranslated region (UTR) variants are a type of intron variant specifically involved in RNA transcription, stability, and translation (Steri, 2018). It has been shown that intronic variations in many genes are strongly linked to certain genetic conditions, including, but not limited to, Alzheimer's disease, bipolar disorder, breast cancer, pancreatitis, and cystic fibrosis (Matias, 2001; Steri, 2018). Rs174546 and rs174545 of the FADS1 gene are both 3'UTR variants which have been associated with alterations in PUFA status. The minor allele (T) for rs174546 was found to be highly associated with gamma linolenic acid (GLA) ($p = 5.18 \times 10-171$) in a Singapore population (Dorajoo). Hermant et al. also proposed that rs174546 acts as a functional variant, and the minor allele triggers downregulation of the FADS1 gene by creating an miR-149-5p binding site within the FADS1 3'UTR. MiR-149-5p is a microRNA which plays a role in the regulation of physiological processes including inflammatory response (Ren, 2021). Rs174546 is located downstream from missense variant rs751206996, which was predicted in the PROVEAN analysis of this study to be highly significant for protein functionality.

The reported finding has important implications for athletes and sports medicine practitioners. Literature on genotype vs ancestral interactions discussed throughout this paper suggest certain races may be predisposed to lower n-3 PUFA status. Increasing evidence suggests that n-3 PUFAs, specifically EPA and DHA, are valuable factors in the occurrence and outcomes of TBI. It is plausible that ancestry data can be used to assess individual risk for n-3 PUFA deficiency more accurately. Subsequently, this may allow athletes and sports practitioners alike to make better informed decisions about individual supplementation of EPA and DHA in preventing or treating TBI. Athletes may be encouraged to adopt a preventative approach to brain health by making dietary choices in accordance with individual risk for n-3 deficiency. Improved risk assessment may also be valuable in the event of undiagnosed TBI, as athletes who supplement with prophylactic n-3s may experience better recovery in the absence of formal post-TBI treatment.

Study Limitations

Limitations of this study include a small study cohort (20 participants). The sample size was not very diverse, with subjects being all male and of the same age range. 23andMe results show that the majority of subjects are of African American and European ancestry. Some lab errors did occur with 23andMe, and three of the saliva samples came back inconclusive. However, new kits were provided free of charge, and these samples were successfully redone. The 23andMe genome database was somewhat limited, therefore only 9 variants could be compared to serum PUFA levels. Furthermore, none of the SNPs identified as deleterious using PROVEAN were available in 23andMe or other literature. All subjects participated in the T1 and

T2 blood draws, and the 23andMe genetic testing. However, 3 subjects from the study were unable to return for the T3 blood draw for various reasons. This limited the ability to fully assess changes in fatty acid concentrations in those 3 subjects. Concentrations of PUFAs were measured in only blood serum for Part 1 subjects, and in serum and erythrocytes for Part 2 subjects. Previous research confirms that erythrocyte fatty acids are more accurate in reflecting long-term fatty acid status (Sun, Q., et. al, 2007). Unfortunately, since Part 1 subjects only had serum blood data, erythrocyte fatty acid measurements could not be utilized.

Conclusion

As discussed throughout this paper, there is preliminary evidence that genetic variations in the FADS1 gene influence fatty acid metabolism. The extent of this influence and subsequent consequences for athlete health and performance is still somewhat unknown, as genetic research is a highly complex and growing field. Results of this exploratory study suggest the minor allele for SNPs rs174545, rs174546, rs174547, rs174548, rs174549, rs174550, rs174555, rs174556, and rs174561 decrease baseline serum levels of EPA. These results support earlier findings that certain genotypes in FADS1 variants impact baseline PUFA levels, specifically for EPA. Further research is needed to determine if SNVs/SNPs, specifically in non-coding regions of the FADS1 gene, are responsible for observed changes in fatty acid metabolism. Additional research exploring the relationship between PUFA status, brain health, and TBI is also recommended. Currently, there is very little research on genetics in athletic populations, and even less on the influence of genetic variability on fatty acid metabolism and how this may influence brain

health. Further research investigating genetic variability in athletic populations, specifically college athletics, is recommended.

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Appendices

Appendix A

23andMe, Inc., Sunnyvale, CA, USA

23andMe is a trademarked company based in Sunnyvale, California (23andMe, 2022). Permission and use of 23andMe data for this study was obtained and approved through the Institutional Research Board (IRB) prior to collection of data. All participants signed a written consent form prior to the start of this study.

Appendix B

Pre-Survey Dietary Recall Results

Food	0	1-3 servings/wk	4-6 servings/wk	1 serving/day	1-3 servings/day	>4 servings/day
Cereal (1 cup)	13	5	0	1	0	1
Milk (whole, lowfat, skim, chocolate) (per 1 cup)	14	4	0	1	0	1
Milk (any) over cereal (per 1/2 cup)	13	6	0	1	1	0
Milk (any) or cream in coffee (per tablespoon or oz)	13	6	0	0	0	1
Yogurt (flavored or fruited)(per 8 ounces yogurt/ 6 ounces Greek yogurt)	18	2	0	0	0	0
Yogurt (plain) (per 8 ounces yogurt/ 6 ounces Greek yogurt)	19	1	0	0	0	0
Ice Cream (per 1/2 cup)	14	5	1	0	0	0
Frozen Yogurt (1 cup)	19	1	0	0	0	0
Ice Cream Bar/Frozen Fudge Bar (per 1 item)	18	2	0	0	0	0
Cheese: American or Mozzarella (per 1 ounce/slice)	5	9	4	1	1	0

Cheese (hard): Cheddar, Swiss, Provolone, etc. (per 1 ounce/slide)	8	7	2	2	1	0
Cottage cheese (per 1 cup)	20	0	0	0	0	0
Cheese dip or cheese spread (per 1 ounce)	16	3	1	0	0	0
Pudding made with milk (per 1/2 cup)	19	1	0	0	0	0
Creamy soup or sauce (per 1 cup)	13	7	0	0	0	0
Broccoli (per 1/2 cup)	7	12	0	1	0	0
Greens: mustard, turnip, collard, spinach, etc. (per 1/2 cup)	7	8	1	3	1	0
Calcium-fortified juice (orange, others) (per 8 ounces/1 cup)	7	6	2	4	0	1
Bread (white, wheat, pita, English Muffin) (per slice)	2	8	3	5	2	0
Bagel or muffin (per item)	13	7	0	0	0	0
Biscuit or cornbread (per item, 2" diameter)	10	9	0	1	0	0
Pancakes or waffles (frozen) (per	14	4	0	2	0	0

item, 4" diameter)						
Pancakes or waffles (homemade) (per item, 4" diameter)	12	8	0	0	0	0
Beans: red, pinto, lima, etc. (per 1 cup)	15	4	1	0	0	0
Tofu, regular (per 1 cup)	20	0	0	0	0	0
Pasta (per 1 cup)	9	7	2	1	0	1
Eggs, cooked any style (each)	1	8	7	4	0	0
Hamburger (per 4 ounces/1/4 pound)	11	8	0	0	1	0
Oysters, shrimp, crab, crawfish, herring (3 ounces)	9	7	4	0	0	0
Canned salmon (with bones) (per 3.75 ounce can)	13	4	1	1	1	0
Sardines (per 3.75 ounce can)	18	2	0	0	0	0
Cake (slice, 3”X3”X2” piece)	20	0	0	0	0	0
Almonds (per 1/4 cup)	18	2	0	0	0	0
Milk Chocolate (per 1.6 ounce bar)	19	1	0	0	0	0
Recovery shake, nutrition shake, meal replacement formulas	17	2	0	1	0	0
Sports bars	2	5	0	7	6	0

Appendix C

Individual Serum Polyunsaturated Fatty Acid Levels at T1

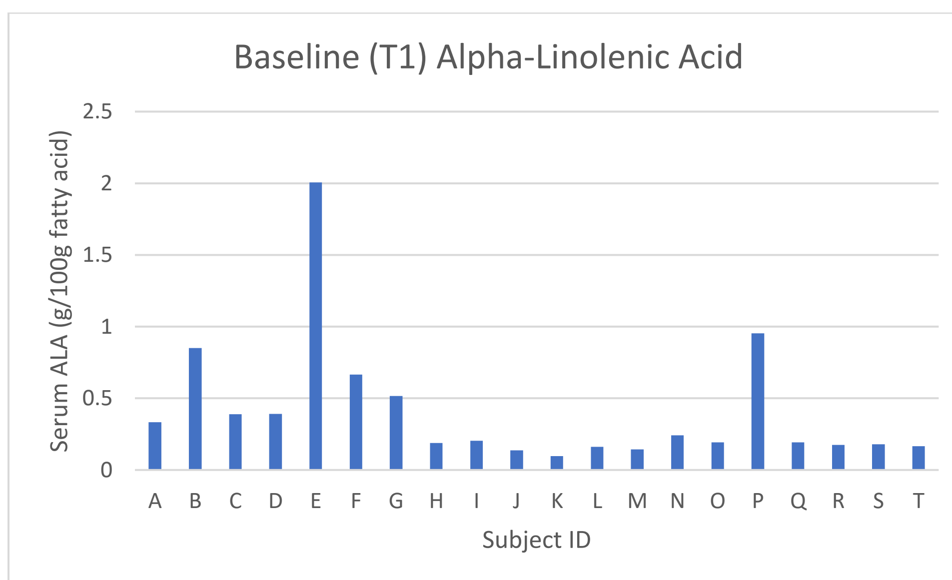

Figure C1: Baseline (T1) alpha-linolenic acid for all subjects (n=20)

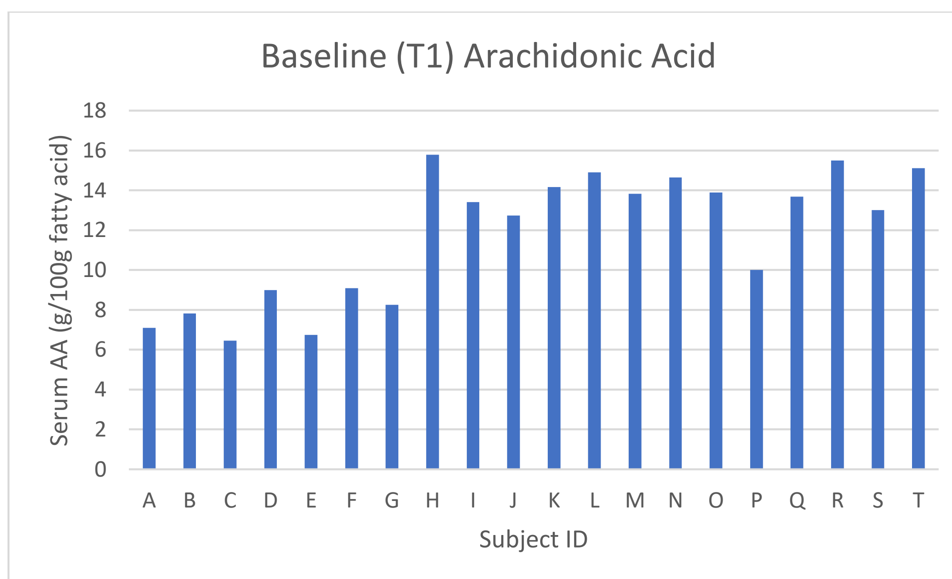

Figure C2: Baseline (T1) arachidonic acid for all subjects (n=20)

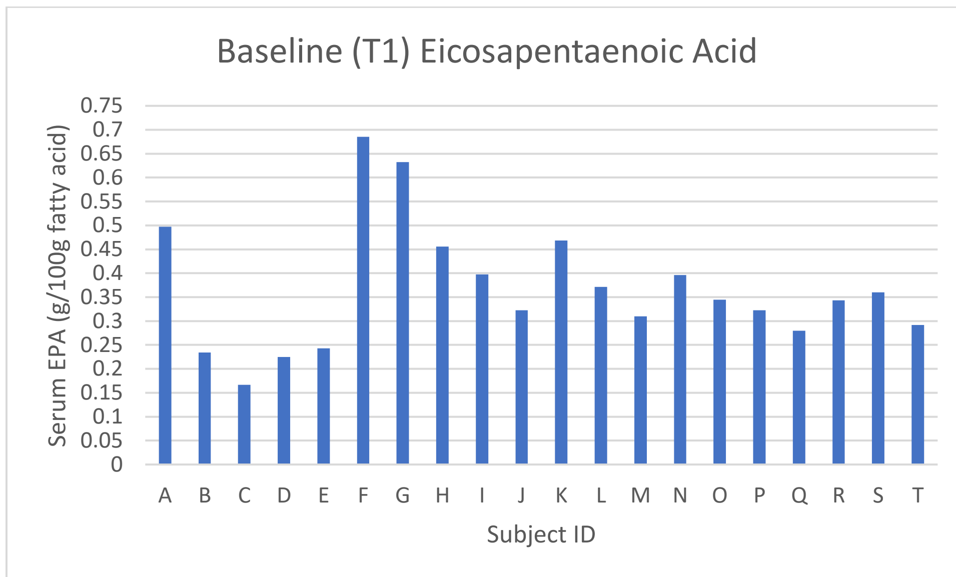

Figure C3: Baseline (T1) eicosapentaenoic acid for all subjects (n=20)

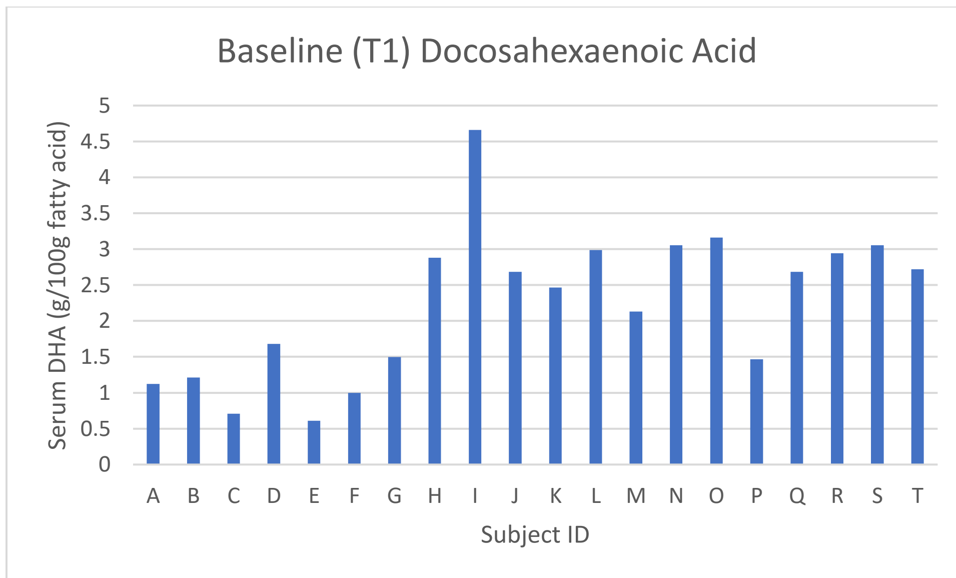

Figure C4: Baseline (T1) docosahexaenoic acid for all subjects (n=20)