Exploration of the perceived and actual benefits of omega-3 fatty acids and the impact of *FADS1* and *FADS2* genetic information on dietary intake and blood levels of EPA and DHA

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ABSTRACT

Exploration of the perceived and actual benefits of omega-3 fatty acids and the impact of *FADS1* and *FADS2* genetic information on dietary intake and blood levels of EPA and DHA

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From a global health perspective, increased intake of omega-3 fatty acids (FAs), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are beneficial for human health. However, the consumption of EPA- and DHA-rich foods such as fatty fish is low in the Western diet. Therefore, finding new ways to motivate people to increase their consumption of omega-3 FAs is essential. In order to find effective ways to motivate individuals, understanding people's awareness of omega-3 FAs and how they obtain their knowledge about nutrition and health is critical. Consequently, we developed an online survey to assess awareness and self-reported intake of omega-3 FAs and supplements in young adults.

EPA and DHA are also produced endogenously to a limited extent through a pathway regulated by fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) genes. Of relevance, single nucleotide polymorphisms (SNPs) in the *FADS* genes influence levels of omega-3 FAs, where minor allele carriers have lower levels compared to major allele carriers. Accordingly, we conducted a clinical trial to investigate FA levels in response to dietary EPA and DHA supplementation in young adults stratified by SNPs in *FADS1* and *FADS2*.

The level of reported awareness of omega-3 terminology varied depending on an individual's field of study and thus providing all participants with the same set of nutrition information could be an effective tool to increase knowledge and motivate behavior change.

Additionally, the variation in FA levels in accordance to SNPs in *FADS1* and *FADS2* could be used to create tailored nutritional recommendations which may improve lifestyle habits. The results discovered in the first two studies regarding awareness of omega-3 FAs and genetic variation were subsequently used to design a nutrigenetics intervention in young adults. Individuals who received their *FADS1* genetic information were more aware of different omega-3 FAs and reported fewer barriers to their consumption by the end of the study, compared to those who did not receive their personal genetic information. All participants increased their intake of EPA and DHA, which was reflected in blood RBC levels.

Overall, this thesis demonstrates the power of combining nutritional and genetic information as motivators to increase omega-3 consumption.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
APOE	Apolipoprotein E
BDNF	Brain derived neurotrophic factor
BF_3MeOH	Boron trifluoride-methanol
BMI	Body mass index
CONSORT	Consolidated standards of reporting trials
CVD	Cardiovascular disease
D5D	Delta-5-Desaturase
D6D	Delta-6-Desaturase
DAG	Diacylglycerol
DGLA	Dihomo-γ-linolenic acid
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
DRI(s)	Dietary reference intake(s)
EE	Ethyl ester
ELOVL2	Elongase 2
ELOVL5	Elongase 5
EPA	Eicosapentaenoic acid
ERK	Extracellular signal-regulated kinases
FA	Fatty acid
FADS1	Fatty acid desaturase 1
FADS2	Fatty acid desaturase 2
FAO	Food and Agricultural Organization
FFA	Free fatty acid
GC	Gas chromatography
GWAS	Genome wide association study
HCPs	Health Care Professionals
HDL-c	High-density lipoprotein cholesterol
HETE	Hydroxyeicosatetraenoic acids
hsCRP	High sensitivity C-reactive protein
HOMA-IR	Homeostatic model assessment of insulin resistance
IQ	Intelligence quotient
LA	Linoleic acid
LD	Linkage disequilibrium
LDL-c	Low density lipoprotein cholesterol
MAPK	Mitogen activated protein kinase
MetS	Metabolic Syndrome
MUFA(s)	Monounsaturated fatty acid(s)
n-3 PUFA	Omega-3 polyunsaturated fatty acid
n-6 PUFA	Omega-6 polyunsaturated fatty acid
NHANES	National Health and Nutrition Examination Survey

PL(s)	Phospholipid(s)
PPARα/δ/γ	Peroxisome proliferator-activated receptor alpha/delta/gamma
PUFA(s)	Polyunsaturated fatty acid(s)
RBC(s)	Red blood cell(s)
RDs	Registered Dietitians
RNA	Ribonucleic acid
ROUT	Robust regression and outlier removal
RT PCR	Real-time polymerase chain reaction
SEM	Standard error of the mean
SFA(s)	Saturated fatty acid(s)
SNP	Single nucleotide polymorphism
SREBP-1c	Sterol regulatory element-binding protein 1
T2D	Type-2-Diabetes
TAG(s)	Triglyceride(s)
TFAs	Trans fatty acids
Total-c	Total-cholesterol
VLDL	Very low density lipoprotein
WHO	World Health Organization
yrs.	Years

CHAPTER 1: REVIEW OF THE LITERATURE

1.1 Introduction

Throughout evolution, the foods consumed by humans on a daily basis have changed dramatically. The current diet of North Americans is generally referred to as the "Western diet", which consists of an excess of processed foods, high amounts of refined sugar, high dietary fat, low fruit and vegetable intake, and low intake of fish and seafood (72). Consequently, the intake of different types of dietary fat has changed radically, with individuals now consuming more saturated fatty acids (SFAs) and trans fatty acids (TFAs), and less monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) than even 100 years (yrs.) ago (72). With the reduction in fish and seafood intake, this means that two specific PUFAs, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are particularly low in the diet. Recent global reports show that intake of EPA and DHA are low across the world (111, 223), which poses an important public health concern. Little is known about the long-term impact of low EPA and DHA consumption.

Nutrigenetics / nutrigenomics are emerging fields in science that combine information about nutrition and genetics. Current dietary recommendations are made at a population level, with limited extension for personalization. Nutrigenetics takes into consideration an individual's genetic makeup, and in particular the single nucleotide polymorphisms (SNPs) that contribute towards variations in response to diets and/or specific nutrients. These SNPs and resulting responses to foods and nutrients could be considered when building a personalized diet to optimize health. The vast majority of studies to date have focused on assessing people's perceptions of nutrigenetics rather than behaviour change in a nutrigenetics intervention. In order

to assess the value of nutrigenetics, an appropriate dietary and genetic target must be selected. Omega-3 FAs are attractive candidates as they are modified endogenously and genetic variation contributes to differences in FA levels in the body. Therefore, tailoring an individual's diet with their genetic information related to omega-3 FAs may encourage individuals to increase their intake of EPA and DHA.

This literature review is composed of three major sections: 1) an overview of dietary fats, with an emphasis on omega-3 FAs, focusing on the importance of these FAs in the diet and the crucial role they play in the body and their connection to human health; 2) basic and genetic insights regarding the fatty acid desaturase (FADS) pathway activity, and the implications of genetic variation in *FADS* genes; and 3) current evidence demonstrating the potential value of nutrigenetics.

1.2 Dietary Omega-3 FAs

1.2.1 Types of Dietary Fats

Consumption of the Western diet (described above) is associated with increased risk of chronic metabolic diseases, including cardiovascular disease (CVD), type-2 diabetes (T2D), and obesity (72). Further, North America is also experiencing an obesity epidemic, with most people consuming an abundance of high energy foods coupled with low levels of physical activity (90). The global burden of non-communicable diseases is expected to reach 57% of all diseases by 2020 (325). However, diet and lifestyle modifications are viable options for the prevention and treatment of these lifestyle-related diseases. An examination of the diet across the globe, along with making clear dietary and lifestyle recommendations will be critical to reduce this health care burden.

Fats are a key part of the diet and are the most energy dense nutrient found in foods (255). Typically, the information North Americans receive regarding dietary fats is predominantly negative, with the "low fat era" (beginning in the 1980's) convincing the public that all fats should be avoided (312). However, this message is overly simplistic because dietary fats are diverse in structure and function and cannot be referred to synonymously. To be specific, there are four main types of fats: TFAs, SFAs, MUFAs and PUFAs, each of which have unique physicochemical properties. Moreover, these different fats also have broad, but distinct, health effects, i.e., a high intake of TFAs are "harmful" to health and a high intake of MUFAs are "beneficial" to health (228). Therefore, guidelines on the intake of dietary fats should focus more on the types of fats, rather than the total amount consumed (171, 188).

1.2.2 Recommendations for Dietary Fat Intake

Nutritional recommendations were historically developed to prevent malnourishment, deficiency, and disease using young adult men as the subjects in these studies. Further, these recommendations are homogenous, assuming that across the population the nutritional requirements would be the same (100, 181, 243). Current recommendations for dietary fat intake suggest that fats should provide 20-35% of a person's daily caloric intake (312). Further, it is recommended that for overall health, individuals should increase their intake of MUFAs and PUFAs, while decreasing their intake of SFAs. Altering the ratio of the types of dietary FAs consumed may be more important to overall health than reducing total fat (312). Specifically, SFAs are recommended to be consumed at less than 7% of daily total energy, and TFAs are recommended to be consumed at less than 1% of daily energy (223). The intake recommendations for omega-3 FAs are made for the combination of EPA and DHA, as most foods contain both of these FAs (129). The majority of advisory boards suggest that people

should consume two servings of fish per week, with the preference for consumption of fatty fish (54, 120, 170, 171, 279, 312). If this advice was followed, this would correspond to an intake of approximately 300-500 mg EPA and DHA per day (**Table 1.1**).

Table 1.1. Summary of Global Recommendations from Expert and Governing Groups.

Organization	**Recommendation**
American Heart Association (2016) (193)	General population / Adults - Two servings of fatty fish / week (for general health) For CVD health - 1000 mg/day of EPA and DHA preferably from oil fish
Dietitians of Canada (2013) (54)	General adult population – 300-450 mg/day EPA and DHA / two Food Guide servings of fatty fish per week
European Food Safety Authority (2012) (4)	0.7-2 yrs. – 100 mg/day DHA 2-18 yrs. – 250 mg/day EPA and DHA Adults – 250-500 mg/day EPA and DHA Pregnant/lactating women – 250-500 mg/day EPA and DHA (plus 100-200 mg/day DHA)
International Society for the Study of Fatty Acids and Lipids (2004) (76)	Adults – minimum 500 mg/day EPA and DHA for adults for CVD health Pregnant/lactating women – minimum 200 mg/day DHA
Joint Food and Agricultural Organization (FAO) of the United Nations/World Health Organization (WHO) expert consultation on fats and fatty acids in human nutrition (2010) (151)	0.5-2 yrs. – 10-12 mg/kg body weight per day of DHA 2-4 yrs. – 100-150 mg/day of EPA and DHA 4-6 yrs. – 150-200 mg/day of EPA and DHA 6-10 yrs. – 200-250 mg/day of EPA and DHA Adults – 250 mg/day of EPA and DHA Pregnant/lactating women – 300 mg/day EPA and DHA (200 mg should be DHA)
United States Dept. of Agriculture and Department of Health and Human Services (2010) (212)	General population - 8 ounces/week of seafood, which would provide an average of 250 mg/day of EPA and DHA Pregnant/lactating women - 8-12 ounces/week from fish and marine animals (choices focused on those lower in methyl mercury)

The Global Organization for EPA and DHA Omega-3s (GOED) has provided a comprehensive review of EPA and DHA recommendations (2014). Some groups are represented within this table. Many advisory/expert committee recommendations have specifications for adults, children, and pregnant/lactating mothers (10, 244). Adapted from: GOED (120).

1.2.3 Current Intake of Dietary Fat

There are significant differences in dietary fat intake across the globe (223, 294), highlighting that geographical location impacts dietary intake. In addition, age, sex, allergies and physical activity levels have also been shown to significantly influence dietary fat intake (80).

The global consumption rates for dietary fats were estimated as a percentage of total energy intake, based on data collected from food surveys and food frequency questionnaires (FFQs) (111, 223). A recent report analyzed FFQs from n=1,630,069 adults (≥ 20 yrs.) across the world. On average, adults reported to be consuming 1.4% of their energy as TFAs and 9.4% of their energy as SFAs (223). The intake of TFAs was generally higher at younger ages (≤50 yrs.), while the intake of omega-3 FAs were generally higher at older ages (≥51 yrs.) (223). MUFA levels were not calculated in this analysis, while PUFA levels were reported separately for omega-6 FAs, and further separated for omega-3 FAs alpha-linolenic acid (ALA; 18:3n-3), EPA, and DHA (223). Omega-6 FA intake was reported to be 5.9% of total energy, the average intake of ALA was 1371 mg ALA/day and average intake of EPA and DHA was 163 mg EPA and DHA/day (223). Notably, ~67% of the world's adult population had EPA and DHA intakes lower than 100 mg EPA and DHA/day (223). DHA intake is generally lower in developing countries, with a median intake of ~96 mg DHA/day; the exception to these low intakes being several small island states which consume a very high amount of fish, providing ~1,000 mg EPA and DHA/day (111). In comparison, developed countries consume a range of 184-473 mg DHA/day (111). Countries with low gross national incomes and high birth rates had the lowest levels of DHA intake (111). Specific to Canada, males and females had similar intakes of DHA which were ~200 mg/day and ~260 mg/day, respectively from two studies (114, 248), although the averages differed between reports, with ~86 mg/day DHA in adults reported in work by Denomme *et al* (86). In another report of Canadian adults (n=5785, 20-79 yrs.) blood levels of EPA and DHA were reported as a percentage of red blood cell (RBC) FAs and the differences in percentages were investigated (184). The mean Omega-3 Index (sum of EPA and DHA calculated from % FA in red blood cells (RBC)) was 4.5%; however, 45% of the population

scored less than 4%, and due to these low circulating EPA and DHA levels, were considered to be in the "high risk" category for coronary heart disease (184).

1.2.4 Dietary Reference Intakes for Individual Omega-3 FAs

Currently, there is an adequate intake (AI) for ALA (107, 168, 199, 276), which is listed as 1,600 mg/day for men and 1,100 mg/day for women, with no defined upper limit (54, 312). There are no AIs for EPA and DHA, and there are no dietary reference intakes (DRIs) set for any of these PUFAs (107). In the case of EPA and DHA, these recommendations focus on ideal intake, as most countries have low intake of EPA and DHA. Therefore, DRI recommendations are made by several different organizations based on the positive health benefits seen with increased intake of EPA and DHA (120) (**Table 1.1**). Several organizations propose specific recommendations for EPA and DHA intake during pregnancy, lactation, and infant development (181). Some recommendations also address intake for individuals who have hypertriglyceridemia, or those at risk for CVD (120). Although the recommendations between governing groups are similar (minimum intake of EPA and DHA ~250 mg/day), many experts suggest that these values should actually be higher, and the variability in recommendations makes the creation of a global DRI very difficult (107, 168). An additional complication in determining an appropriate DRI for EPA and DHA is related to the endogenous conversion of ALA into EPA and DHA that occurs in the body (107, 168, 282). For this reason, EPA and DHA are not considered essential nutrients by classical definition as the human body is able to convert these nutrients endogenously.

1.2.5 Epidemiological Studies Related to Dietary Intake and Disease Occurrence

In relation to dietary fat, the epidemiological studies that pioneered omega-3 FAs and health research occurred between 1960-1970 with the study of Greenland Eskimos (94). This population has a very high intake of dietary fat, predominantly animal fat from fish and seal, while having low rates of heart disease (13, 87, 94). After this literature was published, global epidemiological studies were completed to compare dietary intake between different countries; Greenland and Japan (for example) had significantly greater consumption of fish and seafood, and lower rates of coronary heart disease, compared to North America, which has low fish and seafood consumption and higher rates of coronary heart disease (334). Therefore, fish and seafood intake were inversely correlated with CVD.

Recently, Stark *et al.* completed a systematic review comparing EPA and DHA blood levels in healthy adults across the globe (294). The highest levels of EPA and DHA (i.e., Omega-3 Index >8%) were reported in Japan, Scandinavia, and other areas with significantly different dietary intake compared to Westernized societies (294). Additionally, similar intake levels (Omega-3 Index >8%) continue to be found in Inuit populations (such as the Greenland Eskimos and other indigenous populations) (294). In contrast, very low blood levels of EPA and DHA (i.e., Omega-3 Index ≤4%) were observed in most of the world including North America, Central and South America, Europe, the Middle East, Southeast Asia, and Africa (294). Generally, the blood FA data matched the FFQ data from these countries (293), and by this comparison it can be assumed that countries with higher levels of EPA and DHA are likely getting these FAs through high fish and seafood intake (223).

1.2.6 Food Sources of Omega-3 FAs

Several types of foods that are naturally high in omega-3 FAs are summarized in **Table 1.2**. ALA-rich dietary sources include some types of oils (canola, flaxseed, rapeseed), walnuts, ground flaxseeds, some leafy green vegetables, and soy products (55, 222). As reported from epidemiological studies, fish and seafood are rich sources of EPA and DHA; however, not all fish and seafood provide the same amount of EPA and DHA (55). Fish with darker flesh such as salmon, herring, and mackerel have a higher overall fat content, with higher EPA and DHA compared to white fish such as cod and pollock. Other food sources enriched with EPA and DHA include seaweed and algae (124). Seaweed, algae, and phytoplankton are the original sources of EPA and DHA; when fish consume these plants, these omega-3 FAs are incorporated within their tissues (9, 233).

Apart from whole food sources, oils from cod, fish, krill, and algae exist as supplements and are high in EPA and DHA (124). Typically, both EPA and DHA are part of the supplement composition, with a ratio of 2:1 EPA:DHA; however, supplements containing only EPA or DHA are also available (93, 229). In addition to various ratios of EPA:DHA, these FAs also come in various forms, i.e. as free fatty acids (FFAs), phospholipids (PLs), triglycerides (TAGs), or ethyl esters (EE). The bioavailability of each of these different forms has been compared to determine which form of FA delivery would be superior. EPA and DHA are reported to be most bioavailable in the FFA form, and the least bioavailable from is EE (115). However, the most common forms of supplements are found in either the PL or TAG form, and no conclusion on superior bioavailability can be determined to date, as animal studies and human studies are not consistent (115).

Kris-Etherton *et al.* reported that in order to increase intake of EPA and DHA to the recommended levels, significantly more fish and marine products would need to be consumed (171). However, increased consumption of fish would increase the burden on aquaculture industry and the fish farming industry and the consequences on the ecosystem currently unknown (9, 148, 240, 294). In order to address these sustainability issues, yet increase EPA and DHA intake in the general population, the past 15 yrs. has seen the development of foods fortified with EPA and DHA, including milk and eggs. In the production of EPA- and DHA-rich eggs, hens are fed ALA-rich diets (typically high in flaxseed). Research on omega-3 fortified eggs shows that hens efficiently convert ALA into EPA and DHA when fed an increased amount of flaxseed (159). Thus, fortified eggs represent an important and alternate source of EPA and DHA other than marine sources. In addition, using algal biomasses and micro-encapsulated algae have been suggested for aquaculture and animal feed, in order to increase EPA and DHA content, with possible extension to human food (294). Further, the concentration of EPA and DHA may be increased through genetically modified oilseed crops, such as canola or soybeans, which could aid in the supply of EPA and DHA to people across the world (171, 294). Therefore, the incorporation of algae into products, and the development of new crop strains, may help increase dietary intake of EPA and DHA without compromising aquatic life.

Food companies have begun to fortify other products such as yogurt, cereal bars, juices, *etc.* with omega-3 FAs. One reason for additional food fortification was in recognition of the health benefits associated with increased intake, as well as the acknowledgement that many individuals are consuming minimal fish and seafood in their typical diets. However, to date, the fortification of food products with EPA and DHA results in only minimal increases in their intake. For example, there is ~20 mg DHA per serving of omega-3 enriched milk ("Dairy Oh!™"

(330)) and 150 mg EPA / DHA per omega-3 egg; compared to 1,000-1,500 mg EPA / DHA per serving of salmon (55). Raising levels of EPA and DHA in the body through fortified foods alone would be very difficult strategy in order to reach the amounts suggested for CVD risk reduction, as many of these products have very small amounts (10-150 mg of EPA and DHA/serving) (140, 249). Although, small increases in DHA intake may be important for brain health and cognitive development (146, 236). For example, the Joint FAO and WHO recommendations suggest that infants between 6 months and 2 yrs. should consume 10-12 mg/kg of body weight per day of DHA, and the European Food Safety Authority recommends 100 mg/day DHA (120). In contrast to these recommendations, infants are currently consuming ~18 mg/day DHA and thus one serving of Dairy Oh! ™ would double their current intake (86, 198, 200, 330).

Table 1.2. Summary of Food Sources High in Omega-3 FAs.

ALA	**EPA and DHA**
Chia Flaxseeds (ground) Hemp Some leafy green vegetables Soy products Vegetable oils - Canola, flaxseed, rapeseed, soybean Walnuts	Fatty fish and seafood - Herring, salmon, sardines, trout, tuna Functional foods - Some cow's milk, some soy milk - Some cereal bars, eggs, juices, oils, spreads, yogurts Supplements - Algal oil, cod liver oil, fish oil (typically made with anchovy, menhaden, salmon, sardine), krill oil, squid oil

Whole foods naturally high in ALA, EPA, and DHA, as well as supplements and fortified foods enriched with EPA and DHA. Dietitians of Canada provides an extensive list of many products and the corresponding milligrams of ALA, EPA and DHA amounts per serving, based on the Canadian Nutrient File (2010) (55). Adapted from: Vannice *et al*. (312), Meyer *et al*. (222), and the Dietitians of Canada (55).

1.2.7 Health Effects Associated with Omega-3 FAs

ALA is often associated with neutral health effects in comparison to EPA and DHA (6, 14, 174, 258, 326). Consequently, the majority of this section outlining the possible health benefits of omega-3 FAs is focused on EPA and DHA. The following sections will briefly review cardiovascular health, brain health, and metabolic health in relation to EPA and DHA omega-3 FA intake.

1.2.7.1 Cardiovascular Health

Over the last 30 yrs., several studies have associated the increased intake of EPA and DHA with improvements in heart health (52, 140, 214). Studies show that an increased intake of EPA and DHA reduce risk of stroke, myocardial infarction, heart attack, and cardiac mortality and morbidity (88, 214). In contrast, inadequate intake of EPA and DHA is associated with increased risk of sudden cardiac death (227). The proposed mechanisms behind the cardio-protective effects have been explored by many groups, with several findings speculative and little confirmed due to the differences between rodent and human models (2, 306). Briefly, EPA and DHA have an anti-thrombotic effect through the action of oxylipin synthesis, and specifically, act to reduce the production of thromboxane A_2 and prostacyclin I_2 from omega-6 derived oxylipins (52). Additionally, animal and cell culture models have shown that EPA and DHA can promote electrical stability in the heart through the stabilization of ion channels, which helps to regulate heart rate (52). In regards to CVD and atherosclerosis, researchers have shown that the incorporation of EPA and DHA into a plaque renders it more stable and less likely to rupture (52).

In addition, blood lipids including TAG, total cholesterol, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c), can be altered depending on the amounts of EPA and DHA in the diet (191, 213, 289). There are consistent reports of TAG lowering after EPA and DHA supplementation, and inconsistent results regarding their effects on cholesterol parameters (289). Both lower doses (300-1000 mg EPA and DHA/day) (127, 172) and higher doses (>3,000 mg EPA and DHA/day) (214) are effective for TAG lowering in a dose dependant manner (review (128)). Lower doses can also contribute towards changes in cardiac physiology (<1,000 mg EPA and DHA/day) (214). Health Canada recently supported that at least 1,500 mg EPA and DHA/day results in a significant reduction in TAG levels (58). To follow, Health Canada and the Food and Drug Administration has approved 5,000 and 4,000 mg EPA and DHA/day, respectively, for treatment of hypertriglyceridemia (57, 213). In terms of the well-established TAG lowering effect, EPA and DHA decrease very low-density lipoprotein (VLDL) TAG secretion rates from the liver (130, 306). There are several proposed mechanisms by which EPA and DHA reduce VLDL TAG synthesis, including: a) increased lipoprotein lipase activity which hydrolyses TAG found in VLDL (262), b) decreased hepatic VLDL synthesis in part through the suppression of apolipoprotein B (130, 162), c) decreased hepatic glycogen and hepatic FA synthetase, resulting in a subsequent diminished FA synthesis and TAG formation (3, 127, 162, 333), and d) increased FA oxidation in peroxisomes and in mitochondria via the activation of peroxisome proliferator-activated receptor alpha/delta (PPARα/δ) (106).

Despite substantial literature on the positive cardiac benefits associated with high EPA and DHA intake, recent meta-analyses suggest a neutral response across several studies for the prevention of CVD and overall mortality with a moderate versus high dose of EPA and DHA

(268). However, McLennan proposes that one reason for the discrepancy in these results is the inconsistent doses of EPA and DHA in the different fish oil supplements, with moderate doses providing up to 1,800 mg EPA and DHA/day, and higher doses providing up to 5,000 mg EPA and DHA/day (214). Additionally, some studies compare fish oil supplements to whole fish, and moderate consumption of whole fish could provide 300-500 mg EPA and DHA/day, where a higher intake of fish may provide up to 1,500 mg EPA and DHA/day (214). Moreover, some of the clinical trials with neutral results may have been underpowered, provided a low supplement dose of EPA and DHA, did not account for baseline FA levels, had inadequate intervention periods, and / or overestimated compliance and adherence (264, 294). All in all, high EPA and DHA intake has shown strong inverse associations with risk of CVD and lowering of blood lipid levels, while the effects of low levels of EPA and DHA remain to be determined.

1.2.7.2 Cognitive Development and Brain Health

The composition of the brain is 60% fat, with a large volume of water (66, 236). Specifically, a large percentage of the FAs in the brain are PUFAs, and ~30% of the total FA composition is made up of DHA (66, 236). This suggests an important role for DHA in brain functioning and mental health. EPA and DHA are associated with the proper structural formations of the brain, eyes, and central nervous system (205). Moreover, research in primates suggests that DHA is more efficiently incorporated into the fetal brain and retina compared to ALA (123). Most of the research in relation to EPA and DHA and brain health has been investigated in the context of fetal and infant development (35, 146, 236), as well as mood and depression (158, 216, 301). In terms of mechanisms, EPA and DHA are incorporated into cell membrane PLs, which are part of the axons and synapses. Indeed, DHA-rich synapses appear to be crucial for a well-developed central nervous system (157). Additionally, EPA and DHA

increase the fluidity of membranes, allowing for efficient transfer of neurotransmitters to enhance the electrical basis for memory formation (96, 157). DHA can also be found in higher concentrations in retinal tissue (50% FAs are DHA), neuronal cells in the central nervous system, liver, and testes (66, 307, 312).

In consideration of more broad correlations, there have been associations between the levels of EPA and DHA in the blood and measurements of brain function that include intelligence quotient (IQ) scores (134, 146). When examining mood and mental health, several reports show that an increased intake of EPA and DHA (range from 600-6,600 mg EPA and DHA / day) results in improved symptoms of major depressive disorder (216, 301), and reductions in cases of schizophrenia (252) and attention deficit hyperactivity disorder (267). Gispert-Llaurado *et al.* reported mixed results regarding fish consumption in European children (n=500, 7-9 yrs.) and found no association between fish consumption and neuropsychological outcomes; however, inverse associations were found between fish consumption and social skills, focus and attention, rule-breaking, and aggressive behaviours (118). A recent meta-analysis of both breastmilk and formula fed infants with varying amounts of EPA and DHA showed that the increased intake of EPA and DHA had no effect on visual acuity, language development, or cognition in infants (263). However, several other systematic reviews report significant benefits for increased EPA and DHA intake of cognitive (149), visual (261), and immune function (266).

1.2.7.3 Metabolic Health

There have been several studies conducted which suggest varied improvements to metabolic health with increased intake of EPA and DHA. Briefly, low EPA and DHA intake has been associated with higher self-reported weight (n=7556, 45-69 yrs., women from Norway) (309). Dietary intake of EPA and DHA has an indirect relationship with obesity and insulin

resistance through the insulin signalling pathway (40, 235). One way in which EPA and DHA can improve insulin sensitivity is through the increase of adiponectin by activating PPARγ (a master transcriptional regulator in adipocytes) (152, 196). In addition, EPA and DHA produce anti-inflammatory oxylipins which can act to increase insulin sensitivity, particularly in adipose tissues (152). The specific mechanisms by which increased EPA and DHA intake improve metabolic health, at times lower fasting glucose and insulin levels, and enhance weight loss are not clearly established (196). However, associations exist between increased EPA and DHA intake and improvements in metabolism (180) and reduced risk for diabetes (53, 57, 302). Some of the improvements to insulin resistance may be related to TAG lowering, as described previously in the cardiovascular health section (196). Another proposed mechanism shown in rodents is related to the incorporation of EPA and DHA into cell membranes of various tissues (adipose, liver, and skeletal muscle), which may improve insulin-stimulated glucose transport (196). In order to improve insulin sensitivity, EPA and DHA are reported to down-regulate lipogenic genes like sterol regulatory element-binding protein 1 (SREBP-1) (84, 196). The results of improved insulin sensitivity and glucose lowering are inconsistent with some studies reporting changes and others reporting neutral metabolic responses with increased EPA and DHA intake (106). In regards to weight and adipose tissue accumulation, EPA and DHA have been shown to reduce fat pad mass, hypertrophy of fat cells, and can alter adipocyte size by activating PPARγ (196). Importantly, in connection to metabolic health, inflammation underlies many chronic conditions (CVD, T2D, obesity). Considerable research has demonstrated a role of EPA and DHA in the mitigation and reduction of inflammation (96, 302), specifically occurring through an increased production of anti-inflammatory oxylipins that include resolvins and protectins (40, 50, 96, 302).

1.3 Omega-3 FAs in the Body

1.3.1 ALA as an Essential FA

The FADS pathway which will be described later, works to endogenously convert ALA into EPA and DHA. ALA must be consumed from the diet and can't be produced endogenously in the body (275). The role of the essential FAs ALA and linoleic acid (LA; 18:2n-6) were first demonstrated in the causation of skin conditions (scaly dry tail with lesions) in rats, whose diets were devoid of ALA and LA (48). The importance of ALA was highlighted in rodent studies were dams were supplemented with ALA before and during pregnancy. These results demonstrated a significant impact on pups brain function, memory, and learning (182, 183). Additionally, in a case study with one human patient from 1982, it was reported that neurological symptoms were reversed when an appropriate amount of ALA was supplemented to the diet (138).

1.3.2 Structure of Omega-3 PUFAs

FAs are made up of two main components, the carboxyl group and the hydrocarbon chain. The hydrocarbon chain differs in the number of carbons and the position of double bonds, which results in each FA having a unique structure (234). FAs with more double bonds become more "kinked" in structure, and thus need more physical space when they are incorporated into PLs or TAGs (81, 234). The incorporation of these kinked FAs into PLs tend to make the membranes more fluid, as the kinked structure prevents a close alignment of PLs in lipid bilayers (133, 229). The alterations to lipid bilayers and cell membranes play a role in determining the functional properties of these FAs within the body (133, 312).

Highly unsaturated PUFAs have at least 18 carbons and more than two double bonds (312). PUFAs can be further classified into subspecies such as omega-3 and omega-6 FAs (234). Omega-3 and omega-6 FAs are the primary PUFA subspecies which are the focus of this thesis, while omega-7 and omega-9 FAs, which are non-essential FAs, will not be discussed further. The three main dietary omega-3 FAs are ALA, EPA and DHA. Omega-3 FAs are defined as having the first double bond in their structure occurring at the third carbon from the methyl end of the hydrocarbon chain. For example, EPA has 20 carbons and five double bonds, with the first double bond occurring at the third carbon in the FA chain (**Figure 1.1**).

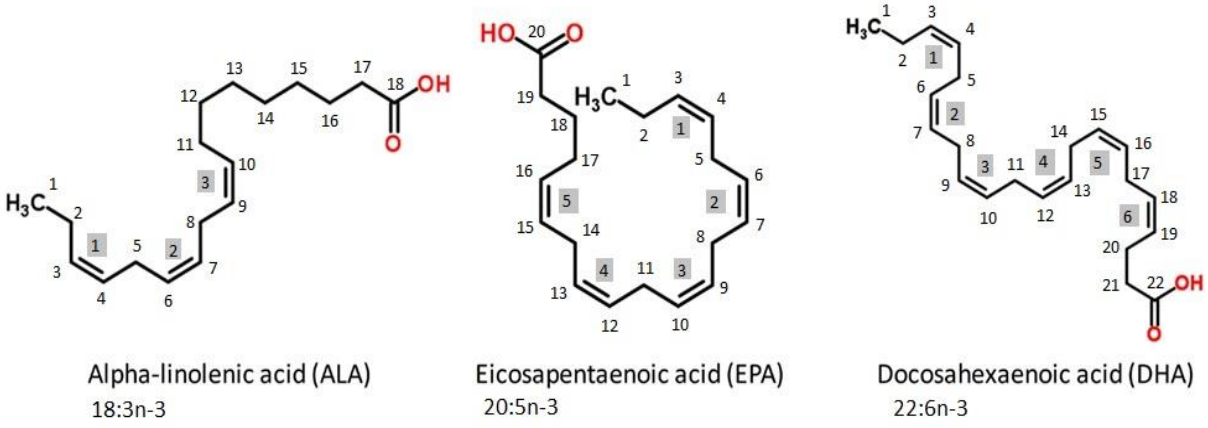

Figure 1.1. Chemical Structure of ALA, EPA, and DHA.

ALA; alpha-linolenic acid; 18:3n-3, EPA; eicosapentaenoic acid; 20:5n-3, and DHA; docosahexaenoic acid; 22:6n-3. Carbon atoms are numbered, starting from the methyl end (H_3C/CH_3). Numbers within a grey box indicate position and number of double bonds. Adapted from: Moreno *et al*. (225).

1.3.3 Incorporation into Cell Membranes in the Body

Although FAs are commonly discussed as FFAs, they are not often found "free" in foods or in the body. Indeed, the vast majority of FAs consumed in foods are present in dietary TAGs. The proportion of FAs within the food matrix is very important in determining which FAs are incorporated into the body (312). During the digestion of dietary fats, TAG are hydrolyzed by

lipases to release FFAs and monoglycerides (234). Subsequently, these FAs can be oxidized, incorporated into PLs, chylomicrons/lipoproteins, and/or re-esterified and stored as TAG (312). When there is an abundance of a certain type of dietary FA (e.g. EPA), this could displace and replace other FAs in PLs or TAGs, resulting in alterations to signalling and transport within the tissue (84, 234). Further, the displaced FAs would move into circulation and may be converted into other metabolites (e.g. oxylipins and eicosanoids), or accumulate in other tissues (adipose, liver, skeletal muscle) (312). EPA and DHA play a role in cell membrane structure and composition, and contribute to the fluidic properties of a membrane (307). Specifically, EPA tends to remain dynamic with the ability to move and re-locate as necessary (190, 260). In contrast, DHA tends to be more static in its position within the PL membrane. Supplementation studies show that EPA and DHA incorporate into various fractions including TAGs, PLs, diacylglycerols (DAGs), and cholesterol esters within the liver, adipose, and skeletal muscle (209).

1.3.4 Metabolic Fates of Omega-3 FAs in the Body

In addition to storage, FAs have many metabolic functions in the body. The primary fate of ALA in the body is β-oxidation (37, 287, 307). Rates of ALA oxidation, which are measured through CO_2 production and excretion on breath, are reported to be 20-33% of the ingested ALA (41, 46, 47). Original studies report that there were sex differences in the amount of carbon-labeled ALA recovered as $^{13}CO_2$ in breath; specifically ~33% of ALA was recovered in $^{13}CO_2$ in breath in young men and 22% of ALA was recovered in $^{13}CO_2$ in breath in young women (46, 47). Other studies report an average ~20% $^{13}CO_2$ was recovered in breath in both men and women (85, 315). The other work done to determine the percentage of ALA distributed to various processes occurred with respect to the ALA conversion into long-chain PUFAs. It is

estimated that ~1-9% of ALA is converted into EPA and ~1-4% is converted into DHA (43). The remainder of the ingested ALA has other fates including carbon recycling by FA synthesis, storage in PLs or TAGs, and transformation into other FAs including SFAs and MUFAs (43, 123). The exact percentages of ALA which are distributed into these other fates are currently unknown.

Compared to ALA, less work has been done with EPA- and DHA-tracers. EPA and DHA can be found in circulation in the blood as part of PLs or TAGs, oxidized, converted into other PUFA (195, 257), and some DHA can be retro-converted to other long-chain PUFAs, such as EPA and DPA (51, 69, 70, 296). EPA and DHA can also be converted into other metabolites, such as oxylipins, which can act as direct and secondary messengers (53, 307). EPA and DHA produce anti-inflammatory oxylipins such as prostaglandins (EPA gets converted into series three prostanoids PGE_3, PGD_3 and T_XA_3), leukotrienes, and hydroxyeicosatetraenoic acids (HETEs) (308), resolvins, and DHA-derived neuroprotectins, and maresins (33). These oxylipins and their proposed functions are described in other work (50, 53, 308, 324). As a result of oxylipin production, EPA and DHA have been reported to partly inhibit several inflammatory processes including: leukocyte chemotaxis, adhesion molecule expression, leukocyte-endothelial adhesive interactions, and others (49, 50). It is unclear what percentage of EPA and DHA are converted into these oxygenated metabolites.

Overall, EPA and DHA have an important structural role within the body and are converted into several other metabolically active metabolites. The next section will discuss the conversion of ALA into EPA and DHA in more detail.

1.4 FADS Pathway

1.4.1 FADS Pathway Enzymes

1.4.1.1 FADS1 and FADS2 Genes and Enzyme Activity

FADS1 and *FADS2* genes are found side-by-side on chromosome 11 in an opposite 5'-to-3' orientation (98). *FADS1* and *FADS2* are responsible for producing the delta-5-desaturase (D5D) and delta-6-desaturase (D6D) enzymes, respectively. These enzymes desaturate PUFAs by removing a hydrogen atom and adding a double bond (307). In order for desaturase activity to occur, a cytochrome-b5-like domain and two membrane spanning domains, in addition to oxygen and nicotinamide adenine dinucleotide phosphate (NAD(P)H), are necessary (98, 232). Desaturase activity occurs in the microsomal membrane fraction of the endoplasmic reticulum, with the binding of non-heme iron (66). Molecular research of the *FADS* genes has shown 87% amino acid sequence homology between mouse and human, which makes the mouse model very useful for FA research (66). Currently, the *FADS3* gene shares 52-62% sequence identity with *FADS1* and *FADS2* genes and encodes for a yet unidentified protein (232). More work continues to be done to gain a greater understanding of the *FADS3* gene and enzyme activity (336).

Several noteworthy studies in mice have elucidated and described the fundamental roles that *FADS1* and *FADS2* play in metabolism and health (102, 125, 232, 300). Historically, *FADS1* and *FADS2* are reported to have evolved before the appearance of gnathostomata (jawed vertebrates) and have played a crucial role in FA metabolism for millions of yrs. (63). Interestingly, birds and reptiles have many other *FADS* genes, and not all of these genes are specific to either D5D or D6D activity (63). D5D appears to be mammalian specific, and is thought to have evolved from D6D (63). *FADS2* deficient mice are viable, but both sexes are sterile, have no synthesis of long-chain omega-3 FAs, no subsequent oxylipin production, and

have disturbed platelet aggregation (297, 300). In contrast, *FADS1* knockout mice die before the age of 12-weeks and these mice are acutely sensitive to inflammatory challenges (102, 307).

1.4.1.2 Elongase 2 (ELOVL2) and Elongase 5 (ELOVL5)

The elongase genes, *ELOVL2* and *ELOVL5*, are found on chromosome 6. ELOVL enzymes are responsible for adding two carbon atoms to the hydrocarbon chain of the FA for elongation. Elongation takes place mainly in the endoplasmic reticulum with a range of ELOVL enzymes, from ELOVL1-7, which are not all related to the FADS pathway (307, 320). ELOVL2 and ELOVL5 specifically elongate PUFAs (305).

1.4.2 FADS Pathway Conversion

A substantial amount of research has been completed to understand and characterize the pathway involved in PUFA conversion and metabolism. The description below will focus on omega-3 FAs, although the same series of desaturation and elongation steps occur with omega-6 FAs (320) (**Figure 1.2)**. The omega-3 FA ALA is consumed in the diet and is the first omega-3 FA in the FADS pathway. D6D catalyzes the first reaction, through the addition of a double bond to the aliphatic chain of ALA to create stearidonic acid (SDA, 18:4n-3). ELOVL5 then elongates the aliphatic chain of SDA into eicosatetraenoic acid (ETA, 20:4n-3), which is then further desaturated by D5D to form EPA (320). In the production of DHA, EPA is first elongated with ELOVL2 to form DPA. DPA is then further elongated with ELOVL2 to form tetracosapentaenoic acid (TPA, 24:5n-3), which is then desaturated by D6D to form another intermediate FA called tetracosahexaenoic acid (THA, 24:6n-3). THA moves from the endoplasmic reticulum to the peroxisome where it undergoes β-oxidation to form DHA (78, 291, 300). After DHA is created, DHA moves back into the endoplasmic reticulum and is then

incorporated into the cell membrane to undergo esterification and/or lipoprotein packaging and secretion into the blood (92, 104).

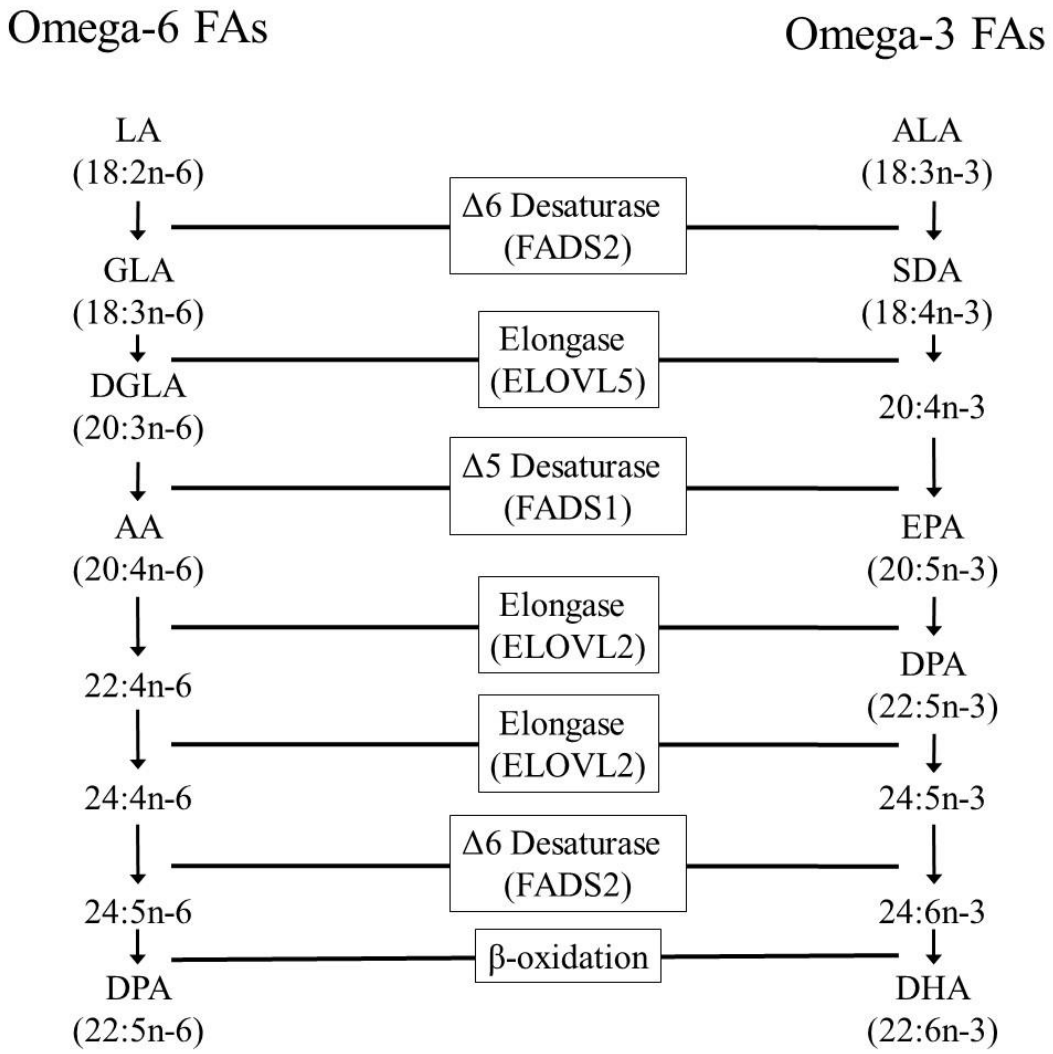

Figure 1.2. FADS Pathway: Conversation of Omega-3 and Omega-6 FAs.

AA; arachidonic acid, ALA; alpha-linolenic acid, DGLA; di-homo gamma linolenic acid, DPA; docosapentaenoic acid, ELOVL2 and ELOVL5; elongase 2 and elongase 5, EPA; eicosapentaenoic acid, DHA; docosahexaenoic acid, FADS1 and FADS2; fatty acid desaturase 1 and fatty acid desaturase 2, GLA; gamma-linolenic acid, LA; linoleic acid, and SDA; stearidonic acid. Adapted from: Robertson *et al*. (269).

1.4.3 FADS Pathway Conversion Efficiency

D6D is the rate-limiting step in PUFA conversion (258, 291), and is well recognized as the main determinant of PUFA levels in the body (66, 307). The rate of ALA being converted to EPA is estimated to be between 0.2-8%, and from ALA to DHA is even less, with estimates of ~1% (0.05-4%), with higher estimates calculated in women (37, 42, 44, 83, 251, 258). The conversion rates have been studied by several groups, with original reports estimating the conversion rate using orally ingested ALA stable isotopes (44, 92, 315). More recent work has re-investigated these conversion rates using more advanced methods (steady-state infusion of ALA stable isotopes) and reported that these conversion rates may be three times higher than previously estimated (92). To determine the conversion of ALA, a stable isotope ([*U*-^{13}C] ALA) was administered and measured over a period of 21 days. In men, 84% of ALA remained as ALA, 7.9% of ALA became EPA, 8.1% became DPA and no detectable amounts of labelled carbon from ALA were found to be converted into DHA (46); and in women, 63.7% remained as ALA, 21.1% became EPA, 5.9% became DPA and 9.2% of labelled carbon from ALA was found in DHA (47).

As omega-3 and omega-6 FAs are converted with the same series of enzymes (**Figure 1.2**), the quantity of these FAs consumed from the diet can influence FADS pathway conversion efficiency. In North America, omega-6 FAs are consumed in greater quantities compared to omega-3 FAs (286). In a meta-analysis (n=11,668, 32-83 yrs., multi-ethnic), an overall estimate of global LA intake was reported to be almost 10-fold higher than ALA intake (290). The proportion of LA to ALA intake is estimated to be similar in other reports, where the ratio of LA:ALA was calculated to be ~16:1 (286). Researchers in the field of FAs recommend that individuals should strive towards a consumption ratio of 4:1 LA:ALA by significantly increasing

the intake of omega-3 FAs and significantly reducing the consumption of omega-6 FAs (171, 282, 283). Due to the unbalanced ratio of dietary PUFA consumed, more omega-6 FA are produced through the FADS pathway. However, research has shown that desaturase enzymes have a preference for omega-3 FAs versus omega-6 FAs (81, 258, 299). During EPA and DHA supplementation, D5D and D6D activity tends to be reduced (65, 66). Due to the limited conversion of ALA into EPA and DHA, as well as the proportionally greater intake of omega-6 FAs from the diet, the importance of an adequate dietary intake of EPA and DHA is highlighted.

1.4.4 Estimated Enzyme Activity

ALA-tracer studies are important in fundamental FA metabolism research. However, tracer studies using stable isotopes are very expensive and are not feasible to conduct on a large scale. Additionally, the majority of the FADS pathway activity takes place in the liver, and collecting liver biopsies for activity estimates is not feasible in human studies (27). Therefore, alternative options for studying conversion rates and FADS pathway activity are necessary. Estimated enzyme activity using product:precursor ratios have been developed and serve as a surrogate measure of enzyme activity (31, 207, 337). Estimated FADS pathway activity ratios are usually calculated using omega-6 FAs. For example, the ratio of AA/LA could be used to generate an aggregate estimate of both D5D and D6D activity. In an omega-3 supplementation study, it is best to use omega-6 FAs for these calculations to avoid confounding the estimation of FADS activity (206). Additionally, omega-6 FAs are more abundant and less variable in comparison to omega-3 FAs (207, 288). Estimated activity has been compared between serum and skeletal muscle, and estimated activity levels were also moderately correlated with estimated activity levels in the adipose tissue (288). Davidson *et al.* (2016) found a high correlation of estimated FADS pathway activity between RBC and across all tissues (in rodents), suggesting that using

FAs in RBC to estimate FADS pathway activity ratios is an accurate and non-invasive proxy for tissue PUFA metabolism (82).

As previously stated, ALA conversion into EPA and DHA is low. However this limited conversion may be sufficient to maintain normal physiological functions by ensuring adequate amounts of EPA and DHA in the necessary organs (brain) and tissue pools (PLs) to ensure basic health and functioning (92). In relation to dietary consumption, when mice were fed a diet deficient in essential FAs, D6D increased and subsequently decreased when PUFAs were added to the diet (66). The conversion of DHA in the liver increases during omega-3 deprivation, suggesting that liver synthesis may be (in part) responsible for maintaining whole-body DHA levels (92).

1.4.5 Additional Factors That Influence FADS Pathway Activity Efficiency

It is noteworthy that several additional factors can influence the efficiency of the FADS pathway activity. The following sections will provide further details regarding the influence of sex (163), age (319) and genetic variation in *FADS1* and *FADS2* (186). To date, human research has primarily focused on the association between FA levels between sex and age, while less research has investigated FADS pathway activity.

1.4.5.1 Sex Differences Between Males and Females

Differences in FADS pathway activity have been explored between males and females in both rodent and human studies. Although studies using rodent models more consistently report a sex effect, several human studies also report that females have a higher rate of FADS pathway activity compared to males (42, 98). At a whole body level, estrogen plays a key role in FA metabolism and storage in females (163, 277). Thus, estrogen likely plays an indirect role in

increasing FADS pathway activity and the subsequent increased synthesis of DHA (163). Estrogen can increase the activity of PPARα by increasing the activity of the ERK-MAPK (extracellular signal-regulated kinases – mitogen activated protein kinase) signaling pathway. Estrogen can also increase the cellular concentrations of PUFAs and oxylipins, which are PPARα ligands, which in turn can stimulate FADS pathway activity (163). In a rat model, significant differences in FA levels existed between males and females; in particular regarding omega-3 FAs. For example, DHA content in PLs was higher in plasma and liver of females compared to males (98). Additionally, both estimated FADS pathway activity was higher in female than in male rats (98). The mRNA content of *FADS1* and *FADS2* genes were significantly greater in the livers of female rats (98).

FADS pathway activity directly relates to PUFA levels, and thus it is also relevant to discuss sex differences in relation to FA levels. Previous reports suggest that females tend to have higher levels of DHA in RBC compared to males (114, 221). In populations consuming low levels of EPA and DHA, women also tend to have higher levels of EPA and DHA compared to males (163). After supplementing EPA and DHA for 12 months (3,270 mg/day EPA and DHA, n=73 female, 76 male, 20-79 yrs.), EPA levels were significantly higher in females than males (319). On the other hand, other studies have suggested that both women (pre-menopausal) and men tend to have very similar levels of EPA and DHA in their RBC (143, 319). Apart from the role of estrogen, it is hypothesized that the main reason females have higher levels of EPA and DHA in their body compared to males is largely for the benefit of a developing fetus (45, 86, 123, 163). Indeed, DHA is critical during pregnancy and fetal development (146, 285). There have also been significant positive correlations between PUFAs in breast milk and RBC PUFA levels in the blood (38, 224, 273, 331). Mothers consuming low amounts of EPA and DHA during

pregnancy and lactation have infants with lower levels of EPA and DHA in their RBCs, compared to mothers who have a higher intake of EPA and DHA during fetal and infant development (285, 302). In order to avoid the confounding effects of estrogen and other hormones, the menstrual cycle (230), and contraceptive use (in humans), the majority of omega-3 research has been completed using male rodents and humans (327, 338).

1.4.5.2 Age

The relationship between age and FADS pathway activity remains under investigation (37, 41, 270). The majority of omega-3 FA research has been conducted in older adults and populations with CVD, hypertriglyceridemia, or neurological disorders (12, 97, 112, 147, 153). The FADS pathway activity may be subjected to age-related effects and thus studies across different age groups may not provide consistent results, making comparisons difficult (163). In older adults (~55 yrs., men and women), D6D activity was positively associated with TAG levels, blood pressure, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), while D5D activity was negatively associated with TAG levels and blood pressure (160). Additionally, increased age has been associated with increased levels of DHA in the blood (163). Zulyniak *et al.* demonstrated that older men had higher DHA levels compared to younger men (340). Walker *et al.* compared the FA composition between young (20-39 yrs.) and older (60-79 yrs.) adults and reported that older adults had higher EPA and DHA in adipose tissue compared to younger adults (young (0.29±0.07% versus older 0.44±0.012) (319). Otherwise, the FA compositions between the various blood fractions (plasma TAG, plasma cholesterol esters, RBC) were comparable between ages (319). One study comparing estimated FADS pathway activity in older and younger men suggested that older men are less capable of converting ALA into longer-chain PUFA than younger men (45). It is unclear what the estimated conversion rates are in

younger and older women, although younger women of child-bearing age may be able to more effectively convert ALA into DHA than older women (42, 86). A lower FADS pathway activity in older adults is in contrast to the FA work above, where it was reported that older individuals had higher levels of EPA and DHA. It has been hypothesized in the literature that EPA and DHA status in older individuals is primarily dependent on dietary intake of EPA and DHA, rather than FADS pathway activity (45).

1.4.5.3 Genetics

Genome wide association studies (GWAS) have highlighted *FADS1* and *FADS2* as critical genes contributing to the variability in blood PUFA levels (144, 304). In 2009, Tanaka *et al*. reported that *FADS1* and *FADS2* contributed ~18% to the variation in plasma lipid levels between individuals (n=1453, >65 yrs., living in Italy) (304). In another report (n=727, 20-64 yrs., Caucasians from Germany), variability in blood FA levels ranged from ~10% overall in the omega-6 FAs, and up to ~28% in AA levels, in accordance to SNPs in *FADS1* and *FADS2* (278). Recently, a large study (n=~188,000, 20-80 yrs., European ancestry) found SNPs in *FADS1* and *FADS2* to be associated with several lipid traits, including TAGs, cholesterol etc., as well as individual blood PUFA levels (71). Hu *et al.* confirmed the association between SNPs in *FADS1* and *FADS2* with blood PUFA levels in a Chinese population (n=2865, 50-70 yrs.) (144).

Several papers from multiple research groups confirmed the relationship between *FADS1* and *FADS2* SNPs and blood FA levels in various populations (31, 186, 203, 207, 218). Specifically, minor allele carriers for SNPs in *FADS1* and *FADS2* are consistently reported to have lower levels of both EPA and AA, and lower estimated FADS pathway activity, compared to major allele carriers (5, 73, 113, 116, 208, 218, 271, 290, 304). Measurements from breast milk (119, 224, 331) and adipose tissue (18, 288) show similar results, where minor allele

carriers have lower EPA and AA compared to major allele carriers. This work summarizes a strong association between SNPs in *FADS1* and *FADS2* and altered FADS pathway activity and FA levels in the body. Due to this consistency, this thesis hypothesized that *FADS1* and *FADS2* genes would be ideal targets for research in the fields of genomics, nutrition, and health.

1.5 Nutrigenetics and Health

1.5.1 What is Nutrigenetics / Nutrigenomics?

Nutrigenetics / nutrigenomics are emerging fields that combine information about nutrition and genetics. The terms nutrigenetics and nutrigenomics are often used interchangeably, as they investigate the same research paradigm but from different angles (181). Specifically, nutrigenomics investigates dietary components such as nutrients and vitamins and how these components subsequently influence metabolism and gene expression (231). Nutrigenetics investigates how genetic make-up influences the response to a diet and/or a specific nutrients and vitamins (231). The addition of genetic information to nutritional studies can be powerful, as it can provide another explanation for variability in datasets. In the large nutritional studies conducted to establish responses to various nutrients, there was, perhaps not surprisingly, a distribution in the responses with a set of "responders" and "non-responders". Thinking back to these studies, it is postulated that, if genetic information could have been available this may have provided reasons why "responders" and "non-responders" were identified (32, 242, 274). Therefore, future nutritional intervention studies should ensure that genetic information is collected in order to get a better understanding of variability in responses to the diet or nutrient. Due to the power of this information, nutrigenetics is expected to revolutionize dietary recommendations, making them personalized, with extensive potential for improvements in health by promoting behaviour change (100, 231). In 2010, McBride *et al*.

reported a gap in the field of personalized nutrition and nutrigenetics, largely that few studies examined how genetic information affects lifestyle behaviors had been conducted to date (210). Additionally, McBride *et al.* summarized that studies up until 2010 had small and self-selected samples, self-reported outcomes, inappropriate lengths of intervention or lack of follow up, and a narrow focus on risk perception and fatalism (210).

1.5.2 Challenges in Nutrigenetics

Within the field of nutrigenetics, the majority of the work to date has focused on gathering input from various stakeholders, including the general public, health care providers (HCPs), and registered dietitians (RDs). Before nutrigenetics information can be implemented and/or utilized in practice, gaining an understanding of the perceptions, acceptance, and knowledge of various stakeholders is critical.

For nutrigenetics/nutrigenomics to gain traction, it is important to establish familiarity of this research with HCPs and the general public. The majority of this work has been completed within the last six yrs., investigating the perceptions of nutrigenetics among RDs, as these HCPs focus on dietary information in their practice. In 2010, Weir *et al*. asked physicians and RDs what they knew about nutrigenomics. The results showed that RDs had heard about nutrigenomics, but most physicians were unfamiliar with the term (323). In 2014, Cormier *et al*. reported specifically on RDs in Quebec (n=373,~38yrs., 95%women) and found that 77% of RDs knew about nutrigenomics, and the majority (59-77%) said they would like to learn more (75). Additionally, increased age of RDs was associated with less knowledge about nutrigenomics (75). In another study by Bouchard-Mercier *et al.*, only 13/141 (9.2%) of RDs actually talked to their patients about nutrigenomics (34). HCPs reported that, as of 2010, they lacked the

professional competency to properly discuss this information and justify the use of nutrigenomics to their patients (323). Apart from comfort and knowledge, HCPs were concerned that direct-to-consumer genetic testing may create a false reason for patients to self-medicate and this direct route of health care information may de-legitimize their professional role (323).

The suggestions to discuss nutrigenetics information with a HCP, or genetic counsellor, are concerning because HCPs report that they are insufficiently knowledgeable, prepared, or comfortable to discuss this type of information with a patient or client (75, 145, 323). Hunter *et al*. proposed that HCPs should encourage their patients to enroll in formal scientific studies to discuss details with a researcher in order to learn more about their genetics in relation to their health (145). HCPs also reported that more regulations for the provision of genetic information to patients and the general public should be in place (323). Moreover, it would be useful that additional information to be created to enhance understanding of the information regarding genetics, health, and nutrigenetics information for use in the general public and for HCPs (323).

In addition to creating resources to enhance the understanding of genetics and health, the financial aspect of genetic testing should be considered. To date, some genetic testing kits can be purchased online, and some can be ordered through a physician or RD. From a consumer perspective, cost is likely a barrier to many individuals despite potential interest in the topic (75, 100, 101). However, in the case of an independent consumer purchase, cost would be incurred by the individual and would not affect the health care system. On the other hand, now that genetic information is more widely accessible, it is possible that individuals will choose to discuss their genetic information with their HCP, thus adding a burden to the health care system (34, 75, 141, 142). Additionally, any genetic test ordered through a physician would be a cost to the health care system. Interestingly, there are reports which suggest that genetic testing may be cost-saving

and the advanced technologies have allowed costs to be significantly reduced over recent yrs. (155).

Apart from awareness of this field, the current perception on the use of this information is also valuable to consider. Individuals who receive information about their genes conferring risk to a certain disease may feel anxious and seek out health interventions without consulting a HCP (211). On the other hand, access to genetic information may motivate individuals to adopt healthier lifestyle and behavioral changes to reduce disease development (30, 210). Patient groups, including those with Metabolic Syndrome (MetS) and T2D, reported to be willing to undergo genetic testing and 27% would be willing to adhere to a personalized diet (255). Further, the results by Nielsen and El-Sohemy suggested that young adults (18-30 yrs.) are also interested in their genetic information and receiving this information was associated with higher ratings in understanding the dietary advice given to them (237). The human genome was sequenced and published in 2001 (217, 313), and thus it is interesting to speculate that young adults within the past two decades may have an increased awareness and acceptance of genetic information due to the accessibility and discussion of this information. Apart from individual reactions, it is important to consider how genetic information may affect families. Some researchers have proposed that genetic risk information may be more powerful when shared among families, in order to create a shared solution and strategy (210). Overall, there are reports that individuals may experience anxiety upon receiving their genetic results; however, there have been several studies that report many people are interested in their personal genetics information, especially how this information might relate to their diet and health.

1.5.3 Nutrigenetics Studies with Omega-3 FAs and *FADS* Genes

As discussed above, SNPs in *FADS1* and *FADS2* contribute to variation in blood FA levels and estimated FADS pathway activity. All of these previously discussed studies examined SNPs in *FADS1* and *FADS2* in cross-sectional comparisons. This research was essential to understand the variation in FA levels as a result of genetic variation; however, until recently it was unclear what the effect of these SNPs would be in response to a dietary supplementation of omega-3 PUFA. Three studies have examined the effects of dietary supplementation of FAs (ALA, EPA and DHA) and the influence of SNPs in *FADS1* and *FADS2* on omega-3 FA levels (5, 73, 116). These studies did not assess behavioural changes, but rather the specific diet-gene interactions at a molecular level.

Firstly, Cormier *et al*. reported that SNPs in both *FADS* and *ELOVL* genes play a role in estimated enzyme activity. After a fish oil supplementation (5,000 mg/day EPA/DHA, n=208, 30.8 ± 8.7 yrs.) D5D activity was increased and D6D activity was decreased (73). This data indicates that the minor allele carriers for most SNPs in *FADS1* and *FADS2* tended to have higher D5D activity and lower D6D activity compared to the major allele carriers (73). Secondly, Gillingham *et al.* supplemented ALA in the form of flaxseed oil in a randomized crossover design (20,600 mg/day, 2,400 mg/day or 1300 mg/day of ALA, n=36, 47.5 ± 11.9 yrs.) for four weeks. Minor allele homozygotes (from the rs174537 SNP in *FADS1*) were shown to have lower plasma levels of EPA (116). Thirdly, Al-Hilal *et al.* investigated response to different doses of fish oil supplements, specifically 450 mg/day, 900 mg/day and 1,800 mg/day of EPA and DHA in adults (n=310, 55.37 ± 6.96 yrs.), to evaluate the influence of *FADS* SNPs (5). Specifically, minor allele carriers for SNPs in *FADS1* and *FADS2* had lower plasma and RBC levels of EPA, and had lower estimated D5D and D6D activity compared to major allele carriers (5). These

three studies were the first examples of omega-3 nutrigenetics studies, where the response to a dietary intervention was evaluated in relation to an individual's genetic make-up. This work highlighted the applicability of nutrigenetics with a focus on omega-3 FAs and *FADS* genes.

1.5.4 First Studies Examining the Delivery of Genetic Information

To date, few studies have examined the delivery of personal genetic information related to lifestyle and nutrition. Of note, there have not been any studies that have investigated genetic information about *FADS1* and *FADS2* in relation to omega-3 FA intake or blood levels. Thus the examples discussed here are unrelated to omega-3 fats, but serve to demonstrate the potential of personal genetic information to motivate behavioural changes. In general, these studies follow a similar design, where there is a Genetic (intervention) group and a Non-Genetic (control) group. Individuals in the Genetic group received their personal genetic information related to the study investigation, whereas the Non-Genetic group received the basic study treatment. In nutrigenetics interventions, all participants would receive information about the specific health or nutrition concept of interest, while only some of the participants would receive their genetic information. In the first example, young adults (n=125, 27 ± 3 yrs., 77% women) were provided their personal genetic information related to four dietary components (vitamin C, caffeine, sodium and added sugars) (238). Participants in the control group received general nutritional recommendations for these four nutrients (238). It was unclear to the reader if any information was provided about the value or detriment of these nutrients to health, although all information appeared to be risk-related rather than benefit-related (238). Those in the Genetic group did not make any significant changes (measured with a FFQ) to vitamin C, caffeine or added sugars, although at the 12-month follow up, the Genetic group (with the risk version of the angiotensin-

converting enzyme gene for sodium intake) did make significant reductions in sodium intake compared to the Non-Genetic group (238).

In relation to dietary fat, recent work by Hietaranta-Luoma *et al.* reported on the provision of genetic information related to Apolipoprotein E (*APOE*) variation, and the subsequent changes to dietary fat intake and cardiometabolic markers of health (136). Genetic information regarding variations in *APOE* (ε2, ε3 and ε4) was given to participants (n= 107, ~47 yrs. (20-67 yrs.) 69.2% women). Variations in *APOE* are related to the risk of developing CVD (136, 179). Three months into the study, the Genetic group with the highest risk allele (ε4 group) had reduced intake of SFAs and increased intake of PUFAs. However, by the end of the one yr. intervention, dietary fat quality was not significantly different between the groups (136). Results from this study suggest that personal genetic information is a motivator, but may not be powerful enough for permanent and persistent lifestyle changes (136). This time-line hypothesis is opposite to what Arkadianos *et al.* found when studying how personal genetic information could influence weight-loss in adults (7). The nutrigenetic intervention (19 genes, related to 7 dietary components) was related to the low-glycemic Mediterranean diet (focusing on folic acid and vitamin B6 and B12, cruciferous vegetables, antioxidants, caffeine, low-fat dairy and oily fish) (7). Patients (n=93, 46 ± 12 yrs., 43% women) with a history of unsuccessful attempts at weight loss were enrolled in a program to follow this diet, complete regular exercise routines and attend regular follow-up visits at the clinic (7). Information about differences in genetic make-up and how the nutrigenetic information was made specific to each allele was not clearly described by the authors in the manuscript. After 100 days, no differences in weight loss were measured between the Genetic group and the Non-Genetic group (7). Interestingly, after 300 days, 57% of the Genetic group had maintained their weight lost (measured at 100 days) and showed

improvements in glucose levels compared to 25% of the Non-Genetic group who had maintained their weight lost (7). Thus, more work needs to be done to determine the appropriate amount of time for follow-up time-points in nutrigenetic interventions.

Overall, many studies have reported on the perceptions, knowledge, and awareness of concepts related to nutrigenetics. In relation to omega-3 FAs, there are three nutrigenetic studies to date that have investigated response to a FA supplementation in a population stratified by SNPs in *FADS1* and *FADS2*. Only a handful of studies to date have provided genetic information to participants, and intervention studies in this field are lacking. There have not been any intervention studies providing genetic information about SNPs in *FADS1* and *FADS2* to participants in order to measure behaviour change.

1.6 Summary

Omega-3 FAs represent an ideal nutrient to examine in the context of a nutrigenetics intervention for several reasons. From a global health perspective, it is widely recognized that EPA, and DHA are beneficial for cardiovascular, metabolic, developmental and cognitive health (22, 83, 161). However, the consumption of EPA- and DHA-rich foods such as fatty fish is low, in particular in the Western diet (111, 184). Therefore, finding new ways to motivate people to increase their consumption of omega-3 FAs are necessary. From a genetic perspective, EPA and DHA are endogenously produced to a limited extent through a well-characterized pathway that desaturates and elongates the essential omega-3 FA, ALA. *FADS1* and *FADS2* play critical roles in this pathway (219, 232). Specifically, minor allele carriers have reduced desaturase activity, resulting in lower levels of EPA (290, 304). This suggests that giving individuals their personal *FADS* genotype may yield a new approach to encourage increased omega-3 FA intake.

CHAPTER 2: RATIONALE AND AIMS OF THE THESIS

2.1 Rationale for Studying Emerging Adults

Emerging adults are not well studied in nutrition and health research, as the majority of research to date has focused on older adults and the treatment, rather than the prevention, of lifestyle-related diseases (67, 161, 201). The term "emerging adults" is used to define a period of psychological and physiological development between adolescence and young adulthood, specifically 18-25 yrs. of age (8, 25). Most emerging adults are in a transition period out of their parental households, resulting in significant changes to dietary and lifestyle habits (25). Furthermore, lifestyle habits developed in emerging adulthood have been shown to continue throughout middle and older adulthood (19). For these reasons, emerging adults are an important demographic to study because of their psychological and physiological stage of development. Targeting lifestyle habits in emerging adults could provide a means to improve well-being and long-term health (1, 332).

2.2 Rationale for Studying Preventative and Personalized Nutrition and Health

The prevalence of lifestyle-related diseases (T2D, obesity, CVD) continue to rise in North America, highlighting an imperative need for preventative health care approaches (325, 334). Providing nutrigenetics information is one viable strategy which could be used for the prevention, screening, and identification of risk factors related to chronic diseases (121, 210). The knowledge of one's personal genetic information related to nutrition and health may allow for specific dietary and/or lifestyle modifications to be made (121, 210). Despite the availability of personal genetic information, many individuals continue to rely more on family history data to determine their susceptibility for disease development. Research by Forsyth and Goetsch

investigated emerging adults with a family history of T2D or hypertension to determine risk perception related to possible health threats (110). Individuals who knew their family history were no more likely to schedule physician visits and take protective health care measures than those without lifestyle-related diseases in their family (110). In consideration of those results, genetic information may be a factor/strategy to encourage emerging adults to develop healthy behaviours (19). As future health concerns do not seem to resonate with emerging adults (8), perhaps being more connected to their health through personal genetic information could play a role in behaviour change. Interestingly, young adults now and in the future would have grown up during a time after the human genome was mapped (217, 313). Due to this historical timeline, it is possible that emerging adults are possibly more interested and less fearful of genetic information (79, 99, 318); however limited work has been completed to date. From a health care standpoint, HCPs support the idea that genetic testing may allow for more precise measurements of disease susceptibility instead of family history data (323). Thus, the field of nutrigenetics is well-positioned to play a critical role in personalizing dietary and lifestyle advice to motivate individuals to adopt health protective behaviours.

2.3 Overall Thesis Rationale and Objective

Over the last decade, substantial advancements have been made in the field of genomics and, more recently, nutrigenetics. However, the majority of nutrigenetics research to date has been prospective, with a limited number of empirical studies testing the applicability of nutrigenetics. In nutrigenetic intervention studies, it is important to focus on a nutrient that can be measured within the human body, and a nutrient whose levels vary within the population due to genetic variation. Therefore, omega-3 FAs are key nutrients for nutrigenetic investigations due

to the health benefits associated with increased EPA and DHA intake, as well as their consistent and reproducible changes in the body related to genetic variation.

The overall objective of this thesis was to investigate the perceived and actual health benefits associated with omega-3 FA intake in a population of emerging adults, and then evaluate whether this could be influenced with personal *FADS1* genetic information. The overall hypothesis of this thesis is that emerging adults would have a low level of perceived awareness associated with omega-3 FAs, although they would experience health benefits upon consumption, and both awareness and health would be positively influenced by providing emerging adults their personal *FADS1* genetic information. To address the objective and hypothesis of this thesis, three independent studies were conducted.

2.4 Specific Aims of the Thesis: Objectives and Hypotheses

Study 1

The specific objectives of Study 1 were to:

1. Examine the current level of awareness in emerging adults regarding terminology used to describe omega-3 FAs and awareness of the possible health effects associated with increased intake of EPA and DHA;
2. Determine whether this awareness of terminology and health effects was reflected in self-reported consumption of omega-3-rich foods and supplements.

Given the objectives of Study 1, it was hypothesized that:

1. Emerging adults would have a low level of recognition of individual omega-3 FAs, especially the full scientific names, and few would be familiar with specific health benefits;
2. Emerging adults would report a low level of consumption of omega-3 foods and supplements.

Study 2

The specific objectives of Study 2 were to:

1. Measure changes in cardiometabolic markers from the blood after a moderate dose of fish oil supplements (1,800 mg EPA and DHA / day) was provided;
2. Examine if the variation in the *FADS1* and *FADS2* gene cluster affected the response to fish oil supplementation.

Given the objectives of Study 2, it was hypothesized that:

1. The consumption of a moderate dose of fish oil would lower lipid levels (specifically TAG) in the blood by the end of the supplementation period;
2. Individuals who carry the minor alleles for SNPs in the *FADS1* and *FADS2* gene cluster would have lower levels of EPA and DHA in their blood compared to those who carry the major alleles.

Study 3

The specific objectives of Study 3 were to:

1. Examine if emerging adults would change their dietary consumption of omega-3 fats after receiving their personal genetic information for a SNP in *FADS1*;
2. Determine if emerging adults in the genetic (intervention) group showed genotype-specific differences in omega-3 intake and perception of genetic information, based on whether they were a major or minor allele carrier.

Given the objectives of Study 3, it was hypothesized that:

1. Individuals who receive their genetic information would consume more omega-3 fats in their diet compared to the non-genetic (control) group;
2. Individuals who are minor allele carriers would consume more EPA and DHA compared to major allele carriers.

The methodologies and results associated with each of these studies are presented in the forthcoming chapters.

CHAPTER 3:

AWARENESS OF OMEGA-3 FATTY ACIDS AND THEIR POSSIBLE HEALTH EFFECTS AMONG EDUCATED EMERGING ADULTS

Roke, K., Rattner, J., Brauer, P., and Mutch D.M. 2016. Awareness of Omega-3 Fatty Acids and Their Possible Health Effects among Educated Emerging Adults. **Submitted for Peer-Review July 2016.**

3.1 Abstract

Objective: Determine the awareness surrounding omega-3 fatty acids (FAs) and their possible health effects, and self-reported consumption of omega-3 foods and supplements, among educated emerging adults.

Design and Participants: Focus groups and cognitive interviews were conducted to develop and refine survey questions and response options. An online survey (using Qualtrics™) was created and participants between 18-25 yrs. were recruited.

Main Outcome Measures and Analysis: Degrees of awareness and self-reported consumption were assessed using Pearson's χ^2 tests, Z-tests, and binary logistic regression.

Results: Of the 834 survey completers, 73% reported a strong interest in health. More respondents recognized the omega-3 FA abbreviations EPA (~51%) and DHA (~66%), relative to ALA (~40%; $p<0.01$). Respondents with a biological science background were more aware of omega-3 FA terminology compared to other disciplines. Most respondents (~83%) recognized that EPA and DHA are linked to heart and brain health. Respondents who used Academic/Reputable sources, Health Care Professionals and/or Social Media to obtain nutritional knowledge were more aware of these health effects. Finally, 48% and 21% of respondents reported consuming omega-3 foods and supplements, respectively.

Conclusions and Implications: This assessment of awareness, in conjunction with self-reported consumption of omega-3 FAs, provides valuable insights for future knowledge translation efforts.

3.2 Introduction

Possible wide-ranging health effects associated with increased omega-3 FA consumption include, but are not limited to, reduced risk for CVD and metabolic diseases (obesity and T2D (197)), as well as improved cognitive development (146). Most of the beneficial health effects associated with omega-3 FAs are related to EPA and DHA, and less so with ALA (215). While a substantial body of research has developed over the past 40 yrs. and supplements are widely used, the extent of consumer knowledge regarding omega-3 FAs is uncertain. For example, only 30% of Belgian adults (n=429, ~41 yrs., 66% women) were aware that omega-3 FAs have a positive effect on human health (314), and 51% of American adults (n=1,798, ~49 yrs., 60% women) recognized that omega-3 FAs lower the risk of heart disease (194). Moreover, awareness of the differences between individual omega-3 FAs is likely to be even lower (292). There have been few studies conducted in other groups.

Due to changes in dietary patterns over time, many countries are consuming less fish (the best natural food source of EPA and DHA) than ever before (223, 314). Indeed, recent data from the National Health and Nutrition Examination Survey (NHANES) showed that only 11.6% of American adults were consuming the recommended amounts of fish and shellfish (265). Various studies examining omega-3 supplement consumption reported that 9% of American adults (11), 39% of Australian university students (16), 24% of Danish adults (166), and 20% of adults in Europe (280) were taking fish oil supplements. However, even when considering omega-3 supplement intake, recent global surveys suggest most individuals in Westernized societies are consuming insufficient amounts of EPA and DHA in their diets (111, 294). It is therefore important to understand consumers' current awareness for future knowledge translation efforts.

Targeting educated emerging adults to assess awareness is a valuable first step for several reasons. First, emerging adults are often health conscious and can influence overall nutrition trends (246). Second, understanding their level of awareness will aid in the development of preventative health messages that could improve long-term health initiatives (161). However, emerging adults acquire knowledge from multiple sources. In 2001, parents were reported to be the primary source of information regarding omega-3 FAs for adolescents (126). Recent advances in technology (e.g., smartphones and social media) mean that emerging adults can be expected to obtain information about omega-3 FAs from a wider variety of sources, old and new (303).

We report on the development and results of a new online survey which we used to analyze the awareness of educated emerging adults regarding omega-3 FA terminology, the health benefits associated with increased EPA and DHA intake, and the sources of information used to learn about omega-3 FAs and health. We also investigated self-reported consumption of omega-3 foods and supplements to determine if reported intake reflected reported awareness.

3.3 Methods

3.3.1 Study Design

The questionnaire for the cross-sectional survey of emerging adults was developed de novo, based on concepts of knowledge translation, by researchers in the area of omega-3 nutrition to assess awareness, self-reported consumption, and information sources. Ethical approval was granted by the University of Guelph Human Research Ethics Board (REB#:14SE027) and all participants provided consent.

3.3.2 Sampling Frame and Recruitment

Emerging adults (18-25 yrs.) participated in both the survey development (including focus groups and cognitive interviews) as well as the online survey reported here. Participants were recruited through i) a poster campaign around the city of Guelph, ii) the University campus through classroom announcements and iii) advertising the study on the recruitment page on the University website. In addition, the study poster was uploaded onto social media (e.g., Facebook, Twitter) to increase access for other emerging adults in South-Western Ontario.

3.3.3 Survey Development

Potential knowledge domains were first identified based on researcher expertise of the content area. Possible survey items were created to address content by adapting some previously developed questions along with new researcher-generated questions. We used the Canadian Community Health Surveys (59, 60), and a general nutrition knowledge questionnaire developed by Parmenter and Wardle (247), as models to structure our survey questions. The work of Dillman was consulted for question format (91). Draft questions were first reviewed for face validity and clarity with focus groups. After revision, draft questions were reviewed in detail using cognitive interviewing with 10 additional participants. The online version of the survey was then pilot-tested to ensure ease of completion. Qualtrics survey software (V13.28.05, ©2015, UT, USA) was used to host the online survey.

3.3.3.1 Focus Groups. We conducted five focus groups (n= 4-6 per group, n=25 total) from the target population to confirm and extend concepts regarding awareness about omega-3 FAs and health (122, 175). The same probing questions and same facilitator were used in each session to ensure consistency between groups (122). Sessions were 60 minutes and were audio recorded with permission. Recordings were reviewed and transcribed verbatim by a research assistant.

After each focus group, issues were documented and questions were revised prior to the next focus group. Upon completion of all five focus groups, answers were compiled and informed the overall questions and response options of the questionnaire.

3.3.3.2 Focus Group Analysis. Participants found it difficult to name any health effects associated with increased EPA and DHA intake without guidance. When prompted with larger topics on health (heart, metabolism, and brain health), participants seemed more familiar with these associations. Therefore, the first health question in the survey inquired about a relationship between EPA and DHA intake and general health. Subsequently, we asked about three well-researched health effects linked with omega-3 FAs (i.e., heart, metabolic, and brain health). It was decided that providing some detail in the online survey could reduce discomfort associated with not being able to sufficiently or confidently answer health questions.

3.3.3.3 Cognitive Interviews. Ten separate cognitive interviews were conducted by the same facilitator (20). This number of interviews was previously reported to be sufficient to identify major wording problems (29). Sessions lasted 60 minutes and were audio recorded with permission. Participants were asked to bring in their own personal electronic device (laptop, tablet, or smartphone) for completion of the online survey to mimic the testing environment (329). A think-aloud protocol was used (175, 329), with prompting as needed. Participants were first trained on the think-aloud protocol, as recommended by Blair and Brick (28). Issues of format and functionality (drop-down menus, skip-logic, etc.), as well as issues related to understanding and interpretations of questions, were addressed after each cognitive interview. Questions were further simplified and re-worded as necessary and the final survey was pre-tested by another 20 individuals.

3.3.3.4 Online Survey. The final online survey was live from January 5 – April 4, 2015. Participants could complete the survey on their own time on an electronic device of their choosing. There were questions of various types (multiple choice, select all that apply, sliding bar scales and open text boxes) and selected questions pertaining to omega-3 FAs (n=9) and demographics (n=7) were included in this analysis. The entire questionnaire took between 5-10 minutes to complete. Survey questions investigating omega-3 FA awareness and self-reported consumption are provided in **Appendix 1**. There were questions related to four primary areas of interest: i) awareness of terminology and types of omega-3 FAs (2 questions); ii) sources of information used to learn about omega-3 FAs (1 question); iii) awareness of omega-3 FAs and their possible health effects (4 questions); and iv) self-reported consumption of omega-3 foods and supplements (2 questions). The full survey developed with additional questions asking about attitudes and behaviors can be found in **Appendix 2.** Based on the cognitive interview results, we included several text reminders to discourage guessing. For example, "If you are not sure what to select, please select "don't know" when available"; and "Please answer the question based on what you currently know and try to avoid guessing the answer".

3.3 Data Analysis

Data were descriptively analyzed using SPSS (IBM Corporation, Version 22/23, NY, USA) and associations were explored using Pearson's chi-squared tests (χ^2), Z-tests with Bonferroni post-hoc comparisons, and binary logistic regression. Variables for sub-group investigation included: *gender* (male, female), *field of study* (field of study/area of work grouped into Biological/Physical Sciences, Social Sciences and Other Fields) and *source of information* (Academic/Reputable, Family/Friends, Food Derived, Health Care Professionals (HCPs), Social Media, Text, and TV Derived).

3.4 Results

3.4.1 Participant Characteristics

A total of 961 individuals responded to the survey. Participants who hadn't heard of omega-3 FAs (n=12) followed a skip pattern and didn't complete the full survey and were not considered further. There were 834 individuals (87%) who completed the full survey. Participant characteristics are shown in **Table 3.1**. When data were analyzed according to different ethnic groups and different levels of education, there were no significant differences based on these subgroups. Therefore, the effect of ethnicity and level of education were not considered further.

Table 3.1. Characteristics of Respondents.

Characteristic	**n (%)**
Gender	
Female	655 (78.5%)
Male	179 (21.5%)
Self-Reported Ethnicity	
Asian	83 (10.0%)
Caucasian/White	596 (71.4%)
European	71 (8.5%)
Other Ethnic Groups*	84 (10.1%)
Highest Level of Education	
High school diploma/trade certificate	172 (20.6%)
Currently enrolled in University undergraduate program	483 (57.9%)
Bachelor's degree/graduate degree[∞]	179 (21.5%)
Field of Study / Area of Work[#]	
Biological/Physical Sciences	542 (65%)
Social Sciences	192 (22%)
Other Fields	100 (12%)
Interest in Overall Health[&]	
8-10/10	614 (73.6%)
5-7/10	207 (24.8%)
0-4/10	13 (1.6%)

Numbers represent frequency (n) and percentage (%) of respondents.
*Other ethnic groups. Aboriginal, Arabic, Black or African Canadian, Hispanic or Latino, Middle Eastern, Don't know, not listed, comprising less than 5% of the total study population.
[#] Individual categories which make up the *field of study* subgroups are found in **Appendix 1, Table 3.1**.
[&]Respondents used a sliding bar scale to indicate their interest in overall health (0=low and 10=high).

3.4.2 Survey

3.4.2.1 Awareness of Omega-3 FA Terminology. When given a list of omega-3 terminology, fewer respondents (40.5%) had heard of the ALA abbreviation compared to the full name alpha-linolenic acid (60.2%). The trend was the opposite for EPA (51.3%) and DHA (65.8%) compared to the full names eicosapentaenoic acid (35.9%) and docosahexaenoic acid (35.3%) (**Table 3.2**). *Gender* did not influence these responses. *Field of study* did have a significant influence, with respondents in Biological/Physical Sciences being more aware of both the abbreviations and full names for all three omega-3 FAs ($p<0.01$) compared to those in Social Sciences or Other Fields (**Table 3.2**).

Table 3.2. Awareness of Omega-3 FA Terminology.

Term	Full Sample (N=834)	Biological/Physical Sciences (n=542)	Social Sciences (n=192)	Other Fields (n=100)
ALA	338 (40.5%)	294 (54.2%)[a]	35 (18.2%)[b]	9 (9%)[b]
Alpha-linolenic acid	502 (60.2%)	396 (73.1%)[a]	78 (40.6%)[b]	28 (28%)[b]
EPA	428 (51.3%)	349 (64.4%)[a]	51 (26.6%)[b]	28 (28%)[b]
Eicosapentaenoic acid	299 (35.9%)	270 (49.8%)[a]	22 (11.5%)[b]	7 (7%)[b]
DHA	549 (65.8%)	407 (75.1%)[a]	94 (49%)[b]	48 (48%)[b]
Docosahexaenoic acid	294 (35.3%)	258 (47.6%)[a]	29 (15.1%)[b]	7 (7%)[b]

Numbers represent the frequency (n) and percentage (%) of respondents who answered "Yes" to having heard of the abbreviation or full name of individual omega-3 FAs. χ^2 analyses assessed differences between *field of study*. Percentage values within a row with different superscript letters were significantly different in pairwise comparisons by Z test ($P \leq 0.05$), using a Bonferroni adjustment for multiple comparisons. Groups sharing letters are not different from one another. Answer responses to Appendix 1 Q1.

When asked which of these terms they had heard of the most, the majority of respondents (~70%) selected the general term "omega-3", while 21% indicated the combined "EPA+DHA" term (**Appendix 1 Q2**). Significantly more respondents in Biological/Physical Sciences,

compared to Social Science and Other Fields, reported being more familiar with the combined "EPA+DHA" term ($p<0.01$).

3.4.2.2 Sources of Information. Participants most frequently reported using Academic/Reputable sources (79%), whereas HCPs were selected the least (26%). Significantly more females (81%) selected Academic/Reputable sources than males (71%) ($p<0.01$). Respondents in Biological/Physical Sciences (84%) reported using Academic/Reputable sources to learn about omega-3 FAs more than other disciplines ($p<0.01$). Additionally, individuals in Biological/Physical Sciences (30%) indicated that they used HCPs significantly more than those in Social Sciences (18%; $p<0.01$) and moderately more than Other Fields (19%; $p=0.07$). Those in Social Sciences and Other Fields used Family/Friends as a *source of information* ($p=0.02$) more than those in Biological/Physical Sciences; however, this was no longer significant after correction for multiple comparisons **(Appendix 3 Table 3)**.

3.4.2.3 Awareness of Possible Health Effects Linked with Increased EPA and DHA Intake. All respondents completed questions asking about general health and specific health effects. Half of the respondents (52%) agreed that increased consumption of EPA and DHA is linked to general health (**Appendix 1 Q4)**. However, when prompted, more individuals responded "Yes" to knowing about specific health effects (84% heart, 83% brain, 62% metabolic) (**Appendix 1 Q5-Q7**). *Gender* did not influence responses about general, heart, or metabolic health effects; however, we found that significantly more females knew about brain health effects compared to males (85% versus 77%, $p=0.04$) (**Table 3.3**). While more respondents in Biological/Physical Sciences reported to know about the link between EPA and DHA intake and general health compared to other disciplines, it was interesting to note that *field of study* did not influence awareness about specific health effects (**Table 3.3**). Respondents who indicated using

Academic/Reputable sources, HCPs, and/or Social Media were significantly more likely to be aware of a relationship between omega-3 FAs and general health (**Table 3.3**). However, only respondents who selected Academic/Reputable sources were significantly more aware of the links between EPA and DHA with heart, metabolic and brain health (**Table 3.3**).

Table 3.3. Sources of Information on Awareness and Health Effects Related to EPA and DHA.

	General Health			Heart Health			Metabolic Health			Brain Health		
	OR	**95% CI**	***p***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***
Sources of Information												
Academic/Reputable	**2.15**	**(1.49-3.09)**	**<0.01**	**1.95**	**(1.28-2.98)**	**<0.01**	**1.92**	**(1.36-2.72)**	**<0.01**	**1.67**	**(1.09-2.54)**	**0.02**
Family/Friends	0.91	(0.67-1.24)	*ns*	1.11	(0.75-1.66)	*ns*	0.86	(0.63-1.17)	*ns*	1.17	(0.79-1.73)	*ns*
Food Derived	0.94	(0.68-1.32)	*ns*	0.76	(0.49-1.19)	*ns*	1.00	(0.71-1.39)	*ns*	1.02	(0.67-1.56)	*ns*
Health Care Professionals	**2.17**	**(1.53-3.09)**	**<0.01**	1.24	(0.77-1.98)	*ns*	1.16	(0.82-1.64)	*ns*	1.53	(0.93-2.50)	*ns*
Social Media	**1.50**	**(1.10-2.07)**	**0.01**	0.87	(0.58-1.32)	*ns*	1.32	(0.97-1.81)	*ns*	0.97	(0.65-1.45)	*ns*
Text	1.11	(0.80-1.54)	*ns*	1.25	(0.82-1.91)	*ns*	1.06	(0.76-1.47)	*ns*	1.31	(0.85-2.02)	*ns*
TV Derived	0.85	(0.61-1.18)	*ns*	0.68	(0.43-1.07)	*ns*	0.77	(0.55-1.08)	*ns*	0.96	(0.63-1.47)	*ns*
Other Variables												
Gender	0.95	(0.67-1.36)	*ns*	1.08	(0.69-1.68)	*ns*	0.97	(0.68-1.38)	*ns*	**1.55**	**(1.01-2.36)**	**0.04**
Field of Study	**0.72**	**(0.61-0.86)**	**<0.01**	1.11	(0.88-1.40)	*ns*	0.96	(0.81-1.15)	*ns*	0.94	(0.75-1.18)	*ns*

Binary logistic regression was used to determine the odds ratio (OR) of answering "Yes" to the health effect question (in comparison to "No"). Each health effect question was analyzed separately, where the health effect was the dependent variable. For the independent variables, "No" was used as the base for *sources of information*, "Female" was used for *gender*, and "Social Sciences" was used for *field of study*. *Sources of information* (all 7 sources), *gender*, and *field of study* were added as covariates in the model. Values indicated are adjusted for co-variates. Significant differences ($p<0.05$) are indicated in bold. CI; confidence interval, *ns*; not significant. Answer responses to Appendix 1 Q4-Q7.

3.4.2.4 Self-Reported Consumption of Omega-3 Foods and Supplements. Almost half (47.5%) of respondents reported consumption of foods with omega-3 FAs. Additionally, 20.7% of respondents reported taking EPA and DHA omega-3 supplements. Neither gender nor field of study influenced consumption of omega-3 foods or supplements.

Table 3.4: Self-Reported Consumption of Foods and Supplements Rich in Omega-3 EPA and DHA FAs.

Consumption of omega-3 foods or supplements	Full Sample (N=834)	Biological/Physical Sciences (n=542)	Social Sciences (n=192)	Other Fields (n=100)	Female (n=655)	Male (n=179)
Foods	381 (47.5%)	260 (48%)[a]	75 (39.1%)[a]	46 (46%)[a]	300 (45.8%)[a]	81 (45.3%)[a]
Supplements	173 (20.7%)	101 (18.6%)[a]	49 (25.5%)[a]	23 (23%)[a]	134 (20.5%)[a]	39 (21.8)[a]

Numbers represent the frequency (n) and percentage (%) of respondents who answered "Yes" to consuming foods or supplements with omega-3 FA. χ^2 analyses assessed differences between *field of study*. Percentage values within a row with different superscript letters were significantly different in pairwise comparisons by Z test ($P \leq 0.05$), using a Bonferroni adjustment for multiple comparisons. Groups sharing letters are not different from one another. Answer responses to Appendix 1 Q9 and Q10.

3.5 Discussion

We found that awareness of omega-3 FA abbreviations versus full names varied in educated emerging adults. Approximately 4 out of 5 respondents reported being aware of a link between increased EPA and DHA consumption with heart and brain health, suggesting that these health messages have been translated to this sample. Additionally, those who used Academic/Reputable sources, HCPs, and/or Social Media to acquire information about omega-3 FAs were more likely to know that EPA and DHA were associated with general health. Almost half (48%) of the respondents self-reported consumption of omega-3 foods, and 21% reported consumption of EPA and DHA omega-3 supplements. Interestingly, while emerging adults in Biological/Physical Sciences generally showed greater awareness of omega-3 terminology and their possible health effects, the self-reported consumption of omega-3 foods and supplements was not different in comparison to respondents in other disciplines. This suggests a potential disconnect between awareness and behavior, and that more in-depth research on determinants of behavior (such as barriers to omega-3 FA consumption) is warranted.

3.5.1 Participant Characteristics

If gaps in awareness are identified in an educated population, then it would be expected that greater gaps in awareness would exist in the general public (246). Therefore, we felt that targeting educated emerging adults for this first investigation regarding awareness of omega-3 FAs was ideal. Our findings revealed several predictors of awareness of omega-3 FAs and their associated health effects, but not self-reported consumption.

Ethnicity was not found to influence any variables in our study; however, our population was predominantly comprised of Caucasian/European respondents. Therefore, ethnicity may have a

greater influence in a more diverse sample. Further, we found little evidence that awareness of nutritional concepts varied between genders, similar to Barzegari *et al.* (17). However, we did find that respondents in Biological/Physical Sciences had greater awareness about omega-3 FAs and possible health effects compared to those in Social Sciences or Other Fields. In previous reports, individuals in Health Sciences showed greater awareness about dietary fats and the connection with heart disease (194), and reported to use a greater proportion of Academic resources (62), which aligns with our findings. Consequently, future knowledge translation efforts about omega-3 FAs should target emerging adults outside of Health Sciences in order to ensure public health goals are achieved for all individuals.

3.5.2 Survey Questions

3.5.2.1 Awareness of Omega-3 FA Terminology. Lin *et al.* reported that most adult consumers recognize the categorical names of different fats (e.g., saturated, monounsaturated, *etc.*), but that fewer were familiar with polyunsaturated fat compared to saturated fat (194). In our study, the DHA abbreviation was the most well-known of all the omega-3 terms. This information may have value for marketing and promotion of foods and supplements enriched with specific omega-3 FAs. A study by Li *et al.* reported on knowledge related to omega-3 FAs in registered dietitians (RDs) (n=262, 95% women, 60% MSc degree or higher). Of those RDs who could name omega-3 FAs, DHA (84%) was more frequently identified, followed by EPA (80%) and then ALA (46%) (192). Our findings further highlight the varying degree of awareness that exists in the population regarding different omega-3 FAs and suggests that additional research-based education is needed (192).

3.5.2.2 Sources of Information. Social Media was used by 60% of emerging adults to learn about nutrition and health, and was associated with knowing more about omega-3 FAs and

general health. This highlights the importance and value of this tool to disseminate nutritional information (62). Although HCPs were reportedly less often used by our sample, using HCPs was associated with greater awareness about omega-3 FAs and their possible health effects. Interestingly, Cash *et al.* reported that HCPs were the preferred source of information for nutrition and health; however, time needed to make an appointment was the primary deterrent for using HCPs (62). These findings highlight a potential opportunity for qualified HCPs to disseminate nutritional knowledge via social media.

3.5.2.3 Possible Health Effects Associated with Increased EPA and DHA Intake. Over half (52%) of our sample indicated that increased intake of EPA and DHA was related to general health, compared to 30% of middle-aged adults reported by Verbeke *et al*. (314). The majority (~83%) of our sample reported knowing about the links between omega-3 FAs and heart and brain health, whereas other studies reported that only 59% of adolescents (126), and 51% of adults (194) knew about the association between omega-3 FAs and prevention of heart disease. It is interesting that participants in our study knew more about the links between omega-3 FAs and specific health effects versus general health; however, this aligned with the focus group data where more individuals associated EPA and DHA intake with specific health effects when questioned directly. These results confirm the importance of question design and the common observation that vague awareness generally greatly exceeds specific knowledge among consumers.

3.5.2.4 Self-Reported Intake of Omega-3 Foods and Supplements. While 48% of our respondents indicated that they purchase or consume foods with omega-3 FAs, this initial survey did not investigate the quantity, frequency, or types of omega-3 foods consumed. Regardless, our findings highlight an important disconnect that exists between awareness of the possible health

benefits and dietary intake. With respect to self-reported EPA and DHA omega-3 supplement consumption, our findings that 21% of emerging adults used supplements aligns with other reports (11, 16, 166). It is commonly recognized that individuals with a higher education have a higher quality diet and also consume omega-3 supplements (89). We report that ~80% of individuals know about the benefits to heart and brain health with increased EPA and DHA consumption, however, ~50% and ~80% of all of our respondents reported that they do not consume high omega-3 foods, or EPA and DHA supplements, respectively. This work highlights the weak link between awareness, knowledge, and behavior. Consequently, further work is needed to gain a better understanding of how to translate awareness about nutrition and health into healthy eating behaviors.

3.5.3 Considerations for Future Research

This survey was developed and used for the first time in this study, and has generated valuable information for omega-3 stakeholders in academia, industry, and public health. However, there are several areas for future investigation, including further development of the survey questions to confirm awareness and knowledge using psychometric methods. Volunteer/recall bias (105) and an unequal gender distribution is common in health survey research (117) and could have influenced our results. Online delivery of this survey allowed greater access to respondents outside of the region the research institute is located; however, a multi-center, multi-country study could be conducted for comparisons and analysis in future research. The majority of our respondents were currently enrolled in an academic institution, providing limited perspective on the awareness of omega-3 FAs among individuals who are working or who did not attend post-secondary school. As such, future investigations should include a greater representation of young adults in the general population.

3.5.4 Implications for Research and Practice

Omega-3 terminology and field of study are important to consider for improving knowledge translation of the developing evidence about omega-3 FAs and, ultimately, to increase their consumption. For example, mentioning DHA on social media in association with a product or a health claim may increase awareness, compared to using the full scientific name. Furthermore, social media may be a preferable format to deliver nutritional knowledge to emerging adults, and one that could be more widely used by HCPs. Future knowledge translation efforts can be designed to enhance current nutrition and health messages regarding omega-3 FAs to improve impact.

3.5.5 Conclusions

This study represents one of the largest investigations to date regarding the awareness of omega-3 FAs and health in emerging adults. This work highlights the weak links between awareness, knowledge, and behavior. As such, further research-based education is needed to gain a better understanding of how to translate awareness about nutrition and health into healthy eating behaviors.

CHAPTER 4:

THE ROLE OF *FADS1* and *FADS2* POLYMORPHISMS ON CARDIOMETABOLIC MARKERS AND FATTY ACID PROFILES IN YOUNG ADULTS CONSUMING FISH OIL SUPPLEMENTS

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4.1 Abstract

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 fatty acids (FAs) known to influence cardiometabolic markers of health. Evidence suggests that single nucleotide polymorphisms (SNPs) in the fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) gene cluster may influence an individual's response to omega-3 FAs. This study examined the impact of a moderate daily dose of EPA and DHA fish oil supplements on changes to cardiometabolic markers, FA levels in serum and red blood cells (RBC), and whether these endpoints were influenced by SNPs in *FADS1* and *FADS2*. Young adults consumed fish oil supplements (1,800 mg total EPA/DHA per day) for 12 weeks, followed by an 8-week washout period. Serum and RBC FA profiles were analyzed every two weeks by gas chromatography. Two SNPs were genotyped: rs174537 in *FADS1* and rs174576 in *FADS2*. Participants had significantly reduced levels of blood triglycerides (−13%) and glucose (–11%) by week 12; however, these benefits were lost during the washout period. EPA and DHA levels increased significantly in serum (+250% and +51%, respectively) and RBCs (+132% and +18%, respectively) within the first two weeks of supplementation and remained elevated throughout the 12-week period. EPA and DHA levels in RBCs only (not serum) remained significantly elevated (+37% and +24%, respectively) after the washout period. Minor allele carriers for both SNPs experienced greater increases in RBC EPA levels during supplementation; suggesting that genetic variation at this locus can influence an individual's response to fish oil supplements.

4.2 Introduction

The Western diet is characterized as being rich in omega-6 polyunsaturated fatty acids (PUFA) and poor in omega-3 PUFA, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (68, 72). Individuals can increase their EPA and DHA levels by consuming fatty fish, omega-3 fortified foods, or dietary supplements such as fish oil, algal oil, or krill oil (169, 311). It is well documented that consuming fish oil leads to reductions in cardiometabolic markers including blood triglycerides (TAG) and inflammatory mediators, which may reduce the risk for cardiovascular disease (CVD) and metabolic diseases (173, 197, 306). Furthermore, individuals with higher baseline fasting TAG levels experience proportionally greater decreases in TAG with fish oil supplementation (12, 97). In contrast, changes in other cardiometabolic markers such as cholesterol, glucose, and insulin, have shown conflicting results with fish oil supplementation (12, 132). Reasons for these discrepancies include differences in study populations with respect to age, sex, and disease status, as well as differences in study design such as dose and length of supplementation (12, 147, 153, 163). Young healthy adults are one age group in which the changes in cardiometabolic markers with fish oil supplementation remains poorly characterized.

Changes in fatty acid (FA) intake, be it from foods or dietary supplements, are reflected in blood and can be quantitatively measured in distinct blood fractions such as serum, plasma, and red blood cells (RBC) (137, 173). It has previously been reported that FA profiles in different blood fractions are altered in response to fish oil supplements providing between 2,200 mg and 4,800 mg EPA and DHA per day (61, 154, 204, 220). However, there are inconsistent results on the extent by which FA profiles change in various blood fractions during and after the consumption of fish oil supplements providing a moderate dose of 1,800 mg EPA/DHA per day

or less (40, 108, 128, 154, 289). A study by Flock *et al.* investigated five different doses (0, 300, 600, 900 and 1,800 mg/day) of EPA and DHA for five months, to mimic potential intake from the diet in adults 20-45 yrs. (n=125 individuals, n=20-25 per group, 48% females) (108). In the investigation by Flock *et al.* none of the doses caused any significant reductions in cardiometabolic markers of health (108).

Understanding the dynamic changes in FA profiles in various blood fractions will help build knowledge regarding the length of time fish oil supplements are required to be taken in order to achieve and maintain increases or decreases in specific FAs. The changes in these FA profiles may also contribute towards alterations in cardiometabolic markers. From previous studies, EPA and DHA measured from RBC FAs increased in a dose dependent manner (108). Additionally, participants who had the lowest levels of RBC EPA and DHA at baseline experienced the greatest increases in EPA and DHA as a result of their specific omega-3 intervention (108).

In addition to the relationships between FAs and cardiometabolic health, there is also evidence demonstrating that variation in the fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) gene cluster can influence cardiometabolic markers. For example, single nucleotide polymorphisms (SNPs) in the *FADS1* and *FADS2* gene cluster have been associated with diseases such as CVD and Type 2 Diabetes (T2D) (176, 177, 185, 219, 284). However, less research has focused on these SNPs and their effects on individual cardiometabolic markers. Previous research in our lab (272) and elsewhere (207) have shown that SNPs in the *FADS1* and *FADS2* gene cluster are related to changes in circulating high sensitivity C-reactive protein (hsCRP) levels; a marker of whole body inflammation. In addition, Cormier *et al.* (74) have recently examined the influence of *FADS1* and *FADS2* SNPs on blood TAG levels. Evidence

from studies supplementing with flaxseed oil or encapsulated EPA/DHA suggests that genetic variation in *FADS1* and *FADS2* can affect how a person responds to omega-3 FA supplements (5, 116). Further, these studies in middle-aged adults showed that minor allele carriers for SNPs in the *FADS1* and *FADS2* gene cluster have lower blood EPA levels before supplementation (5, 116). Therefore, it is important to see if these findings are also seen in young adults consuming fish oil supplements.

The objectives of the present study were to assess the impact of fish oil supplementation, providing 1,800 mg EPA and DHA per day, in young adults (18–25 yrs. of age) using several approaches. First, we examined whether fish oil supplementation could lead to reductions in cardiometabolic markers. Second, we measured changes in FA profiles in both serum and RBC fractions during a 12-week fish oil supplementation period, followed by an 8-week washout period. Last, we examined to what extent changes in cardiometabolic markers and blood FA profiles were influenced by common SNPs in the *FADS1* and *FADS2* gene cluster. Overall, we anticipate that the findings of this study will help generate further support that knowledge of an individual's *FADS1* and *FADS2* genotype may help guide the development of personalized strategies using omega-3 FA supplements to improve health and prevent disease.

4.3 Methods

4.3.1 Participant Characteristics and Omega-3 Supplementation

Young male adults (n = 12) between 18 and 25 yrs. of age were recruited from the University of Guelph through study posters. An omega-3 diet survey was used during the initial screening to ensure that participants had not consumed fish oil supplements in the past 12 weeks and/or were not frequent consumers of foods rich or fortified with omega-3 FAs. This approach

was also used in previous studies, to recruit participants with a low baseline intake of EPA and DHA (108). Participants were instructed to maintain regular exercise and dietary habits throughout the entire study. All participants were supplemented with fish oil, in order to examine inter-individual effects of EPA and DHA supplementation. The study consisted of a 12-week fish oil supplementation followed by an 8-week washout. During the supplementation period, participants consumed three Clearwater Omega-3 capsules per day (kindly provided by Ocean Nutrition Canada Ltd., NS, Canada) providing a total of 1200 mg EPA and 600 mg DHA per day (>98% purity). Participants were instructed to consume fish oil capsules with meals, as this was previously shown to improve absorption (187). In order to determine if participants have consumed their supplements, EPA and DHA levels in both serum and RBC fractions were analyzed at baseline and at time points throughout the study. Anthropometric measurements (age, weight, height, and calculated BMI) were taken at baseline and at the end of the washout period. This study (Clinicaltrials.gov. identifier NCT02042274) was approved by the University of Guelph Human Research Ethics Board (REB #12JL006).

4.3.2 Blood Collection

Participants arrived to the clinical trials unit (Human Nutraceutical Research Unit) after a 10-12 hour fast. Blood was collected by venipuncture by a trained phlebotomist. Blood samples were collected by venipuncture throughout the 20-week study period at baseline and weeks 2, 4, 6, 8, 12, 14, 16 and 20 (study timeline **Figure 4.1**). Serum blood samples were collected in yellow top vacutainers (BD Vacutainer, 5.0 mL, SST (serum separation tubes)) coated with silicone and micronized silica particles to accelerate clotting. Serum blood samples were used for the analysis of cardiometabolic markers and fatty acid analysis. Plasma and red blood cell (RBC) blood samples were collected in lavender top vacutainers (BD Vacutainer, 6.0 mL), containing

EDTA (ethylenediaminetetraaeteic acid) as an anti-coagulant. RBC samples were used for fatty acid analysis.

After the blood was collected, both types of vacutainer tubes were inverted three times and then left at room temperature for 30 minutes. Tubes were centrifuged for 15 minutes at 3000 rpm. The serum blood samples were kept in the fridge prior to pick-up from LifeLabs (same day). One serum sample and the plasma and RBC samples were aliquoted into Eppendorfs and put directly into the -80˚C freezer.

4.3.3 Analysis of Cardiometabolic Markers

Serum blood samples were used for the analysis of the selected cardiometabolic markers (as mentioned below). These samples were sent to LifeLabs Medical Services the same day as collection and analyzed using standard laboratory procedures. Lipid markers including TAG, total cholesterol, LDL-c, HDL-c, and the cholesterol/HDL-c ratio were measured by LifeLabs at each time point. Glycemic parameters (glucose, insulin, and HbA1c) and an inflammatory marker (hsCRP) were also measured by LifeLabs at baseline, week 12, and week 20.

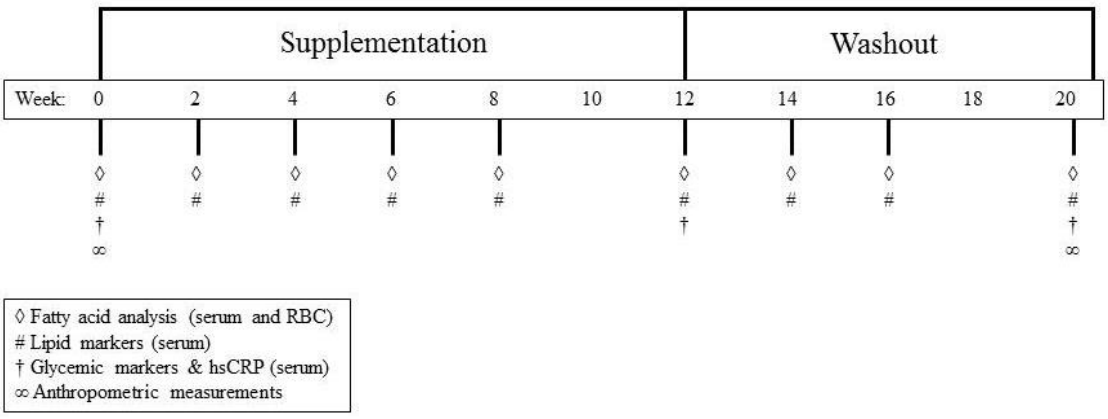

Figure 4.1. Study Timeline and Experimental Design..

All participants received fish oil supplements throughout the duration of the study. The supplementation period lasted for 12 weeks and the washout period for 8 weeks (total duration of 20 weeks). Symbols indicate which measurements were taken at each time-point.

4.3.4 Fatty Acid Analysis

Serum and RBC blood samples were collected at each study visit. Serum samples were collected in separate tubes (as described above), and plasma and RBC were collected using the same tube (as described above).The current analysis was completed as previously described (218), with minor modifications. All solvents and reagents were obtained from Fisher Scientific (ON, Canada). Frozen serum and RBC samples were thawed on ice for approximately 1.5 h prior to extraction. After the addition of 10 µL (0.1 mg/mL stock) of an internal free FA standard (C17:0) (Sigma-Aldrich, USA), total lipids were extracted from 100 µL of serum and/or 100 µL of RBC using chloroform:methanol 2:1 v/v. Samples were flushed with nitrogen and stored at 4˚ C overnight. The next day, samples were centrifuged at 1460 rpm for 10 min. Pooled lipids were saponified using 2 mL of 0.5 mol/L KOH in methanol at 100°C for 1 h. The resulting free FAs were trans-esterified by the addition of 2 mL of 14% boron triflouride in methanol and 2 mL of

hexane at 100 °C for 1.5 h. Double distilled water (2 mL) was added to stop the methylation reaction and samples were subsequently centrifuged for 10 minutes at 1000 rpm. The hexane phase was extracted, evaporated under nitrogen gas, and reconstituted in 100 μL of hexane for analysis. FA methyl esters were separated by gas chromatography using an Agilent 6890B gas chromatograph (Agilent Technologies, California, United States) with a Supelco SP 2560 fused-silica capillary column with flame ionization detector (100m x 0.25mm i.d., 0.2 μm film thickness; Sigma-Aldrich, USA). FA peaks were identified by comparison to retention times of FA methyl ester standards (developed and validated by a collaborating lab within the department). Chromatograms were analyzed individually and the areas for each FA were confirmed. Individual FAs are indicated as a percentage (%) of total FAs. Specific FAs including EPA, DHA, arachidonic acid (AA), docosapentaenoic acid (DPA), alpha-linolenic acid (ALA), γ-linolenic acid (GLA), and linoleic acid (LA) are reported. Differences in % of specific FAs were examined between time points to examine changes relative to the supplementation. FADS pathway activity was estimated by dividing AA/LA, as previously reported (31, 82, 218).

4.3.5 Analysis of Variants in the *FADS1* and *FADS2* Gene Cluster

DNA was extracted using the Qiagen PAXgene blood DNA kit, according to manufacturer instructions (Qiagen, ON, Canada). DNA quality was visually confirmed on a 1% agarose gel. Two SNPS were genotyped: rs174537 in *FADS1* (Chr11:61785208, CEU MAF = 0.30-0.33), and rs174576 in *FADS2* (Chr11:61836038, CEU MAF = 0.36-0.39) (281). These SNPs were selected for the current analysis because several reports (both by us and others) have showed them to be reproducibly associated with blood FA levels and cardiometabolic markers (177, 185, 218, 272, 304). Detection of the rs174537 SNP in *FADS1* and the rs174576 SNP in *FADS2* was carried out using validated TaqMan SNP genotyping assays (Assay ID

C_2269026_10_37 and C_2575520_10_76, respectively; Life Technologies, CA, USA) and each sample was analyzed in triplicate to ensure genotyping accuracy. Amplification was conducted on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, CA, USA) using an amplification protocol of 95 °C for 10 minutes followed by 40 cycles, which included denaturing for 15 seconds at 92 °C and annealing/extending for 1 min at 60 °C. Allelic discrimination was performed using CFX96 software to distinguish between different genotypes: major allele homozygotes (GG for rs174537; CC for rs174576), heterozygotes, and minor allele homozygotes (TT for rs174537; AA for rs174576). Linkage disequilibrium (LD) was determined *post-hoc* to examine the two SNPs used in our study, as well as compared to other SNPs reported in the literature. LD was determined with SNAP (SNP annotation and proxy search (150)) using the CEU population as the reference group, with data generated as per Pettersson *et al.* (254).

4.3.6 Statistical Analysis

Changes in serum and RBC FAs were determined by comparing each time point to baseline values using a non-parametric Wilcoxon paired *t*-test. All genotype analyses were conducted using a dominant model. FA differences according to genotype were assessed at baseline, week 12, and week 20 using a non-parametric Mann-Whitney *U*-test. A genotype × time interaction was used to examine the impact of changes in specific FAs throughout the study period. GraphPad Prism 5 (GraphPad Software, Inc., CA, USA) and JMP Genomics software V5 (SAS Institute, NC, USA) were used for all analyses. A $p < 0.05$ was considered statistically significant.

4.4 Results

4.4.1 Fish Oil Supplementation and Cardiometabolic Markers

All 12 participants completed the study. Anthropometric data and cardiometabolic markers are indicated in **Table 4.1**. The average age of participants was 21.8 ± 1.1 yrs. at baseline. Participants showed a significant decrease in fasted TAG (−13%, $p = 0.03$) and glucose (−11%, $p = 0.03$) levels by the end of the 12-week supplementation period. These changes were not maintained during the washout period, where both fasted TAG and glucose levels rebounded back to baseline values (**Table 4.1**). No significant changes were detected for total cholesterol, LDL-c, HDL-c, the cholesterol/HDL ratio, fasting insulin, HbA1c, or hsCRP during the 20-week study period (**Table 4.1**).

Table 4.1. Participant Characteristics.

Parameter	Baseline	Week-12	Week-20
BMI (kg/m^2)	25.74 ± 4.02	ND	25.76 ± 4.22
Triglyceride (mmol/L)	0.87 ± 0.29	0.76 ± 0.29 *	0.90 ± 0.47
Total cholesterol (mmol/L)	4.02 ± 0.54	4.20 ± 0.70	4.07 ± 0.50
LDL-c (mmol/L)	2.36 ± 0.48	2.53 ± 0.52	2.36 ± 0.38
HDL-c (mmol/L)	1.26 ± 0.17	1.32 ± 0.32	1.30 ± 0.16
Cholesterol: HDL-c	3.24 ± 0.63	3.28 ± 0.69	3.17 ± 0.51
Glucose (mmol/L)	4.83 ± 0.31	4.26 ± 0.50 *	4.62 ± 0.44
Insulin (ρmol/L)	54.50 ± 22.11	46.78 ± 26.24	57.58 ± 28.53
HbA1c (mmol/L)	$0.05 \pm 2.25 \times 10^{-3}$	$0.05 \pm 2.01 \times 10^{-3}$	$0.05 \pm 2.28 \times 10^{-3}$
hsCRP (mg/L)	1.36 ± 1.21	1.40 ± 1.29	1.19 ± 1.00

Average anthropometric and clinical measures are listed at baseline, week 12, and week 20 for study participants ($n = 12$). Values are indicated as mean ± SD. * Indicates $p < 0.05$ compared to baseline. ND, not determined.

4.4.2 Fatty Acid Profiles in Serum and RBCs

Within two weeks of commencing fish oil supplementation, EPA and DHA levels were significantly increased in both serum (+250%, $p = 1.0 \times 10^{-3}$ and +51%, $p = 5.0 \times 10^{-4}$, respectively) and RBC (+132%, $p = 1.0 \times 10^{-3}$ and +18%, $p = 2.0 \times 10^{-3}$, respectively) fractions

(**Figure 4.2A–D**). The levels of EPA and DHA remained significantly elevated throughout the supplementation period. During the washout period, serum EPA levels returned to baseline values within two weeks of supplementation ending. In comparison, serum DHA levels remained significantly elevated within two weeks after supplementation had ceased, but returned to baseline levels by the end of the 8-week washout period. In RBCs, EPA and DHA levels also decreased during the washout period; however, they remained significantly elevated at the end of the 8-week washout period compared to baseline (+37%, $p = 1.0 \times 10{-}3$ and +24%, $p = 4.9 \times 10^{-3}$, respectively). AA levels decreased in serum during supplementation; however, there was considerable inter-individual variability resulting in sporadic significance during the 20 week study (**Figure 4.2E**). In contrast, AA levels in RBCs were significantly decreased by the sixth week in the supplementation period, and were significantly reduced (−13%, $p = 1.0 \times 10^{-3}$) compared to baseline, at week 12 (**Figure 4.2F**). Similar to EPA and DHA, AA levels in RBCs remained significantly altered by the end of the washout period in comparison to baseline, with a significant decrease (−6%, $p = 0.02$).

While EPA, DHA, and AA are the primary FAs that we expected to see changed with fish oil supplementation, we also examined other FAs detected by gas chromatography. By the end of the supplementation period, we noted significant decreases in serum levels of LA and GLA (−9%, $p = 2.4 \times 10^{-3}$ and −34%, $p = 2.4 \times 10^{-3}$, respectively), and significant increases in DPA (+49%, $p = 5.0 \times 10^{-4}$) compared to baseline. After the washout period, serum levels of LA, GLA, and DPA had all returned to baseline values. RBC levels of LA decreased (−13%, $p = 1.5 \times 10^{-2}$), and there were significant increases in RBC DPA levels (+39%, $p = 1.5 \times 10^{-2}$) at week 12 compared to baseline. While RBC LA levels returned to baseline during the washout period, RBC DPA levels remained significantly increased (+22%, $p = 4.9 \times 10^{-3}$). We observed

no significant changes in the levels of ALA in either serum or RBCs during the 20-week study. Moreover, no significant changes were detected for any SFAs or MUFAs during the study.

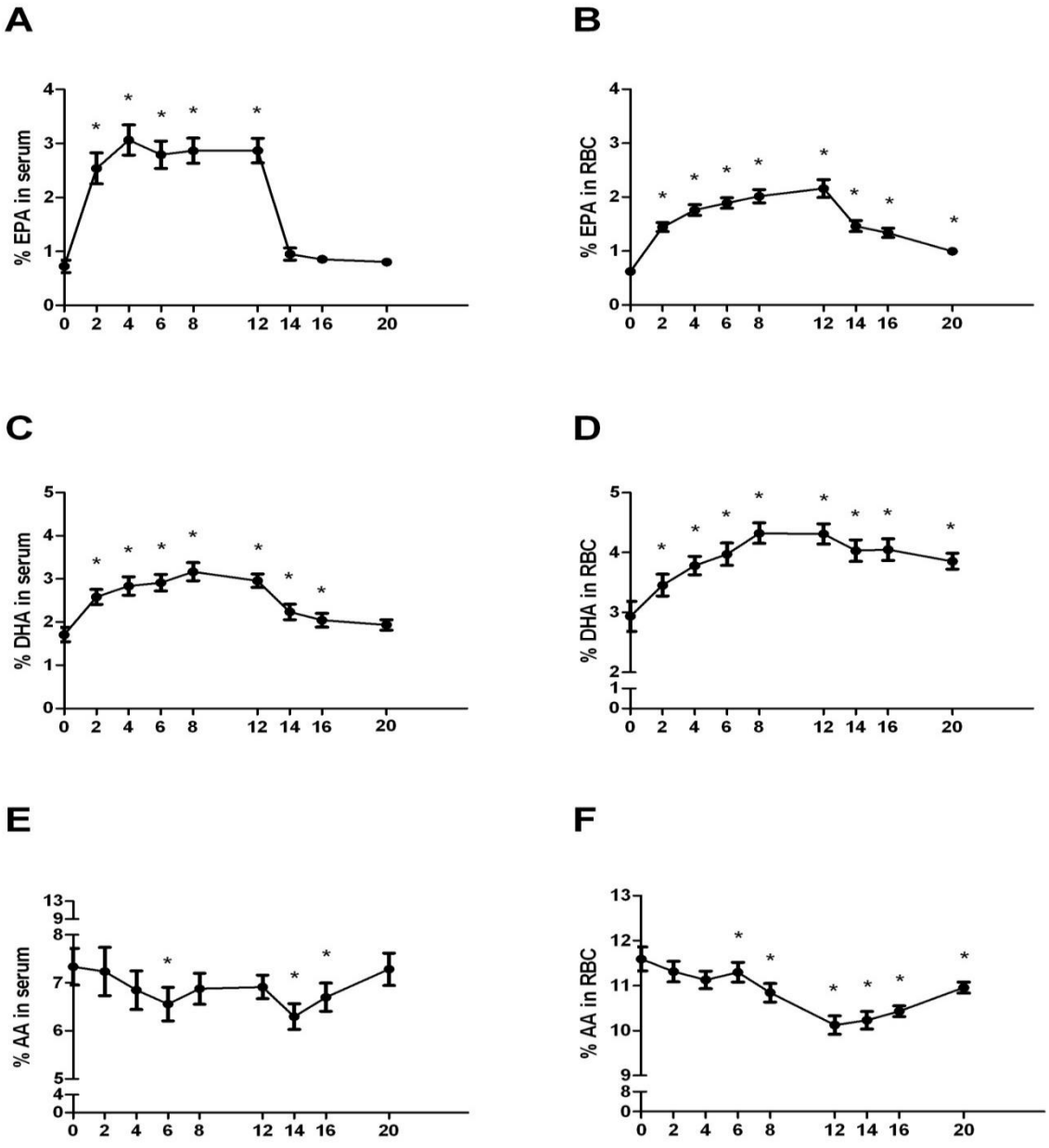

Figure 4.2. Levels of Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), and Arachidonic acid (AA) in Serum and RBCs.

Relative % EPA in (**A**) serum and (**B**) RBCs. Relative % DHA in (**C**) serum and (**D**) RBCs. Relative % AA in (**E**) serum and (**F**) RBCs. FAs are represented as relative % of total FA values. Numbers on the *x*-axis represent time in weeks, where baseline is indicated by 0. * Indicates $p < 0.05$ compared to baseline (0).

4.4.3 Analysis of Variants in the *FADS1* and *FADS2* Gene Cluster

Genotyping results revealed identical allelic distributions for individual participants for the two SNPs analyzed (rs174537 in *FADS1* and rs174576 in *FADS2*). A *post-hoc* examination of LD revealed that these two SNPs are in perfect LD (1.0); thus indicating that identical allelic distribution was not due to chance. As such, we chose to present findings for the rs174537 SNP only. The influence of these SNPs on cardiometabolic markers and FA levels (EPA, DHA, and AA) was examined at baseline, week 12, and week 20 of the study. There were no significant differences in cardiometabolic markers (specifically TAG or glucose levels) between major (GG; $n = 3$) and minor (GT + TT; $n = 9$) allele carriers at any time point. There was a significant difference in serum EPA levels between major and minor allele carriers at baseline, with minor allele carriers showing lower baseline serum EPA levels (−48%, $p = 0.04$; **Figure 4.3A**). A similar trend was observed for EPA levels in RBC, but did not reach statistical significance (−27%, $p = 0.28$; **Figure 4.3C**). While EPA levels increased in both major and minor allele carriers after the 12-week supplementation period, we were unable to detect a significant genotype × time interaction in either serum or RBCs (**Figure 4.3A,C**). However, when expressing changes in EPA levels as a percent (%) change (between baseline and week 12) (**Figure 4.3B,D**), we found that minor allele carriers had a greater increase in RBC EPA levels ($p = 9.1 \times 10^{-3}$, **Figure 4.3D**) during supplementation compared to major allele carriers. Importantly, there was no difference in ALA levels in either serum or RBCs between major and minor allele carriers at any time point; suggesting that changes in ALA intake were not responsible for changes in EPA (data not shown). We observed no significant differences in DHA or AA levels in serum or RBCs at any time point when subjects were stratified by genotype.

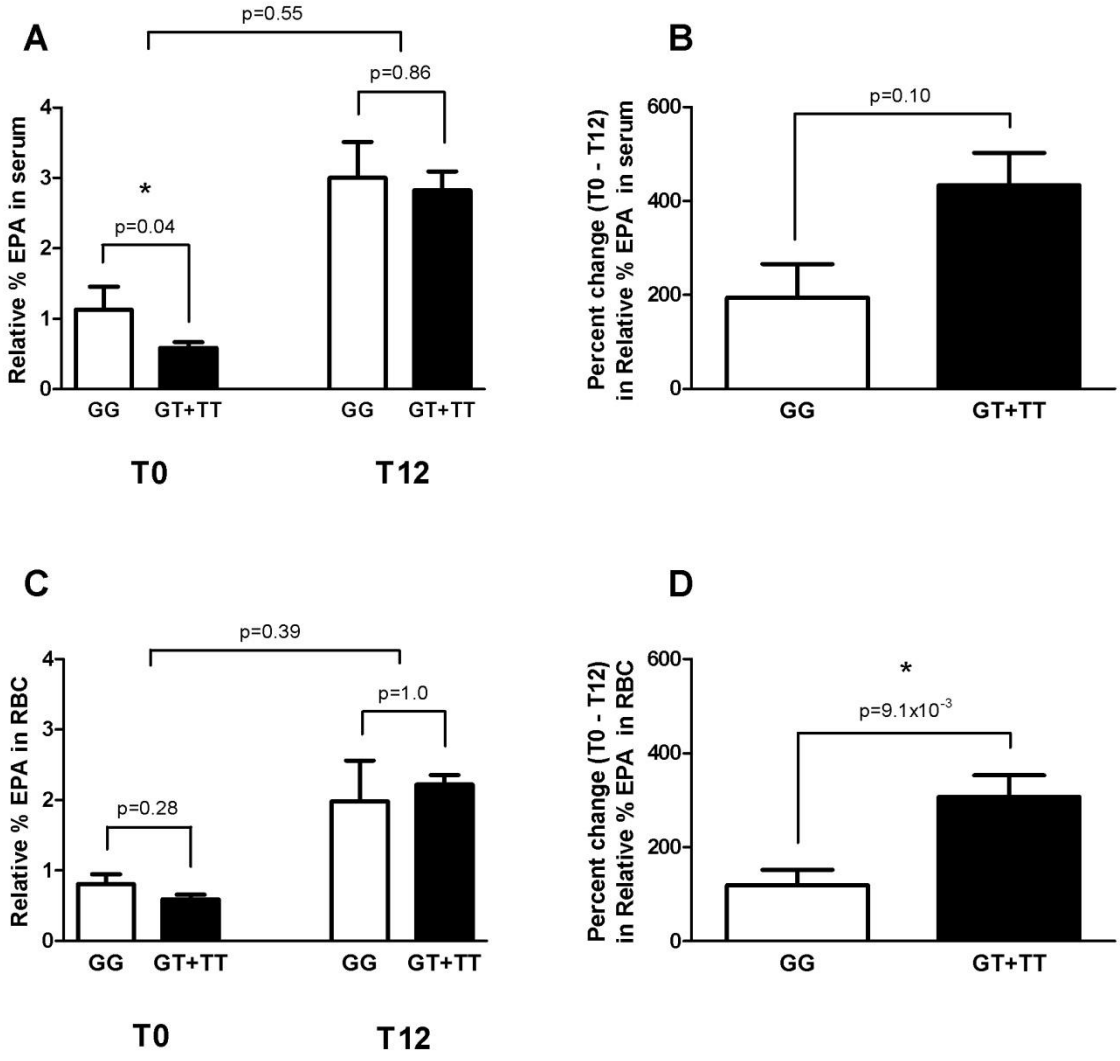

Figure 4.3. Eicosapentaenoic acid (EPA) Levels in Major and Minor Allele Carriers of the rs174537 SNP in *FADS1*.

(**A**) Differences in relative % EPA in serum between major and minor allele carriers at both baseline (T0) and week 12 (T12) as well as the interaction between genotype × time. (**B**) Percent changes (*i.e.*, T0 − T12) for serum % EPA in major and minor allele carriers. (**C**) Differences in relative % EPA in RBCs between major and minor allele carriers at T0 and T12 as well as the interaction between genotype × time. (**D**) Percent change (*i.e.*, T0 − T12) for RBC % EPA in major and minor allele carriers. *p*-values are listed above each of the comparisons. Major allele carriers (GG) ($n = 3$) and minor allele carriers (GT + TT) ($n = 9$). *Indicates $p < 0.05$.

4.5 Discussion

Studies have shown that the increased intake of EPA and DHA from fish oil supplements is linked to reductions in cardiometabolic markers, improvements in overall health, and CVD and metabolic disease prevention (12, 139, 169, 197, 306). It was uncertain if cardiometabolic markers would change in young adults consuming a moderate dose of fish oil supplements (providing 1,800 mg EPA and DHA per day) since these individuals already have normal levels of these markers (131, 189, 253). The results of our study demonstrated that young men experienced reductions in cardiometabolic markers, specifically TAG and glucose, with the daily consumption of a moderate dose of fish oil. There were no significant changes in cholesterol parameters after fish oil supplementation, which is consistent with other studies (2, 12, 131, 256, 317). The present study contributes to the field by showing that young healthy individuals also experience reductions in select cardiometabolic markers with a moderate dose of fish oil supplementation in comparison to the doses more commonly reported in the literature (*i.e.*, >3,000 mg EPA and DHA per day). We also confirmed and extended the findings of previous studies with moderate doses (*i.e.*, <1,800 mg EPA and DHA per day) to show that small but significant differences in some cardiometabolic markers are possible after a fish oil supplementation (40, 108, 128, 154, 289).

It is important to note that the reductions in TAG and glucose were quickly lost upon cessation of supplementation; suggesting that improvements in cardiometabolic markers are only maintained with continuous supplementation. Our findings have potential relevance in the context of future disease prevention, as cardiometabolic markers such as TAG are known to increase in an age-dependent manner; thus reducing TAG levels at a younger age may potentially reduce an individual's risk for CVD (139). Therefore, supplementing with fish oil in early

adulthood could promote a more favorable cardiometabolic marker profile in the longer term. Future longitudinal research is needed in order to determine if the decreases in cardiometabolic markers seen in young adults consuming omega-3 supplements prevents the development of disease later in life.

In addition to changes in cardiometabolic markers of health, it has been previously shown that changes in omega-3 FA intake are reflected in blood FA profiles (137). For example, increasing EPA and DHA intake causes these FAs to be increased in serum, plasma, and RBCs (137, 154). The concentrations of these FAs rise more quickly in plasma and serum in comparison to RBCs, which is expected because RBCs have a turnover rate of approximately 90-120 days (131, 154, 220). As such, RBCs reflect longer-term adaptations in FA intake. We found that EPA levels increased by +250% and DHA increased by +51% in serum within the first two weeks of supplementation compared to baseline. During the washout period, serum EPA and DHA levels decreased back to baseline levels. Specifically, our data showed that serum EPA levels rapidly returned to baseline levels within two weeks of stopping fish oil supplementation, while serum DHA returned to baseline levels only by the end of the washout period. Comparing serum to RBCs, the levels of EPA and DHA in RBCs remained significantly higher by the end of the washout period, most probably due to the 90-120 day turnover rate of RBCs. Overall, DHA maintained slightly higher levels in both blood fractions during and after supplementation compared to EPA, which is consistent with prior studies using either fish oil or cod liver oil supplements providing ~2,000 mg EPA/DHA per day (61, 204, 317). EPA in serum and RBCs increased and decreased more rapidly during the study period than DHA, which also agrees with previous reports studying higher doses of omega-3 FA supplements (*i.e.*, 2,200–4,800 mg EPA/DHA per day) in adults between 21-49 yrs. (204, 220).

While changes in EPA and DHA are expected with fish oil supplementation, it is also interesting to examine if other FAs are changed with supplementation. We found that AA levels were reduced slightly in serum and significantly in RBCs during the supplementation period. This aligns with a previous study from our group in which similarly aged men consumed a fish oil supplement providing 3,000 mg EPA/DHA per day for 12 weeks (339). To expand from this previous work, we have now examined the dynamic changes in AA levels in both serum and RBCs throughout this 12-week supplementation period and 8-week washout period. When examining AA levels throughout the study, we noted that AA levels were more consistently reduced in RBCs compared to serum. In regards to other FAs, we found that serum and RBC DPA levels were significantly increased, and both serum and RBC LA levels were significantly reduced by the end of the fish oil supplementation period. Changes in DPA are likely attributed to the increased amounts of EPA available to be elongated by the fatty acid elongase 2 (ELOVL2) enzyme. Our results are consistent with other studies showing that fish oil supplementation caused increases in serum and RBC DPA (61, 339) and a decrease in both serum and RBC LA (61). The rapid return of serum FAs to initial levels once supplementation ceased highlights the importance of continued supplementation in order to maintain elevated EPA and DHA and reduced AA levels (15, 103). The sustained increases in EPA and DHA levels (particularly in RBCs) during the washout period also demonstrates that care must be taken when designing FA supplementation trials with a cross-over component in order to avoid residual FA changes from the previous supplementation. Importantly, many studies use a washout period of four weeks, which our data suggests would be insufficient to clear changes in FAs such as EPA, DHA, and AA in RBCs (61, 204).

It is now recognized that the fatty acid desaturase enzymes from the *FADS1* and *FADS2* gene cluster, D5D and D6D respectively, influence the FA profile in blood fractions and tissues, as well as cardiometabolic markers. Of relevance to the current study, these parameters can also be modified with fish oil supplements (71). Work by Cormier *et al.* (74) examined the role of several SNPs in the *FADS1* and *FADS2* gene cluster as a potential factor that could influence the changes in TAG levels seen with fish oil supplementation. The authors reported that TAG levels were associated with the rs174546 SNP in *FADS1* [26]. Although the two SNPs examined in our study (rs174537 and rs174576) were found to be in perfect LD (1.0) with the rs174546 SNP used by Cormier *et al.* (74) (*post-hoc* LD analysis outlined in Methods), we were unable to replicate this finding; however, this may simply be due to the small size of our study cohort. Nevertheless, our findings agree with the overall conclusions from Cormier *et al*. (74), as both studies found that fish oil supplementation resulted in a significant decrease in TAG levels that was independent of variation in the *FADS1* and *FADS2* gene cluster (74). In our study, we were unable to show that glucose levels and other cardiometabolic markers were significantly different when stratifying subjects by genotype before, after, or in response to fish oil supplementation. When examining FA levels, we found that minor allele carriers had significantly lower serum EPA levels at baseline ($p = 0.04$), thus agreeing with recent findings reported by Gillingham *et al.* (116) and Al-Hilal *et al.* (5) who used flaxseed oil and encapsulated EPA and DHA, respectively, as sources of omega-3 supplementation. Due to our small sample size, we were unable to show statistically significant genotype × time interactions in serum or RBCs (**Figure 4.3A,C**). To mitigate inter-individual variability in EPA levels, we also reported EPA levels as percent changes (*i.e.*, T0 − T12) (**Figure 4.3B,D**). This approach revealed that minor allele carriers experienced a significantly greater percent change increase in RBC EPA during omega-3

FA supplementation compared to major allele carriers, which agrees with a previous report by Gillingham *et al.* (116). In contrast, we did not observe a significant genotype effect regarding DHA levels in either serum or RBCs. This may be due to a lower amount of DHA (compared to EPA) in our supplements. Overall, the results from our study align well with those previously reported by Cormier *et al.* (74), Gillingham *et al.* (116) and Al-Hilal *et al.* (5). As such, we anticipate that these findings will help to further demonstrate the potential role of variants in the *FADS1* and *FADS2* gene cluster as mediators of the changes in FA profiles and cardiometabolic markers seen with omega-3 supplementation.

Based on the growing body of literature highlighting the relationship between variants in the *FADS1* and *FADS2* gene cluster and FA levels, we believe this locus has potential to be a nutrigenetics target that can be used to help guide the use of omega-3 supplements (139). As reported, minor allele carriers for SNPs in the *FADS1* and *FADS2* gene cluster typically have lower baseline serum and plasma EPA and AA levels (5, 139, 218, 272, 304). Additionally, individuals with lower baseline EPA levels appear to experience greater increases in these FAs with omega-3 supplementation (61, 156). We believe our study contributes to the growing body of evidence suggesting that genotyping the *FADS1* and *FADS2* genes, as well as measuring baseline blood EPA levels, may enable Health Care Providers (HCPs) to use this information to personalize nutritional recommendations in which omega-3 supplements are considered.

We acknowledge certain limitations with our study. Firstly, young males were recruited in order to avoid sex-specific differences in FA metabolism; as females experience changes in lipid levels as well as levels of omega-3 FAs throughout the menstrual cycle and generally have a higher level of DHA in blood than males (163, 230). In future studies, both males and females should be considered. In addition, as this population was considered relatively healthy, it is

possible that some of the cardiometabolic markers may be modified to a greater extent in older individuals or those with metabolic complications such as hyperlipidemia (12, 97, 147, 153). It is also important to note that our study did not include a control group. We acknowledge that the inclusion of a placebo-control group would account for the role of any extraneous variables on the endpoints measured in this investigation. We wanted to directly assess the effect of a fish oil supplementation and therefore inter-individual differences were deemed most important rather than group comparisons to a control group. Due to the quantity of FA analyses performed, we were also limited by personnel and finances which lead to the exclusion of a control group. Subsequent studies completed by our lab group investigating omega-3 FAs have included a placebo-controlled group (270, 340).

We also acknowledge that we have a small sample size; however, we are confident in our data because of the high level of agreement with previous independent studies reporting similar findings in large cohorts. As such, our work lends additional support regarding the biological relevance of the relationship between omega-3 FA supplements, cardiometabolic markers of health, and variants in the *FADS1* and *FADS2* gene cluster. Nevertheless, findings from the present work should be expanded in future studies to continue this line of investigation. A consideration for future omega-3 studies to enhance screening and recruitment into an omega-3 intervention could consider the use of an FFQ in order to more accurately monitor dietary habits. In addition, RNA could be collected to examine the effect of fish oil supplements on D5D and D6D gene expression.

4.6 Conclusions

Our study demonstrated that fish oil providing 1,800 mg EPA/DHA per day caused a reduction in circulating levels of TAG and glucose, and significantly altered levels of circulating FAs in young healthy men. Importantly, normalization of TAG and glucose following the washout period reinforces the necessity of continued supplementation in order to maintain these reductions in cardiometabolic markers. Further, studying dynamic changes in FA profiles during the 20-week study period showed that EPA and DHA enrichment persists for at least eight weeks in the RBC fraction following supplementation; reinforcing the importance of a washout period of sufficient time in future clinical trials. We have also shown that genotype may be a potential mediator of an individual's response to fish oil supplementation, most notably with regards to EPA levels. Overall, our study has demonstrated that young adults provided with a moderate daily dose of fish oil supplements experience reductions in some cardiometabolic markers. This information about effects of omega-3 supplements on cardiometabolic markers, and knowledge of an individual's *FADS1* and *FADS2* genotype, may help guide the development of personalized strategies to improve long-term health.

CHAPTER 5:

EVALUATING CHANGES IN OMEGA-3 FATTY ACID INTAKE AFTER RECEIVING PERSONAL *FADS1* GENETIC INFORMATION: A RANDOMIZED NUTRIGENETIC INTERVENTION.

Kaitlin Roke[a], Kathryn Walton[b], Shannon Klingel[a], Amber Harnett[a], Sanjeena Subedi[c], Jess Haines[b], and David M. Mutch[a]. Evaluating Changes in Omega-3 Fatty Acid Intake after Receiving Personal *FADS1* Genetic Information: A Randomized Nutrigenetic Intervention. **Submitted for Peer Review, August 2016.**

5.1 Abstract

Background: Nutrigenetics research is proposed to lay the foundation for personalized dietary recommendations. However, there have been a limited number of randomized nutrigenetic interventions conducted to date. Therefore, it remains unclear if providing individuals with their personal genetic information encourages meaningful changes in dietary behaviors.

Objective: To evaluate the changes in omega-3 fatty acid (FA) intake and blood levels after the provision of personal genetic information for a common variant in the fatty acid desaturase 1 (*FADS1*) gene to young adults.

Design: 57 females (18-25 yrs.) were randomized into Genetic (intervention) and Non-Genetic (control) groups and measurements were taken at Baseline and Final (12-weeks). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) dietary intake was assessed using a validated omega-3 food frequency questionnaire. Red blood cell (RBC) FA content was quantified by gas chromatography. Implications of participation in a nutrigenetics study, awareness of omega-3 FA terminology, and opinions of receiving genetic information was assessed with online questionnaires.

Results: Upon completion of the study, EPA and DHA intake increased significantly ($p=5.0x10^{-3}$) from below recommended dietary intake levels to those meeting Dietitians of Canada recommendations (≥300 mg EPA and DHA/day) in both the Genetic and Non-Genetic groups. This change was reflected by small increases in RBC %EPA in both groups ($p=0.02$). Compared to the Non-Genetic group, participants in the Genetic group reported increased awareness of omega-3 terminology by the end of the study ($p\leq0.01$), found the dietary recommendations more useful ($p=0.03$), and rated cost as a barrier to omega-3 FA consumption less often (32% versus 61%).

Conclusion: This represents the first nutrigenetics intervention study to examine if personalized genetic information for *FADS1* could promote increased omega-3 FA intake. While genetic information did not appear to influence omega-3 FA intake (or corresponding omega-3 FA blood levels) in the short-term, this information did improve awareness regarding omega-3 FAs and minimize the notion of cost as a barrier to omega-3 FA consumption.

5.2 Introduction

The field of nutritional genomics, or nutrigenetics, aims to unravel the genetic basis for why individuals respond differently to the same nutrients and/or foods (210, 231, 243). The long-term outcomes of this research are expected to lay the foundation for personalized dietary recommendations to help prevent the development of chronic diseases. A more direct outcome of nutrigenetics research may simply entail the use of personal genetic information as an additional factor to help motivate people to adopt healthier dietary behaviors.

To date, the vast majority of nutrigenetics research has focused on the examination and assessment of the perceptions surrounding genetic and health information in various populations (202, 210, 295). When individuals were asked, many reported to be interested in undergoing genetic testing for the prevention of chronic diseases (21, 75, 142, 239). However, there are currently a limited number of randomized controlled nutrigenetic trials assessing the applicability of providing genetic information on changes in dietary behaviors. The few nutrigenetic intervention studies performed to date suggest that individuals who receive personal genetic information may make more changes to their diet compared to controls (7, 136, 238).

Omega-3 fatty acids (FAs) represent an ideal nutrient to examine in the context of a nutrigenetics intervention for several reasons. From a global health perspective, it is widely recognized that increased intake of omega-3 FAs, in particular eicosapentaenoic acid (EPA,

20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), is beneficial for cardiovascular, metabolic, developmental, and cognitive health (22, 83, 161). However, the consumption of EPA- and DHA-rich foods such as fatty fish is low, specifically within the Western diet (111, 184). Therefore, finding new ways to motivate people to increase their consumption of omega-3 FAs are necessary.

From a genetic perspective, EPA and DHA are endogenously produced to a limited extent through a well-characterized pathway that desaturates and elongates the essential omega-3 FA, alpha-linolenic acid (ALA, 18:3n-3). The fatty acid desaturase 1 and 2 genes (*FADS1* and *FADS2*) play critical roles in this pathway (219, 232). It has consistently been demonstrated that single nucleotide polymorphisms (SNPs) in the *FADS* genes influence the degree of endogenous conversion of ALA into EPA and DHA (290, 304). Specifically, minor allele carriers have reduced desaturase activity, resulting in lower levels of EPA (290, 304); however, providing more dietary ALA to minor allele carriers was shown to increase blood EPA to a level equivalent to that observed in major allele carriers (116). This suggests that giving individuals their personal *FADS* genotype may yield a new approach to encourage increased intake and optimize dietary recommendations for omega-3 FAs.

Providing personal genetic information in relation to omega-3 FAs in the context of a randomized intervention represents a novel area of investigation. Therefore, the objective of this study was to test the impact of providing personal genetic information for *FADS1* on the consumption of omega-3 FAs in a population of female adults over a 12-week period. We examined changes in EPA and DHA intake from foods and supplements, analyzed blood omega-3 FA levels, and assessed perceptions of nutrition and genetics.

5.3 Subjects and Methods

5.3.1 Participants and Ethics

Female adults (emerging adults between 18-25 yrs.) were recruited through email and poster advertisements displayed around the University of Guelph campus and throughout the city. Individuals were deemed ineligible if they regularly consumed omega-3 FA supplements and/or consumed fish more than two times per week. Pertaining to eligibility criteria, this included participants who reported having a regular menstrual cycle, and would expect their period (approximately) every 28 days. Baseline and Final (week-12) study visits were scheduled when the participants were menstruating to minimize variability in lipid levels within an individual, as recommended by Mumford *et al.* (230). Study design and participant flow through the study is reported using Consolidated Standards of Reporting Trials (CONSORT) guidelines (**Figure 1**). Ethical approval for the study was granted by the University of Guelph Human Research Ethics Board (REB#:15AP019).

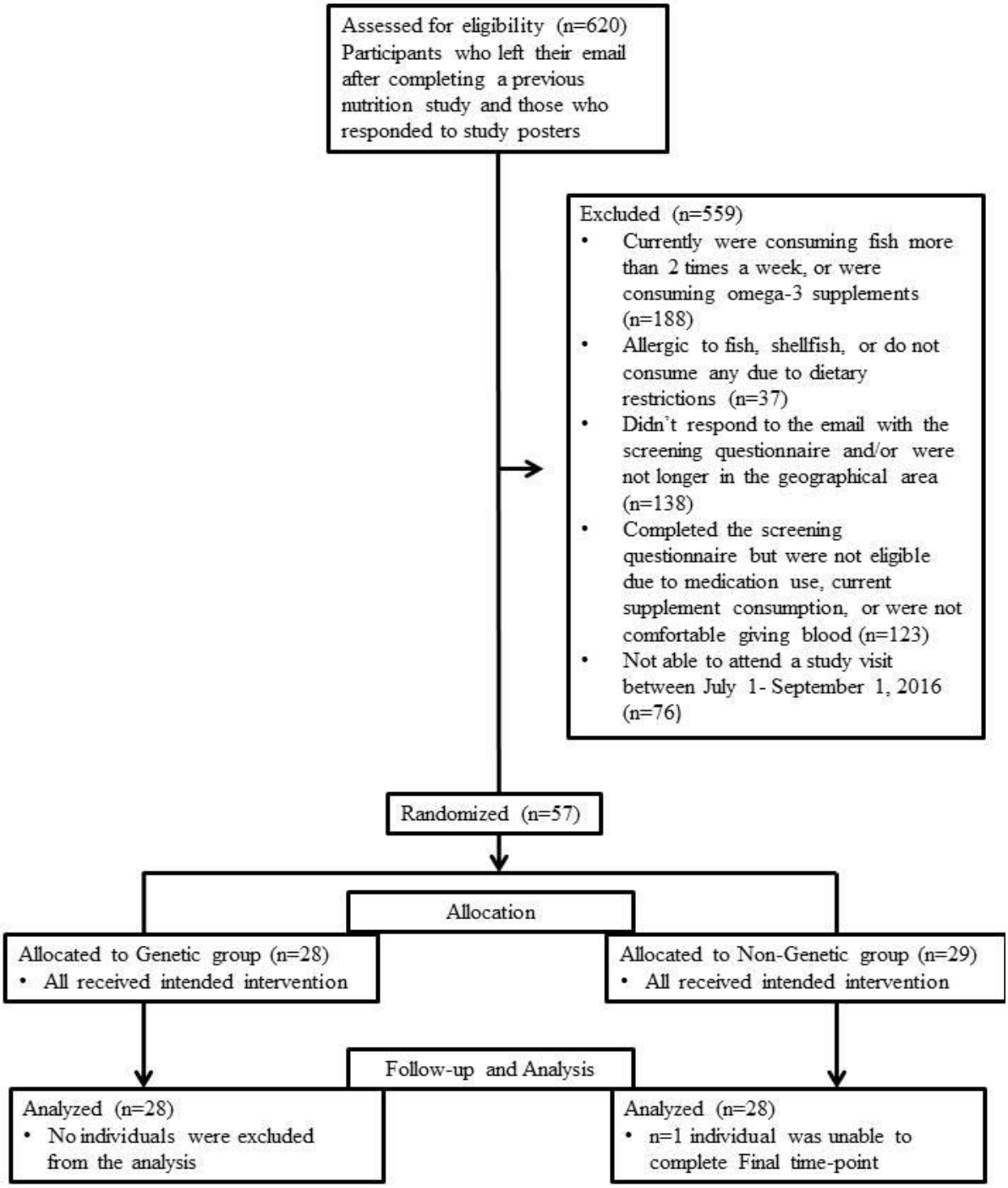

Figure 5.1: Study Flow Chart. CONSORT Guidelines were used for Reporting..

5.3.2 Study Design

Participants attended three study visits: 1) signing the consent form and saliva collection for DNA analysis; 2) Baseline blood draw visit, one-on-one meeting to discuss additional details about the study intervention, distribution of study intervention materials; and 3) Final blood draw visit (week-12). All participants were genotyped and their genetic information was used to randomize an equal number of major and minor allele carriers into control and intervention groups. Randomization of participants into one of two groups (Genetic or Non-Genetic) was completed using a random number generator by two individuals external to the study intervention. The lead investigator for the study intervention was blinded to this assignment.

At visit two, each participant had a meeting with the lead investigator and was provided information (verbal and written) regarding omega-3 FAs and genetic information about SNPs in *FADS1* (**Appendix 4**). Information shared about omega-3 FAs included general nutritional information, foods and supplements that have these FAs, and possible health effects associated with their consumption; compiled from the Dietitians of Canada fact sheets (54, 55). Participants were informed that they had the freedom to change their dietary intake if they chose to, although no dietary changes were required for participation in the study. Information shared about genetics highlighted the difference in omega-3 FA levels between major (GG) and minor (GT+TT) allele carriers for a common SNP in *FADS1* (rs174537). Specifically, we indicated that individuals who are GG allele carriers were reported to have an increased ability to convert ALA into EPA and DHA (31, 73, 203), while individuals who are GT or TT allele carriers were shown to have less EPA in their bodies and a reduced ability to convert ALA into EPA and DHA (290, 304). After the Baseline blood draw and one-on-one meeting, each participant was given a security-style sealed envelope with either their personal genetic information (if they were assigned to the Genetic

Group), or a blank page (if they were assigned to the Non-Genetic group), to open after their appointment.

Participants were contacted throughout the study for questionnaire distribution and appointment scheduling. Participants were not provided with additional omega-3 FA or genetic information throughout the intervention, unless sought out independently. There were multiple links to reputable websites provided, although we did not track how many individuals independently acquired additional information about omega-3 FAs or the genetic information related to *FADS1* and *FADS2* metabolism.

5.3.3 Online Questionnaires.

Participants completed online questionnaires at Baseline and Final (week-12) to assess dietary intake of omega-3 FAs. Data on the perceptions of receiving genetic information and overall perceptions of the study intervention was also assessed through online questionnaires at Final (week-12). Survey development was reported previously (Study 1 Methods). Qualtrics survey software (V13.28.05, ©2015, UT, United States) was used to host the online surveys.

5.3.3.1 Food Frequency Questionnaires. Dietary intake of omega-3 FAs (specifically EPA and DHA) was assessed at Baseline and Final (week-12) using a validated Canadian food frequency questionnaire (FFQ) (248) that was updated to be reflective of the omega-3 enriched foods currently available on the market (e.g., newer brands of eggs and spreads fortified with EPA and DHA). For each category of whole food, functional food, or supplement, the FFQ prompted specific product names/brands, frequency of consumption, and portion size consumed during the past week. The Canadian Nutrient File (version 2015) was used to assess the amount of EPA and DHA in whole foods (e.g., fish, eggs, poultry) (56). Researchers used food labels obtained from Internet searches and conducted visits to local grocery stores to confirm amounts of omega-3

FAs in functional foods and supplements enriched with EPA and DHA. Many food products do not distinguish between the amount of EPA or DHA, and thus EPA and DHA are represented together as a summation of both FAs. To reflect the current Canadian dietary recommendations for both EPA and DHA, the total daily intake of EPA and DHA was combined and calculated for each participant in this analysis.

5.3.3.2 Diet and Genetics Questionnaire. After the final study visit, participants completed a questionnaire regarding perceived dietary changes and perceptions of the study intervention (questions in **Appendix 5)** Questions that focused on general perceptions in nutrition and health were incorporated based on the work from the Canadian Behavior, Attitude and Nutrition Knowledge Survey (BANKS) (178). Questions regarding perceptions of genetic information included some questions from a nutrigenomics survey developed by Nielsen and El-Sohemy (237). We examined the participants' awareness of terminology used to describe omega-3 FAs, including the full scientific names (alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid) and their corresponding abbreviations (ALA, EPA and DHA), at the start and end of the intervention (Study 1 Methods).

5.3.4 Experimental Procedures

5.3.4.1 Genotyping. DNA was extracted from saliva using the Oragene DNA collection kit, according to manufacturer's instructions (DNA Genotek, Ontario, Canada). Participants were genotyped for the rs174537 SNP in *FADS1* using a validated TaqMan genotyping assay (Life Technologies, CA, USA). This SNP was first reported in a large genome wide association study (304), and has been consistently reported by both our lab (270, 271) and others (31, 82, 207, 290, 316) to influence blood FAs and estimated desaturase activity. Due to high linkage disequilibrium

within several SNPs in the *FADS1* and *FADS2* gene cluster, participants were only genotyped for rs174537.

5.3.4.2 Blood Collection. Participants arrived to the clinical trials unit (Human Nutraceutical Research Unit) after a 10-12 hour fast. Blood was collected by venipuncture by a trained phlebotomist. Blood samples were collected by venipuncture throughout the 20-week study period at baseline and weeks 2, 4, 6, 8, 12, 14, 16 and 20 (study timeline **Figure 4.1**). Serum blood samples were collected in yellow top vacutainers (BD Vacutainer, 5.0 mL, SST (serum separation tubes)) coated with silicone and micronized silica particles to accelerate clotting. Serum blood samples were used for the analysis of cardiometabolic markers and fatty acid analysis. Plasma and red blood cell (RBC) blood samples were collected in lavender top vacutainers (BD Vacutainer, 6.0 mL), containing EDTA (ethylenediaminetetraaeteic acid) as an anti-coagulant. RBC samples were used for fatty acid analysis. After the blood was collected, both types of vacutainer tubes were inverted three times and then left at room temperature for 30 minutes. Tubes were centrifuged for 15 minutes at 3000 rpm. The serum blood samples were kept in the fridge prior to pick-up from LifeLabs (same day). One serum sample and the plasma and RBC samples were aliquoted into Eppendorfs and put directly into the -80˚C freezer.

5.3.4.3 Gas Chromatography for FA Analysis. Serum and RBC blood samples were collected at each study visit. Serum samples were collected in separate tubes (as described above), and plasma and RBC were collected using the same tube (as described above). The current analysis was completed as previously described (218), with minor modifications. All solvents and reagents were obtained from Fisher Scientific (ON, Canada). Frozen serum and RBC samples were thawed on ice for approximately 1.5 h prior to extraction. After the addition of 10 μL (0.1 mg/mL stock) of an internal free FA standard (C17:0) (Sigma-Aldrich, USA), total lipids were

extracted from 100 μL of serum and/or 100 μL of RBC using chloroform:methanol 2:1 v/v. Samples were flushed with nitrogen and stored at 4˚C overnight. The next day, samples were centrifuged at 1460 rpm for 10 min. Pooled lipids were saponified using 2 mL of 0.5 mol/L KOH in methanol at 100°C for 1 h. The resulting free FAs were trans-esterified by the addition of 2 mL of 14% boron triflouride in methanol and 2 mL of hexane at 100 °C for 1.5 h. Double distilled water (2 mL) was added to stop the methylation reaction and samples were subsequently centrifuged for 10 minutes at 1000 rpm. The hexane phase was extracted, evaporated under nitrogen gas, and reconstituted in 100 μL of hexane for analysis. FA methyl esters were separated by gas chromatography using an Agilent 6890B gas chromatograph (Agilent Technologies, California, United States) with a Supelco SP 2560 fused-silica capillary column with flame ionization detector (100m x 0.25mm i.d., 0.2 μm film thickness; Sigma-Aldrich, USA). FA peaks were identified by comparison to retention times of FA methyl ester standards (developed and validated by a collaborating lab within the department). Chromatograms were analyzed individually and the areas for each FA were confirmed. Individual FAs are indicated as a percentage (%) of total FAs. Differences in % of specific FAs were examined between time points to examine changes relative to the supplementation. FA methyl esters were separated by gas chromatography using an Agilent 6890B gas chromatograph (Agilent Technologies, CA, USA). FAs of interest were reported as percent FA composition: ALA, EPA, DHA, linoleic acid (LA; 18:2n-6) and arachidonic acid (AA; 20:4n-6). The Omega-3 Index was calculated by adding %EPA and %DHA (129). FADS pathway activity was estimated by dividing AA/LA, as previously reported (31, 82, 218).

5.3.4.4 Clinical Measurements. Following an overnight fast, venous blood samples were collected from all participants at Baseline and Final (week-12) visits. Fasted serum samples were sent to LifeLabs Medical Laboratory Services (ON, Canada) for the analysis of triglycerides (TAG), total cholesterol, LDL-cholesterol (LDL-c) and HDL-cholesterol (HDL-c).

5.3.5 Statistics

R software (R Core Team, VIE, Austria) was used to determine a sufficient sample size prior to commencing the study. A minimum sample size of n=25 individuals per intervention group was calculated using changes in RBC %EPA reported in previous omega-3 FA intervention studies, and differences in RBC %EPA based on SNPs in *FADS1*. The %EPA values used to calculate the effect sizes were based on past work from our lab (271, 339) and others (5, 73). The p-value was set at ≤ 0.05 and power (β) was set at 80%.

Intention to treat analysis was implemented (CONSORT), where participants remained in their original intervention groups throughout data analysis. FFQ and questionnaire data was analyzed using SPSS (IBM Corporation, Version 23, NY, USA). Descriptive statistics were completed for all questions to determine trends and potential question issues before individual question analysis. Independent t-tests were used to determine differences in mean intake of EPA and DHA (based on FFQ data) between the Genetic and Non-Genetic groups at Baseline. A two-way analysis of variance (ANOVA) was used to analyze dietary intakes of EPA and DHA using Group (Genetic versus Non-Genetic), Time (Baseline versus Final), and the Group × Time interaction. The major (GG) and minor (GT+TT) allele carriers within the Genetic group were also compared to determine any differences related to the genetic information provided. A two-way ANOVA was used to analyze the awareness of omega-3 terminology, using Group and Time effects as described above. Pearson's χ^2 tests were used for questions requiring a

determination of difference in proportions between the Genetic and Non-Genetic groups. An independent samples t-test was used to determine differences in the means between the Genetic and Non-Genetic groups when analyzing Likert-style questions (scale from 1-7). In addition, GraphPad Prism V6 (GraphPad Software, CA, USA) was used to evaluate differences in anthropometric, clinical, and FA data at Baseline with a two-way ANOVA, as well as over the intervention, using Group, Time and Group × Time effects as described above. Outliers within the clinical and FA data were identified using a robust regression and outlier removal (ROUT) analysis in GraphPad. A $p \leq 0.05$ was considered statistically significant. R software was used for correction of multiple comparisons using the Benjamini Hochberg approach (23, 24).

5.4 Results

5.4.1 Participant Characteristics

The age of participants ranged from 19-25 yrs. (mean=22.0±1.5 yrs.). On average, participants rated interest in their personal health as 8.5±1.3 out of a possible score of 10. Ethnicity, school or employment status, field of study/area of work, and contraceptive use were not different between the Genetic and Non-Genetic groups (**Table 5.1**).

Table 5.1. Demographics of the Genetic and Non-Genetic groups at Baseline.

	Genetic (n=28)	Non-Genetic (n=29)	χ^2 p-value
Genoptype FADS1 (rs174537)			
Major (GG)	13/28 (46.4%)	13/29 (44.8%)	0.79
Minor (GT+TT)	15/28 (53.6%)	16/29 (55.2%)	
Field of study / area of work			
Life Science	19/28 (67.9%)	21/29 (72.4%)	0.46
Social Science	7/28 (25.0%)	4/29 (13.8%)	
Other	2/28 (7.1%)	4/29 (13.8%)	
Current Position			
Undergraduate student	20/28 (71.4%)	19/29 (65.5%)	0.36
Graduate student	7/28 (25.0%)	8/29 (27.6%)	
Working full-time	1/28 (3.6%)	2/29 (6.9%)	
Ethnicity			
White/Caucasian	18/28 (64.3%)	22/29 (75.9%)	0.47
Asian	4/28 (14.3%)	3/29 (10.3%)	
European	4/28 (14.3%)	1/29 (3.5%)	
Other	2/28 (7.1%)	3/29 (10.3%)	
Contraceptive use			
Not taking contraceptives	8/28 (28.6%)	9/29 (31.0%)	0.89
Oral contraceptive	17/28 (60.7%)	18/29 (62.1%)	
IUD contraceptive	3/28 (10.7%)	2/29 (6.9%)	

N=57 participants completed Baseline measurements and questionnaires. This data is reported as proportions (n/n total) and percentages (%). Percentages are provided in parantheses and are reported as a percentage of either the Genetic or the Non-Genetic group. Pearson's χ^2analysis were conducted to determine a difference in the proportions between the Genetic and Non-Genetic groups for each parameter. $p \leq 0.05$ was considered statistically significant. IUD, intra-uterine device.

All participants were genotyped for rs174537 in *FADS1*. Our population consisted of 26 homozygous major allele (GG), 23 heterozygous (GT), and 8 homozygous minor allele (TT) carriers (**Table 5.1)**. The SNP was in Hardy-Weinberg equilibrium. Due to the low number of minor allele carriers, we combined individuals with GT and TT genotypes into a single group for analysis. Genotypes were evenly and randomly divided between the intervention and control groups.

5.4.2 FFQ Analysis

Total daily intake of EPA and DHA was combined and calculated for each participant as many food products didn't specify between EPA and/or DHA and listed both FAs together (55, 56, 120). At baseline, on average, participants were consuming an average of 200 $\pm$ 29 mg EPA and DHA/day. There were no significant differences in EPA and DHA intake between Genetic and Non-Genetic groups at Baseline **(Table 5.2)**. As indicated by the Group × Time interaction, the nutrigenetic intervention did not differentially influence dietary intake of EPA and DHA (p=0.42) **(Table 5.2)**; however, both groups significantly increased their intake of EPA and DHA during the intervention ($p=5.0x10^{-3}$).

The Genetic group was further investigated to determine if there were any differences in EPA and DHA consumption when stratified according to *FADS1* genotype. Dietary intake of EPA and DHA tended to be higher in GT+TT individuals (272 ± 71 mg/day) than GG individuals (141 ± 38 mg/day) at Baseline (p=0.06). At Final (week-12), dietary intake of EPA and DHA was higher in GT+TT individuals (432 $\pm$ 112 mg/day; ~59% higher than Baseline) than GG individuals (198 $\pm$ 34 mg/day; ~40% higher than Baseline) (p=0.08). However, the Group × Time interaction revealed there was no significant difference in EPA and DHA intake between GG and GT+TT individuals at Final (week-12) (p=0.29).

There were 10/56 (~18%) participants (4 in the Genetic Group and 6 in the Non-Genetic Group) who chose to start taking supplements after the first study visit and who continued taking these supplements up to week-12. Out of these 10 participants, 9 participants took fish oil supplements and 1 participant took an algal supplement. These participants were amongst those with the highest EPA and DHA intakes measured with the FFQ at Final (week-12).

5.4.3 FA Analysis

There were no differences between the Genetic and Non-Genetic groups at Baseline for %ALA, %DHA or the Omega-3 Index (**Table 5.2**).%EPA was higher in the Non-Genetic group compared to the Genetic group at Baseline. At the end of the study intervention, %EPA increased similarly in both groups (p=0.02) (**Table 5.2**). The Omega-3 Index also showed a significant Time effect. The Group × Time interaction analysis revealed the nutrigenetic intervention had no differential effect on these FAs (**Table 5.2**).

We also examined the effect of rs174537 on estimated FADS pathway activity by calculating the ratio of AA/LA at Baseline. In the Genetic group, GG allele carriers had higher desaturase activity (0.98±0.03) compared to the GT+TT allele carriers (0.84±0.15; p=0.01). In the Non-Genetic group, GG allele carriers also had higher desaturase activity (0.93±0.14) compared to the GT+TT allele carriers (0.86±0.08; p=0.08).

5.4.4 Clinical Blood Lipid Analysis

There were no differences between the Genetic and Non-Genetic groups at Baseline for any of the clinical parameters measured (**Table 5.2**). As indicated by the Group × Time interaction, the nutrigenetic intervention did not differentially affect any of the parameters between the two groups (**Table 5.2**). However, both groups experienced small increases in Total cholesterol, the Chol/HDL ratio, LDL-c and Non-HDL from Baseline to Final (**Table 5.2**). The change in the Chol/HDL ratio was not significant after correction for multiple comparisons.

5.4.5 Diet and Genetics Questionnaires

5.4.5.1 Awareness of Omega-3 FA Terminology. We examined the participants' awareness of terminology used to describe omega-3 FAs, including the full scientific names (alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid) and their corresponding abbreviations (ALA, EPA and DHA), at the start and end of the intervention. Overall, the Genetic and Non-Genetic groups were well matched for their awareness of omega-3 terminology at Baseline (**Table 5.2, Appendix 5 Q1**). A Group effect was observed regarding the awareness of docosahexaenoic acid, where more individuals in the Genetic group said they were familiar with the term compared to the Non-Genetic group at Final (**Table 5.2**). However, this difference was no longer significant after correction for multiple comparisons. Awareness of both abbreviations and full scientific names increased in both groups during the intervention and remained significant after correction for multiple testing (**Table 5.2**). Lastly, Group × Time interactions were observed for both the ALA and EPA abbreviations, with individuals in the Genetic group reporting greater awareness of these terms compared to the Non-Genetic group.

Table 5.2: Characteristics of the Genetic and Non-Genetic Groups at Baseline and Final.

	Genetic (n=28)		Non-Genetic (n=28)		Group p-value	Time p-value	Group×Time Interaction p-value
	Baseline	Final	Baseline	Final			
Omega-3 Dietary Intake							
EPA and DHA (mg/day)	211.50 ± 43.16	323. 23 ± 65.27	190.16 ± 39.21	395.82 ± 70.60	0.63	**$5.0x10^{-3}$**	0.42
RBC fatty acid data							
ALA (%)	0.43 ± 0.02	0.44 ± 0.02	0.41 ± 0.02	0.42 ± 0.02	0.66	0.14	0.79
EPA (%)	0.45 ± 0.02	0.51 ± 0.03	0.55 ± 0.02	0.61 ± 0.04	**$3.9x10^{-3}$***	**0.02**	0.89
DHA (%)	3.40 ± 0.11	3.42 ± 0.09	3.42 ± 0.11	3.54 ± 0.11	0.64	0.20	0.36
Omega-3 Index	3.86 ± 0.11	3.97 ± 0.11	3.97 ± 0.12	4.15 ± 0.13	0.34	**0.04**	0.66
Clinical Data							
BMI (kg/m^2)	23.01 ± 0.60	22.87 ± 0.61	23.41 ± 0.50	23.55 ± 0.53	0.50	0.99	0.11
TAG (mmol/L)	0.90 ± 0.07	1.02 ± 0.08	1.02 ± 0.07	1.02 ± 0.06	0.24	0.06	0.06
Cholesterol (mmol/L)	4.40 ± 0.19	4.67 ± 0.20	4.43 ± 0.14	4.72 ± 0.14	0.87	**$2.0x10^{-4}$***	0.85
HDL (mmol/L)	1.76 ± 0.06	1.75 ± 0.07	1.75 ± 0.09	1.78 ± 0.09	0.94	0.66	0.48
Chol/HDL ratio	2.58 ± 0.13	2.70 ± 0.11	2.67 ± 0.12	2.77 ± 0.12	0.60	**0.04**	0.81
LDL (mmol/L)	2.18 ± 0.15	2.31 ± 0.14	2.22 ± 0.13	2.47 ± 0.12	0.58	**$2.6x10^{-3}$***	0.32
Non-HDL Chol	2.65 ± 0.18	2.88 ± 0.18	2.68 ± 0.13	2.94 ± 0.12	0.83	**$2.0x10^{-4}$***	0.92
Questionnaire Data							
Alpha-linolenic acid	16/28 (57.1%)	23/28 (82.1%)	18/29 (62.1%)	20/28 (71.%)	0.73	**$2.0x10^{-3}$***	0.33
Eicosapentaenoic acid	12/28 (42.9%)	22/28 (78.6%)	12/29 (41.4%)	13/28 (46.4%)	0.08	**$1.0x10^{-3}$***	0.07
Docosahexaenoic acid	14/28 (50.0%)	22/28 (78.6%)	12/29 (41.4%)	14/28 (50.0%)	**0.04**	**$5.0x10^{-3}$***	0.21
ALA	12/28 (42.9%)	23/28 (82.1%)	19/29 (65.5%)	20/28 (71.4%)	0.71	**0.01***	**0.04**
EPA	15/28 (53.6%)	23/28 (82.1%)	20/29 (69.0%)	20/28 (71.4%)	0.86	**0.01***	**0.05**
DHA	19/28 (67.9%)	25/28 (89.3%)	19/29 (65.5%)	22/28 (78.6%)	0.46	**0.01***	0.49

For the clinical and FA data, values represent mean ± SEM. The questionnaire data is represented as the proportion of participants who answered "yes". Percentages are provided in parentheses. A 2-way ANOVA was used to evaluate the independent effects of Group (Genetic versus Non-Genetic) and Time (Baseline versus Final), as well as the Group×Time interaction. p-values <0.05 are shown in bold. Clinical, FA and questionnaire data were adjusted for multiple comparisons using a Benjamini Hochberg correction. Values significant after correction for multiple testing using are indicated with a *. Clinical and questionnaire data (n=56), FA data (n=55). ROUT outlier analysis removed 4 individuals from the TAG data, 1 individual from Cholesterol, %EPA, and %ALA data. The question associated with omega-3 terminology data can be found in Appendix 5 Q1.

5.4.5.2 Perceptions and use of Nutritional Information. Overall, there was a significant difference in perceptions of the study intervention between the Genetic and Non-Genetic groups at Final (week-12) (**Table 5.3, Appendix 5 Q2**). Specifically, the Genetic group reported to agree more strongly that the dietary recommendations were knew to them (p=0.05) and that the dietary recommendations were useful when they considered their diet throughout the study (p=0.03), in comparison to the Non-Genetic group. There were no significant differences between groups for the other statements related to use of the nutritional information (**Table 5.3**).

Table 5.3: Rating of Selected Statements Regarding the Study Intervention.

	Genetic (n=28)	**Non-Genetic (n=28)**	
	Average	**Average**	**p-value**
I understood the nutrition information about omega-3 fats provided at the start of the study	6.21±0.26	6.21±0.23	1.0
The recommendations about omega-3 fats that were provided in the document at the start of the study were new to me	4.64±1.75	3.71±0.31	**0.05**
I enjoyed learning about the dietary recommendations related to omega-3 fats	6.14±1.18	5.89±1.07	0.41
The dietary recommendations were useful when I considered my diet throughout the study	5.68±1.21	4.93±1.25	**0.03**
When I am in the grocery store or supplement store, I can confidently determine foods that have been fortified, or have added EPA and DHA omega-3 fats	5.43±1.48	5.32±1.19	0.77
I would like to know more about the dietary recommendations related to omega-3 fats	5.64±1.47	5.39±1.47	0.53
I am interested in the relationship between diet and genetics	6.12±1.45	6.61±0.79	0.11

These questions were asked in the Final study questionnaire. Participants were asked to indicate on a scale from 1-7, how much they disagreed (strongly disagreed = 1) or agreed (strongly agreed = 7) with the corresponding statements (4 was neutral). The average for each question is represented as mean ± SEM. An independent samples 2-sided t-test was used to determine differences between Genetic and Non-Genetic groups. A $p<0.05$ was considered statistically significant and is indicated in bold font. The question and answer options associated with this data can be found in Appendix 5 Q2.

5.4.5.3 Perceived Dietary Changes. At Final (week-12), we asked participants to self-report if they had made changes to their consumption of omega-3 foods, fortified products, or supplements over the course of the study (Appendix 5 Q3-Q6). When asked about consumption of omega-3 foods, there were 8, 5 and 15 individuals in the Genetic Group, and 6, 9 and 13 in the Non-Genetic group who said "yes", "no" and "sometimes" to making changes to overall omega-3 consumption in their diet, respectively. Participants who said "yes" or "sometimes" were specifically asked about the factors contributing to their dietary changes.

There were 8/28 (28.6%) in the Genetic Group and 5/28 (17.9%) in the Non-Genetic group who reported that the reason for their dietary changes were related to the general nutritional information provided to them at the start of the study (**Appendix 5 Q7)**. Additionally, there were 5/28 (17.9%) individuals in the Genetic group who reported that their personal genetic information was the reason they made changes to their diet. Overall, the proportion of responses for reasons to change dietary intake was significantly different ($p=0.05$) between the Genetic and Non-Genetic groups.

We also asked participants to identify obstacles that may have influenced their ability to change their omega-3 dietary habits (**Table 5.4, Appendix 5 Q8**). Response rates between the Genetic and Non-Genetic group were not statistically different (**Table 5.4**), although there was a trend that fewer individuals in the Genetic Group reported that "*Omega-3 foods are expensive*" compared to the Non-Genetic group.

Table 5.4: Obstacles or Barriers to Change Diet and Omega-3 FA Consumption.

	Genetic (n=28)	Non-Genetic (n=28)	χ^2 p-value
Omega-3 foods are expensive	9/28 (32.1%)	17/28 (60.7%)	0.17
When I get busy I don't make time to eat healthy foods	8/28 (28.6%)	4/28 (14.3%)	
I didn't experience any barriers to change throughout this study	3/28 (10.7%)	1/28 (3.6%)	
Other obstacles / barriers[#]	8/28 (28.6%)	6/28 (21.4%)	

These questions were asked at the end of the study in the Final questionnaire. The data is represented as the proportion of participants who answered "yes" to that answer option. This data is reported as proportions (n/n total) and percentages (%). Percentages are provided in parentheses and are reported as a percentage of either the Genetic or the Non-Genetic group. Pearson's Chi-squared analysis (χ^2) was conducted to determine a difference in the proportions between the Genetic and Non-Genetic groups. $p \leq 0.05$ was considered statistically significant.

The question associated with the data can be found in Appendix 5 Q8.

Other obstacles / barriers[#] : It is difficult for me to get to a grocery store (Genetic n=1, Non-Genetic n=0); I am not involved in the grocery shopping in my home (Genetic n=1, Non-Genetic n=0); I eat most of my meals away from home (Genetic n=0, Non-Genetic n=1); I have an allergy to an omega-3 containing food (Genetic n=1, Non-Genetic n=1); I do not buy fortified products (Genetic n=1, Non-Genetic n=1); I do not have time to cook foods high in omega-3 fats (Genetic n=2, Non-Genetic n=1); I do not like fish (Genetic n=1, Non-Genetic n=1); I do not like taking supplements (Genetic n=1, Non-Genetic n=1).

5.5 Discussion

This study represents one of the first nutrigenetics interventions reported in the literature that focuses more on promoting healthy diet behaviours rather than addressing health risks. Our study highlighted the possible health benefits of increased omega-3 FA consumption, rather than the risks related to low intake. Additionally, our investigation wanted to move away from fear-based messaging to increase motivation for a healthy diet, as opposed to potential anxiety upon receiving genetic information. As this study was one of the first benefit-related studies conducted, we wanted to examine the effects of providing individuals with their personal *FADS1* genetic information on the consumption of omega-3 FAs.

We found that in both the Genetic and Non-Genetic groups, intake of EPA and DHA was increased by the end of the study and this was reflected by increases in RBC %EPA and minimal increases in %DHA. Both the Genetic and the Non-Genetic groups were meeting the minimum

dietary reccommendation of 300 mg EPA and DHA/day (according to the Dietitians of Canada) by the end of the study. Providing individuals with their personal *FADS1* genetic information did not lead to significant differences in dietary intake or blood levels of omega-3 FAs compared to controls. However, there were some encouraging signs suggesting that providing individuals with genetic information increased their awareness of omega-3 FAs, rendered the omega-3 nutritional information more useful in the context of their genetic information, and minimized barriers to the consumption of omega-3 FAs.

Our FFQ analysis revealed that Baseline intake of EPA and DHA was ~200 mg/day in our study participants, which is slightly higher than recent global reports of EPA and DHA intake (111, 184, 294), although still lower than the minimum recommendations by Dietitians of Canada (54, 241). Individuals in both the Genetic and Non-Genetic groups increased their EPA and DHA intake during the study, suggesting that providing general nutritional information related to omega-3 fats was sufficient to motivate increased EPA and DHA consumption in emerging female adults. This increased dietary intake was reflected by increased %EPA in RBCs. Interestingly, the GT+TT minor allele carriers appeared to increase EPA and DHA consumption to a greater extent (59%) than GG major allele carriers (40%). No major changes were observed in clinical markers between the Genetic and Non-Genetic groups. Therefore, at least in the context of a 12-week intervention, personal *FADS1* genetic information did not appear to significantly impact omega-3 FA intake.

Our findings regarding the limited impact of providing personal genetic information on dietary behavior appears to conflict with two previous investigations; however, important differences exist between our research and these previous studies. For example, Hietaranta-Luoma *et al.* gave adults (n=107, 20-67 yrs., 69% female) information about their risk for

cardiovascular disease (CVD) in relation to their personal apolipoprotein E (*APOE*) genetic make-up (136). Individuals with the highest risk for CVD showed the greatest improvements in fat quality in their diets (136). However, our study focused on benefits to health rather than risk reduction; thus it may be plausible that individuals may alter their behavior more substantially if they feel it will reduce the risk for disease instead of potentially improving health. Further, it is interesting to speculate that according to the reported psychological behavior of emerging adults, most of these individuals are not considering their future health (8, 19, 110). Additionally, given the age of these participants, it is possible that their parents may not have experienced any lifestyle related symptoms, conditions, or diseases in their lifetime (110). In another study, Arkadianos *et al.* provided genetic information related to the Mediterranean diet to participants (n=93, 46 ± 12 yrs., 43% women) enrolled in a weight loss program (7). After 100 days, there were no differences between the Genetic and Non-Genetic groups; however, after ~300 days, 57% of the Genetic group maintained weight loss compared to 25% who maintained weight loss in the Non-Genetic group (7). Therefore, it is possible that if we continued our investigation over a longer period of time, the impact of personal *FADS1* genetic information on omega-3 intake may have been more pronounced compared to the Non-Genetic group.

While we did not see differences in omega-3 FA intake between the Genetic and Non-Genetic groups, it is interesting to note that participants in the Genetic group rated the nutritional information we provided as new and useful more often than those in the Non-Genetic group. This aligns with findings by Nielsen and El-Sohemy, who found that young adults who received personal genetic information related to four dietary components (caffeine, vitamin C, sodium and sugar) reported to have a greater understanding and utility for the dietary advice provided to them (237). Additionally, we show that participants in the Genetic group of our study reported

greater awareness of omega-3 terminology after the intervention, specifically with regards to the ALA and EPA abbreviations. Similarly, when asked about barriers to omega-3 FA consumption, 60% of the Non-Genetic group rated that *"omega-3 foods are expensive"* compared to 32% of the Genetic group. Interestingly, this suggests that having personal genetic information could change attitudes about the value of healthy eating. Thus, greater awareness and a reduced perception of "cost" as a barrier to omega-3 intake may render these individuals more likely to choose foods with omega-3 FAs in the future. Although these findings were not significant after accounting for multiple testing, this is not unexpected given the stringency associated with this statistical test. Rather, this highlights the need to replicate these encouraging findings in a larger study population. Increasing the length of the study would also provide insight into the long-term impact of personal genetic information on awareness and barriers pertaining to omega-3 FAs.

The present study has some limitations that warrant consideration. First, the current study only included female participants who were primarily of European descent. Gender and ethnicity may influence dietary behavior changes upon receiving personal genetic information; therefore, a more diverse participant population is needed in future studies. Second, our population consisted solely of well-educated emerging adults. Future studies should examine the role of personal *FADS1* genetic information on dietary behavior changes in different subgroups of the population, such as those with health problems and those outside academia. Third, the FFQ used in this study was validated for EPA and DHA (248), but not ALA. Future studies should create an updated FFQ to add ALA-rich foods, fortified products, and supplements in order to better estimate the consumption of this important omega-3 FA. Having an estimation of ALA intake may provide more insight into differences in consumption patterns between individuals stratified according to their *FADS1* genotype. Fourth, increasing the sample size and expanding the analysis to other

age groups would enable a more powerful exploration of personal *FADS1* genetic information. Finally, additional studies to more thoroughly assess the qualitative effect of genetic information (i.e., perceptions, reactions, emotions) using focus groups are warranted.

5.6 Conclusions

The present study represents the first of its kind to explore the provision of *FADS1* genetic information and subsequent changes to omega-3 FA intake. While we found little evidence that this genetic information affected EPA and DHA intake and circulating blood levels in a group-dependent manner, we did find that individuals who received their genetic information had greater awareness of omega-3 terminology, rated cost as a barrier to omega-3 consumption less often than those in the control group, and found their genetic information to be more useful in the context of general information pertaining to omega-3 FAs. Therefore, providing personal genetic information to emerging adults may provide an additional factor to help motivate behavior changes in the consumption of omega-3 FAs.

CHAPTER 6: INTEGRATIVE DISCUSSION

6.1 Study Summaries

The overall aims of this thesis were to investigate the perceived and actual health benefits associated with omega-3 FA intake in a population of emerging adults, and then to evaluate whether this could be influenced with personal *FADS1* genetic information.

These aims were addressed by conducting an online survey and two clinical trials. Study 1 used an online survey to evaluate awareness of omega-3 FAs and their health effects, Study 2 examined the health benefits of omega-3 FAs and the influence of SNPs in *FADS1* and *FADS2* in a clinical trial, and Study 3 assessed changes in dietary intake of omega-3 FAs and perceptions of receiving personal *FADS1* genetic information in a nutrigenetics intervention.

6.1.1 Study 1 Summary (Chapter 3)

Study 1 found differences in awareness between omega-3 terminologies. Specifically, emerging adults were more aware of the EPA and DHA abbreviations than their corresponding full scientific names. People working in the biological and / or physical sciences were more aware of omega-3 FAs and their associated health benefits compared to those working in social sciences and other fields. In addition, most emerging adults knew about the links between EPA and DHA with heart and brain health. Although emerging adults reported a high level of awareness of the health effects, there were only 48% and 21% who self-reported to consume omega-3 foods and EPA and DHA supplements, respectively. Therefore, this study identified that there is a weak connection between awareness, knowledge, and behavior of emerging adults. Lastly, source of information was associated with awareness of health effects related to EPA and

DHA consumption. Social media, academic and/or reputable sources, and HCPs may be used as critical information channels to deliver accurate, evidence-based information about nutrition and health.

The completion of Study 1 addressed the awareness about omega-3 FA terminology and health effects. Next, we wanted to determine what the actual health effects were when emerging adults were supplemented with a moderate dose of fish oil. Additionally, the impact of genetic variation in *FADS1* and *FADS2* on FA levels and estimated FADS pathway activity were investigated.

6.1.2 Study 2 Summary (Chapter 4)

In Study 2, we found that moderate daily fish oil supplementation reduced levels of circulating TAG in emerging adults over a 12-week period. However, once the supplementation was completed, TAG levels returned to baseline levels. Therefore, increased EPA and DHA intake should be a lifestyle habit that is sustained, and supplementation should not stop once TAG levels have been reduced. In relation to changes in FA levels, our results showed that eight weeks after supplementation was finished, circulating EPA and DHA levels were still significantly elevated compared to baseline. Therefore, washout periods, particularly in cross-over studies using FA treatments, need to be longer than eight weeks to ensure FAs have returned to baseline levels. Importantly, we also found that minor allele carriers for SNPs in *FADS1* and *FADS2* had lower EPA levels compared to major allele carriers, which is consistent with previous reports. Minor allele carriers increased their EPA and DHA levels to a greater extent than major allele carriers by the end of the supplementation period.

These results highlight the potential for personalized dietary recommendations related to omega-3 FAs based on SNPs in *FADS1* and *FADS2*. Next, we examined the use of nutrition information about omega-3 FAs in combination with genetic information about a common SNP in *FADS1* to create a personalized nutrigenetics intervention.

6.1.3 Study 3 Summary (Chapter 5)

Study 3 was the first intervention study to deliver genetic information about a SNP in *FADS1* to emerging adults. Overall, we found that emerging adults were eager to receive their genetic information. All participants increased their intake of EPA and DHA according to FFQs, and modestly increased circulating EPA levels by the end of the 12-week study, with no significant differences between the Genetic and Non-Genetic groups. Importantly, on average, participants moved from being below the Dietitians of Canada recommended intake (<300 mg EPA and DHA/day), to being just at or over this recommendation by the end of the study. While we found little evidence that genetic information affected EPA and DHA intake and circulating blood levels in a group-dependent manner, we did find that individuals in the Genetics group had greater awareness of omega-3 terminology, compared to the Non-Genetic group. Additionally, participants informed of their genetic information rated cost as a barrier to omega-3 consumption less often, and found their genetic information to be more useful in the context of general information pertaining to omega-3 FAs, compared to the Non-Genetic group. Therefore, providing personal genetic information to emerging adults may provide an additional factor to help motivate behavior changes in the consumption of omega-3 FAs.

6.2 General Discussion

6.2.1 The FADS Pathway

6.2.1.1 Measurement and Estimation of FADS Pathway Activity

Desaturase enzymes are predominantly active in the liver; however, obtaining liver biopsies to measure FADS pathway activity is not feasible in human clinical trials. As a surrogate, measurement, FADS pathway activity is commonly estimated by calculating the ratio between product:precursor FAs in the FADS pathway. Discussion about actual FADS pathway activity and its correlation to estimated FADS pathway activity is important in order to appreciate the body of research in this area.

The early work reporting estimated FADS pathway activity showed a good correlation between estimated FADS pathway activity in blood and tissue (319), as well as with disease states (321), which likely encouraged the use of this estimate in subsequent studies. A comparison between estimated FADS pathway activity and FADS pathway activity measurements using tracer studies was completed by Gillingham *et al.* (116). A stable isotope ([*U*-^{13}C] ALA) was orally ingested to measure the fate of ALA and the amount of tracer measured in FADS pathway activity conversion products, EPA and DHA (37, 46, 47). The results showed that all estimates of FADS pathway activity (D5D, D6D and aggregate) were positively correlated with plasma ^{13}C-EPA concentrations (116). Therefore, given the methods used to date, the correlations between estimated and actual FADS activity seem to be well correlated.

The first studies to examine FADS pathway activity were completed with oral stable isotope ALA-tracers, which provided important insights regarding the endogenous fate of ALA (36, 44).

There is consensus in the literature that there is a relatively low level of conversion from ALA to EPA (0.2-8%), and even lower conversion from ALA to DHA (~1%) (46, 47, 251). Since these initial oral stable isotope studies, Domenichiello *et al.* recently (2015) estimated FADS pathway activity using steady-state infusion of stable isotope-labeled ALA in rats, to re-investigate FADS pathway activity (92). Although this work was conducted in the rat brain, their findings suggest that conversion of ALA to DHA may be three-fold higher than previously reported (92). These newly developed methods will enrich our understanding of the conversion of ALA into EPA and DHA. However, it is imperative that FADS pathway activity levels are also measured in the liver and whole body, in order to determine the reliability of these brain estimates (92). If a higher FADS pathway activity is also uncovered in other tissues through this new methodology, then human studies using the calculation of estimated FADS pathway activity need to consider how to apply these recent findings. In relation to this thesis, it is too early to predict how these recent studies measuring FADS pathway activity in rat brains relate to human conversion rates and more specifically, how estimated FADS pathway activity using human blood samples reflects actual desaturation activity in the tissues.

The estimated FADS pathway activity has been used for many yrs. as a way to avoid invasive measurements in human subjects (31, 207). As the estimated FADS pathway activity is calculated the same way each time, it is useful for comparisons between studies. In the clinical trials conducted in this thesis, these estimates were calculated to determine the influence of SNPs in *FADS1* and *FADS2* on FADS pathway activity and FA levels in the blood. In other work in the literature, SNPs in *FADS1* and *FADS2* have also been associated with estimated FADS pathway activity in blood in adults with CVD (207) and adolescents (31). The studies in this thesis align with these previous reports (31, 207), where minor allele carriers had lower D5D

activity and higher D6D activity compared to major allele carriers. However, not all studies report consistent findings, where previous work has indicated that an individual's background diet and disease state may contribute to variability in the estimates of FADS pathway activity (39, 259). Consideration of factors such as background diet and disease state should be further examined in future work.

Overall, more work in this field is needed, as the FADS pathway plays a fundamental role in FA metabolism, health and disease. In ALA-tracer studies reported in the past, there were variable conversion rates between males and females (42, 45, 98). Due to this possible discrepancy, it would be important to include both males and females in future tracer work examining FADS activity. Additionally, variation in FADS pathway activity and FA levels is also influenced by age (45, 153, 257, 340), thus including individuals within a specific age range, or stratifying by age for analysis would reduce possible variability in the results. Moving forward, unravelling the various factors that influence FADS pathway activity in humans, as well as re-visiting the conversion of ALA into EPA and DHA using the new steady-state stable isotope methods, are critical.

6.2.1.2 Dietary Intake and Molecular Contributions to FADS Pathway Activity

In the previous section, estimated FADS pathway activity and its implications were discussed. Within the literature review, factors such as age, sex, and genetic variation were reviewed regarding their impact on FADS pathway activity (163, 290, 319). Dietary intake is another important factor which could contribute to the FADS pathway activity, where studies have shown that decreased PUFA intake results in increased FADS pathway activity and vice versa (66, 290).

In addition to factors such as dietary intake, at a molecular level, the majority of desaturase activity occurs when the enzyme is in the endoplasmic reticulum and its activity is dependent on non-heme iron (65, 66). Applying this molecular knowledge to dietary intake, it could be possible that an individual who is iron deficient may have lower FADS pathway activity and may endogenously produce less EPA and DHA. Without the measurement of iron status, it is possible that some participants in clinical trials, including those reported in this thesis, were iron deficient, which could introduce variation into results and reduce power to detect meaningful associations. We asked participants to maintain their regular lifestyle habits, thus we can assume that intake levels of various nutrients (including iron) remained relatively constant throughout the investigation. It is possible that within Study 3, in order to increase intake of EPA and DHA, individuals may have added or replaced certain foods in their diet, with food or products high in omega-3 FAs. It could be possible that while adding more fish to their diet, they may have removed other animal proteins, including red meat which is a rich source of dietary iron. Additional questions incorporated to a nutritional questionnaire to ask about dietary addition and/or substitution could be valuable in future studies to understand how dietary advice impacts diet composition and nutrient intake.

Work in 1987 reported a mild impairment in essential FA metabolism, with lower FADS pathway activity and lower levels of long-chain PUFAs like EPA, DHA, and AA in moderately iron-deficient rats (77). One human study in 2013 supplemented women (n=21, ~24.6 yrs.) with iron, measured plasma FA levels and estimated FADS pathway activity and reported that there was a positive correlation between serum ferritin and estimated D6D activity (335).

The question of iron status could be examined from a molecular perspective, i.e. how do low, moderate and high iron levels affect FADS pathway activity; and from a clinical perspective,

where iron status could be tested at the beginning of the intervention and participants could be stratified by iron status for analysis; or iron supplementation could be given along with a fish oil supplementation. In summary, future work should consider iron status as an additional component to omega-3 clinical trials, and / or re-analyze existing samples for examination of a connection between iron status, FA levels, and FADS pathway activity.

6.2.2 Considerations in Genetic Testing

6.2.2.1 Interest and Participation in Nutrigenetics Interventions

The field of nutrigenetics is still relatively new and the sharing of personal genetic information is important to consider as this field grows (100). Due to the novelty of this field, it is important to understand consumer perceptions and buy-in from the general public and HCPs. As nutrigenetics has been reported to revolutionize dietary recommendations, a more thorough analysis of the progression of this research is needed to identify gaps and directions for future work (231). Study 3 represents the first nutrigenetic study to provide genetic information related to omega-3 FAs to participants. The literature reports that individuals who receive information about their genetic predisposition to certain diseases may feel anxious and seek out health interventions without consulting with a HCP (211). On the other hand, access to genetic information has been reported to motivate individuals with MetS and T2D to adopt lifestyle or behavioral changes to reduce disease development (30, 210). Further, the results by Nielsen and El-Sohemy suggested young adults (18-30 yrs.) are also interested in personal genetic information (237). Therefore, these two studies report that adults with chronic disease and those that are healthy are both interested in learning more about their genetics in relation to their health. In order to prevent the development of chronic diseases, targeting emerging adults within this thesis was a valuable strategy from a well-being and health care standpoint (161, 201).

Our results from Study 3 found that educated female emerging adults were also interested in receiving their genetic information and found this information useful when considering the general dietary information provided. In Study 3, we acknowledge a potential participation bias. In other words, individuals who chose to participate were most likely interested in this type of information from the onset. If participants or patients have a choice to be involved in the reception of their genetic information, it could be expected that people will have a positive attitude about the reception of this data. Other factors to consider in the likelihood of people participating in a nutrigenetics intervention include geographical location, living situation, and access to food (167, 250, 298, 310). One eligibility requirement for Study 3 was that participants would be living at the same address throughout the duration of the study. This allowed us to reduce the possible confounding factors listed above.

Within prospective and qualitative studies on nutrigenetics, HCPs report other concerns including their impression that there is limited scientific data regarding the relationship between diet and genetics (100, 145, 211). Part of this concern may be related to the lack of knowledge and comfort with this material, as reported from other work (75, 226, 323). Additionally, HCPs report logistical concerns about the ethical and legal privacy issues, and HCPs also worry about patient anxiety and confidentiality (75). Thus there should continue to be research-based nutrigenetic interventions conducted to provide a safe and controlled environment for participants to learn about their genetics and health.

To summarize, it seems that participants that are healthy and those that have a chronic disease are both interested in participating in nutrigenetic interventions. It would be valuable to engage both groups in this research, as nutrigenetic information could improve preventative health initiatives in young adults, while nutrigenetic information could improve treatment

options and follow through with older adults and those with a chronic disease. RDs may be the first HCPs to implement these nutrigenetic results (34, 75, 141, 142). However, as part of their job, it is the RDs' responsibility to ensure that ethical and legal issues are managed appropriately. Moreover, RDs are able to work closely with their patients to help create strategies to implement dietary and lifestyle changes and minimize patient anxiety. Therefore, as many participants report to be interested in this information, HCPs and researchers should encourage participation in nutrigenetics research as the reception of genetic information could be an additional factor used to improve health.

6.2.2.2 Delivery and Reactions to Genetic Test Results

To date, most genetic information delivered to patients has been focused on health risks, rather than health benefits (135, 202, 210). One reason why risk-related information may be a problem, is that this may promote patient anxiety (211). Indeed, individuals may be left wondering what to do with their information and may not be directly connected to an HCP to aid in making lifestyle related changes (323). As much of the research to date has been done on risk-related genetic information, little is known regarding the reactions of individuals to the reception of benefit-related genetic information (210). The majority of genetic information delivered to the public by HCPs informs individuals about their risk of developing a particular disease, such as obesity, CVD and/or Alzheimer's (64, 136, 322), as the majority of this information dissemination takes place within a hospital or health care facility. In terms of nutrigenetics research, it is speculated that individuals may have widely different reactions to this information, i.e., feeling motivated to make positive changes, or making no changes as they do not perceive an immediate need to improve their health if they are currently considered "healthy" (165, 231).

Some direct-to-consumer genetic testing companies allow for individuals to order a nutrigenomics test through their RD in order to learn about their genetics in relation to their diet. In nutrigenetics testing, most times when the dietary advice is followed, there is potential for health benefits through the addition of a nutrient (i.e. EPA and DHA) (231). Health claims associated with particular dietary intake and food patterns can be useful for improved health (314, 328). In Study 3, the genetic information about *FADS1* highlighted that (for example) as a minor allele carrier individuals should focus on consuming EPA and DHA directly from the diet because they have lower conversion ability. As well, Study 3 provided information which acknowledged that if EPA and DHA are increased, there is potential for improvements in cardiovascular, metabolic, and cognitive health.

Interestingly, in the nutrigenetic intervention in Study 3, individuals who were major allele carriers (i.e. "higher" converters) rated that they would be more likely to take another genetic test to learn more about themselves compared to the minor allele carriers ("lower" converters). It is possible that those who are willing to take another genetic test about themselves may feel empowered by this personal genetic information and may be encouraged to make additional changes to their lifestyle. More work needs to be done to understand the differences in responses based on personal genetic information. Some of the participants in Study 3 reported that they made changes to their diet based on the genetic information they received. We did not measure psychological changes; however, it is possible that receiving personal genetic information could have influenced ratings of confidence and self-efficacy in making decisions about their diet. To further extend this hypothesis, it is possible that those who chose more health-protective behaviours (i.e. increasing their intake of EPA and DHA) as a result of their genetic information may render themselves less susceptible to develop chronic diseases later in life.

We considered several hypotheses which may occur in the reception of *FADS* personal genetic information. For example, from an intervention and dietary change perspective, we hypothesized that individuals who are "higher" converters may think that they need less EPA and DHA, and may focus more on ALA intake. From a self-efficacy stand point, we hypothesized that these individuals would feel more positive about themselves in relation to omega-3 metabolism as their body was rated as "higher" in this conversion process. Understanding the reactions to this information will help to inform future nutrigenetics interventions, especially related to the language used to describe this genetic information and the impact that this may have on the study participants.

A follow-up study using focus groups would allow for a qualitative perspective to empirically evaluate the impact of receiving *FADS* genetic information. In Study 1, focus groups were conducted to determine emerging adults' awareness about omega-3 FAs and health. This technique could be very powerful for a more in-depth analysis on the reactions to receiving genetic information. In Study 3, we asked participants how receiving their genetic information made them feel, and these responses were collected through an online survey without face-to-face or group discussion. To date, most studies have asked what individuals thought about receiving their genetic information (239), and not about how they reacted to specific genetic information. Focus groups including people who have the same genetic make-up (i.e. all major allele carriers), as well as having mixed groups with both major and minor allele carriers would be interesting in future research.

6.3 Future Directions and Prospective Research Questions

The next section will focus on prospective questions and applicability of the methods and results from the thesis for translation to new investigations. In order to reduce participant, investigator, and financial burden of conducting a nutritional study, the addition of more online surveys will be critical in future research. This will allow study populations to be larger, and will allow individuals from a wider geographical region to be sampled. In addition, the delivery of information about nutrition and health will be very important in an attempt to keep the population healthy. Further educational initiatives focusing on providing information about new and evolving research fields like nutrigenetics will allow for greater acceptance, and less fear and concern surrounding the use and application of this data.

6.3.1 Use of the Study 1 Survey in Future Studies

In Study 1, we developed survey questions using a combination of those that were researcher generated and those previously published. In this thesis, sections of the survey from Study 1 were incorporated into Study 3. We were therefore able to re-use aspects of this survey in a new context; i.e. in Study 3 we determined how awareness changed over time, compared to the cross-sectional examination in Study 1. Before using this survey again outside of a population of emerging adults, work needs to be completed in order to validate the survey questions. These validity measures include psychometric testing, which would help to determine actual versus perceived knowledge and would improve the internal and external validity of the survey (164).

To extend this survey to the larger field, these questions could be adapted and used for the investigation of other nutrients. This extension would involve basic word changes, with the recommendation that some qualitative and psychometric work be conducted concomitantly.

Within the field of omega-3 FAs, the Study 1 survey could be valuable in other clinical trials, public health research, and consumer studies. For example, Study 1 provided insight on educated emerging adults in Guelph, Ontario and thus, investigation of the general public versus adults in an academic institution, different age ranges (children, adults, older adults), and extension across different regions and cultures would be valuable. Due to the online nature of this survey, transfer of the survey and analysis of other populations would be efficient. As recent global reports highlight low intake of EPA and DHA (111, 223), it would be interesting to pair this consumption data with information about awareness of omega-3 FAs and health. In Study 1, we found a high level of awareness, but a low level of consumption of omega-3 FAs. This was not what we hypothesized, and we would expect that low awareness about omega-3 FAs and their possible health effects would be associated with low consumption. We would expect this original hypothesis to extend to other populations.

6.3.2 FFQ Development in Future Studies

In Study 3, we translated the validated FFQ developed by Patterson *et al.* (248) to an online version and added new EPA and DHA-rich foods. The transition of the FFQ to an online version allowed participants to complete this survey on their own time and reduced the need for a researcher to oversee the completion of these FFQs, thus reducing participant and researcher - burden. We acknowledge that there may be value to having a researcher available during the completion of an FFQ, as some participants under-report and incorrectly report their actual consumption (26, 245). However, if participants were given enough detail in person, and prompts were provided online, then the survey should be completed with close correlation to actual dietary intake. Additional analyses could validate the FFQ used in this study and

determine how well the FFQ EPA and DHA values correspond to blood EPA and DHA levels when the survey was completed online versus in person.

An important component missing from the FFQ used in Study 3 was an adequate representation of ALA-rich foods and thus, ALA intake could not be accurately estimated. The addition of ALA foods into this FFQ would allow for a more robust calculation of omega-3 FA intake. In Study 3, the addition of ALA-rich foods to this survey would have allowed for the comparison of ALA intake between the Genetic and Non-Genetic groups. It is possible that there may have been differences in ALA intake between the groups, or between the genotypes within the Genetic group. Not surprisingly, cost was reported to be a barrier to increase omega-3 consumption by several participants. However, ALA-rich food products tend to be less expensive and it is possible that participants may have been consuming more ALA, and not EPA and DHA. It could be speculated that major allele carriers were motivated by their information about being "higher" converters and increased their ALA intake to a greater extent than minor allele carriers. We did find that the minor allele carriers ("lower" converters) tended to have a higher intake of EPA and DHA compared to major allele carriers. We speculate that this increased intake may have been due to their knowledge that targeting EPA and DHA intake may lead to greater health implications for their particular genotype.

It is possible that if our study was carried out over a longer duration, we may have found significant genotype results. In a nutrigenetics study by Arkadianos *et al.* measuring weight loss, there was no difference between Genetic and Non-Genetic groups at 100 days (7), similar to our 12-week intervention in Study 3. Although there were not changes at 100 days, Arkadianos *et al.* reported that the Genetic group maintained significantly more weight loss than the Non-Genetic group at 300 days (7). In summary, as a full omega-3 survey hasn't been developed, this leaves a

gap in the literature which could be filled to improve future studies. The addition of a follow-up point in future nutrigenetics studies could evaluate the longer-term impact of genetic information on dietary behaviour.

6.3.3 Creating Opportunities for Nutrigenetics Education

The development of nutrigenetics educational materials is critical, as more patients will be visiting their HCPs with independently acquired genetic information in the future. Understanding how people respond to knowledge of their genetic information, and how they change their lifestyle accordingly will be critical to the development of these materials. In Study 1, we found that individuals who used HCPs to learn about nutrition and health were more likely to know about the health effects associated with an increased intake of EPA and DHA. These results highlight that HCPs can be important channels for the delivery of accurate and evidence-based information. In other work, it is reported that people prefer to get their information about health from HCPs, although it was previously reported that the time to make an appointment was the primary barrier why HCPs were selected less often than other sources such as the Internet, friends and / or family (62). Thus, as discussions with HCPs are linked to greater awareness and knowledge about health and nutrition, it would be critical for HCPs to learn more about nutrigenomics. There are many ways in which this education could occur, including e-learning, continuing education, and specialized graduate programs (75, 323). Interestingly, a nutrigenetics company called “NutrigenomiX” partners with RDs in order to improve translation of information about diet, health, and genetics (95). These RDs receive training on how to interpret and communicate genetic and nutrition information to their clients (95). For example, if their patient is at risk for hypertension according to their genetic make-up, RDs can support their client in making food choices low in sodium (95). Providing HCPs, including RDs, with

resources on what nutrigenetics is and how to interpret this information will be highly valuable for the general public in the future. HCPs are trusted to provide quality health care messages and could act as key players in the translation of nutrigenetics research to their community and the greater public.

6.4 Discussion Summary

The FADS pathway is critical to omega-3 metabolism and will continue to be a topic of great investigation in the future. Newer methods such as steady-state stable isotope infusion will help to re-define the applicability and usefulness of the estimated FADS pathway activity that currently predominates in most human research. Within the field of nutrigenetics, this fundamental research will be valuable to enhance the quality of personal genetic information delivered to participants. In order to enrol more participants for greater power within studies, online surveys and FFQs will be a way to reduce participant, investigator, and financial burden. Apart from individual research studies, the developing educational initiatives about nutrigenetics will improve both awareness and understanding for HCPs and those in the general public. Overall, improved education and knowledge translation, as well as involving individuals in their own health-care using personal genetic information, represent two strategies to promote an increased intake of EPA and DHA and therefore improve health of the population.

6.5 Concluding Remarks

There is much to be learned in the field of nutrigenetics. The studies in this thesis highlight the importance of perceived and actual health in emerging adults related to omega-3 FAs and the implications of genetic information in *FADS1* and *FADS2*. Emerging adults may begin lifestyle and diet changes during this timeframe, which can continue into adulthood, and thus appropriate content and translation of messages related to omega-3 FAs and their associated health benefits, are critical for the prevention of chronic disease later in life. The provision of genetic information related to omega-3 FAs presents an additional factor which may improve diet and health. Taken together, the results from this thesis provide novel insights to the field and highlight the need for future initiatives to encourage increased omega-3 FA intake through education and provision of genetic information.

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APPENDICES

Appendix 1: Selected Survey Questions and Response Options

Participants were provided the consent form and chose whether or not they wanted to participate in the study. We have included text provided to the participants, as well as survey questions.

We have provided information below to orient you to the topic for this survey.

Macronutrients make up the foods that we eat. There are 3 main categories of macronutrients: fat, protein and carbohydrates. This survey focuses on fat. More specifically, this survey will ask questions about dietary omega-3 fats.

We will be examining your knowledge, awareness, attitudes, and beliefs about dietary omega-3 fats and health.

Please answer these survey questions based on your current knowledge, attitudes, awareness, and beliefs. Please do not use the Internet as an aid to answer the questions.

If you are not sure what to select, please select "don't know" when available. Please answer the question based on what you currently know and try to avoid guessing the answer. This is not a test!

What is your age?

a. 17 or younger *(skip to end of survey)*
b. 18
c. 19
d. 20
e. 21
f. 22
g. 23
h. 24
i. 25
j. 26 or older *(skip to end of survey)*

Q1: Before beginning this survey, had you heard of the following fats:

	Yes	No
ALA		
EPA		
DHA		
Alpha-linoleic acid		
Eicosapentaenoic acid		
Docosahexaenoic acid		

Omega-3 fats are a specific type of polyunsaturated fat.

ALA (alpha-linolenic acid), EPA (eicosapentaenoic acid), and DHA (docosahexaenoic acid) are specific omega-3 fats.

Q2: Which of these omega-3 fats have you heard the most about? *(single answer option)*

a. Omega-3 fats in general
b. ALA
c. EPA
d. DHA
e. EPA+DHA
f. Haven't heard of omega-3 fats of any of the specific fats listed above *(skip to end of survey)*

Q3: Where did you learn about omega-3 fats and health? *(select all that apply)*

a. Academic/Reputable: high school courses, journal articles, research reports, University courses
b. Food Derived: packaging, recipes, supplements
c. Family/Friends: grew up eating omega-3, family, friends
d. Health Care Professionals
e. Social Media: blogs on food and diet, fitness websites, nutrition websites, social media
f. Text: books, magazines, newspaper
g. TV Derived: advertisements, documentaries, TV commercials

Omega-3 is an umbrella term which includes specific fats: ALA, EPA and DHA.

You can find high amounts of ALA in certain vegetable oils, walnuts, flaxseeds and soy products. You can find high amounts of EPA and DHA in fish, seafood, fish oils and some fortified products. You can consume these omega-3 fats from whole foods or from supplements.

The following questions will focus specifically on EPA and DHA omega-3 fats.

Q4: Have you heard of any health outcomes related to eating high amounts of EPA and DHA omega-3 fats?

a. Yes
b. No

Q5: Do you think that increased consumption of EPA and DHA omega-3 fats influence heart health?

a. Yes
b. No
c. Don't know

Q6: Do you think that increased consumption of EPA and DHA omega-3 fats influence diseases or conditions such as Obesity, Type-2 Diabetes or Metabolic Syndrome?

a. Yes
b. No
c. Don't know

Q7: Do you think that increased consumption of EPA and DHA omega-3 fats influence brain growth, development and/or functioning?

a. Yes
b. No
c. Don't know

Q8: Do you choose to purchase or consume foods (such as meat, pasta, milk, dietary oils, *etc*.) based on omega-3 fat content?

a. Yes
b. No

Q9: Do you take EPA or DHA omega-3 supplements?

a. Yes
b. No

The following questions will be used for statistical purposes.

Q10: Slide the bar to indicate your interest in overall health.

0-1: not at all interested in my overall health
9-10: extremely interested in my overall health

Q11: Do you currently attend a school, college, or university?

c. Yes
d. No

Q12: What is the highest degree, certificate, or diploma you have obtained?

a. High school diploma
b. Trade certificate or diploma
c. Non-university certificate or diploma
d. Not completed – but currently enrolled in a University undergraduate program
e. Bachelor's degree
f. Master's or Doctorate
g. Professional school (medicine, dentistry, etc.)

Q13: Which one of the fields below best describes your current area of study / field of work? *(simplified list)*

a. Biological/Physical Sciences
b. Social Sciences
c. Other Fields

Q14: What gender do you most identify with?

a. Male
b. Female
c. Other

Q15: People living in Canada come from many different cultural and racial backgrounds. Do you identify yourself as: *(simplified list)*

a. Asian
b. European
c. Caucasian
d. Other Ethnicity

Appendix 1: Table 3.1. Individual field and area of work used to establish the three major subgroups for *field of study*.

[#]**Field of Study / Area of Work**	**n (%)**	**Contribution of each field of study / area of work**
Biological/Physical Sciences	542 (65%)	Agriculture n=36, Animal Science n=10, Architecture n=5, Biological Science n=193, Computer Science/Mathematics n=8, Engineering n=35, Environmental Science n=20, Food Science n=21, Health Care n=22, Health Science n=108, Nutrition n=61, Physical Science n=23.
Social Sciences	192 (23%)	Education n=10, Psychology n=63, Social and Applied Human Science n=119.
Other Fields	100 (12%)	Arts n=13, Business and Finance n=43, Fine Arts and Music n=7, History n=7, Legal n=13, Marketing and Media n=4, Service Industry n=13.

Numbers represent frequency (n) and percentage (%) of respondents.

Appendix 1: Table 3.2. Individual category contributions to each of the seven major subgroups for *source of information.*

Source of Information	**Categories comprised in subgroup**
Academic/Reputable	high school courses, journal articles, research reports, University courses
Family/Friends	grew up eating omega-3, family, friends
Food Derived	packaging, recipes, supplements
Health Care Professionals (HCPs)	HCPs
Social Media	blogs on food and diet, fitness websites, nutrition websites, social media
Text	books, magazines, newspaper
TV Derived	advertisements, documentaries, TV commercials

Appendix 2: Full Survey Questions and Response Options

CONSENT TO PARTICIPATE IN RESEARCH

Gone Fishing Study - On-line Survey

Thank you for your interest in being a participant for this study. This research project is conducted by Dr. David Mutch and graduate student Kaitlin Roke, from the department of Human Health and Nutritional Sciences at the University of Guelph. This research project has been approved by the Research Ethics Board (REB#14SE027).
If you have any questions or concerns about this research project, please contact:
· Kaitlin Roke - gofish@uoguelph.ca

PURPOSE OF THE STUDY The purpose of this research project is to better understand the knowledge, awareness, attitudes, and beliefs that young adults have about omega-3 dietary fats and health.

PROCEDURES If you volunteer to participate in this study, we would ask you to complete an on-line survey. In this survey you will be asked questions about omega-3 fats and health. The study investigators are interested in what you currently know, not what you can look up on the Internet. Please answer the questions to the best of your ability based on what you currently know and believe. If you do not know the answer a particular question, please select the "don't know" option. Please use the comment boxes to explain your answers when appropriate. This is not a test! The questions are mostly multiple choices, with some opportunities to leave your own answer or opinion. This survey should take between 10-30 minutes to complete.

POTENTIAL RISKS AND DISCOMFORTS There are minimal risks involved in participating in an on-line survey.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY All participants who complete this survey may benefit by learning about nutrition. The results of this on-line survey will be shared within the University of Guelph as well as disseminated to the scientific community through conferences and peer-reviewed manuscripts as part of this larger study. The results of this study will help us to understand the knowledge, attitudes, awareness, and beliefs young adults have about omega-3 dietary fats and health. There are currently no other studies looking at this particular demographic of the Canadian population and this topic; thus this research project will be of significant value for the field.

PRIZE DRAW Upon completion of the survey, you may choose to be entered into a draw to win a prize. The total number of expected participants is approximately 400. There will be 13 prize winners, with 1 prize worth $100, 4 prizes worth $50 and 8 prizes worth $25.

CONFIDENTIALITY The data generated from this survey will be anonymous. Please note that confidentiality cannot be guaranteed while data are in transit over the Internet.

PARTICIPATION AND WITHDRAWAL Your completion of this on-line survey is voluntary. You may choose not to complete the survey. If you choose to not complete the survey part way though, all data from partially completed surveys will be destroyed. Once you have submitted your survey responses, your data cannot be withdrawn, as the data cannot be identified.

RIGHTS OF RESEARCH PARTICIPANTS You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research project. This research project has been reviewed and received ethics clearance through the University of Guelph Human Research Ethics Board. If you have questions regarding your rights as a research participant, contact: Director of Research Ethics - Sandy Auld; (519) 824-4120, ext. 56606; sauld@uoguelph.ca. You are welcome to print this consent form and keep it for your records

By checking this box, you will indicate that you have read this information and have agreed to participate in this research study.

- ❍ Yes I agree to participate in this research study
- ❍ No I do not want to participate in this research study

We have provided information below to orient you to the topic for this survey.

Macronutrients make up the foods that we eat. There are 3 main categories of macronutrients: fat, protein and carbohydrates. This survey focuses on fat. More specifically, this survey will ask questions about dietary omega-3 fats.

We will be examining your knowledge, awareness, attitudes and beliefs about dietary omega-3 fats and health.

Please answer these survey questions based on your current knowledge, attitudes, awareness, and beliefs. Please do not use the Internet as an aid to answer the questions. If you are not sure what to select, please select “don’t know” when available. Please answer the question based on what you currently know and try to avoid guessing the answer. This is not a test!

What is your age?

- ❍ 17 or younger
- ❍ 18
- ❍ 19
- ❍ 20
- ❍ 21
- ❍ 22
- ❍ 23
- ❍ 24
- ❍ 25
- ❍ 26 or older

Experts recommend that we should eat LESS of some types of fats and MORE of others. Please select the options below according to what you think is recommended.

	EAT LESS	EAT MORE	NOT SURE
Monounsaturated fats	❍	❍	❍
Omega-3 fats	❍	❍	❍
Omega-6 fats	❍	❍	❍
Polyunsaturated fats	❍	❍	❍
Saturated fats	❍	❍	❍
Trans fats	❍	❍	❍

Before beginning this survey, had you heard of the follow fats:

	Yes	No
ALA	❍	❍
EPA	❍	❍
DHA	❍	❍
Alpha-linolenic acid	❍	❍
Eicosapentaenoic acid	❍	❍
Docosahexaenoic acid	❍	❍

Omega-3 fats are a specific type of polyunsaturated fat. ALA (alpha-linolenic acid), EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are specific omega-3 fats.

Which of these omega-3 fats have you heard most about?

- ❍ Omega-3 fats in general
- ❍ ALA
- ❍ EPA
- ❍ DHA
- ❍ EPA and DHA
- ❍ Haven't heard of omega-3 fats or any of the specific fats listed above

Based on what you have heard, which of the following omega-3 fats(s) are considered essential to consume? *(check all that apply)*

- ❑ Omega-3 fats in general
- ❑ ALA
- ❑ EPA
- ❑ DHA
- ❑ Ratio of omega-3:omega-6
- ❑ Not sure

Do you choose to purchase or consume foods (such as meat, pasta, milk, dietary oils etc.) based on omega-3 fat content?

- ❍ Always
- ❍ Sometimes intentionally
- ❍ Not intentionally
- ❍ Never

Where did you learn about omega-3 fats? *(check all that apply)*

- ❑ Hadn't heard about omega-3 fats before this survey
- ❑ Advertisements
- ❑ Blogs on food and diet
- ❑ Books on food and diet
- ❑ Documentaries
- ❑ Fitness websites
- ❑ Family
- ❑ Friends
- ❑ Grew up eating omega-3 products
- ❑ High school courses
- ❑ Health care professionals
- ❑ Journal articles
- ❑ Magazines
- ❑ Newspaper articles
- ❑ Nutrition websites
- ❑ Packaging
- ❑ Research reports
- ❑ Recipes
- ❑ Social Media
- ❑ Supplements
- ❑ TV commercials
- ❑ University course(s)
- ❑ Other (fill in option if applicable) _____

The following questions will focus specifically on EPA and DHA omega-3 fats.

Do you think that these products contain EPA and DHA omega-3 fats? Please consider regular, fortified or enriched products.

	Yes	No	Don't know
Beef	❍	❍	❍
Fish	❍	❍	❍
Shellfish	❍	❍	❍
Pork	❍	❍	❍
Poultry	❍	❍	❍
Eggs	❍	❍	❍
Fruit	❍	❍	❍
Vegetables	❍	❍	❍
Nuts	❍	❍	❍
Juice	❍	❍	❍
Milk	❍	❍	❍
Bread	❍	❍	❍
Crackers	❍	❍	❍
Muffins	❍	❍	❍

Which oil below do you think has the MOST EPA and DHA omega-3 fats?

- ❍ Algal / Algae oil
- ❍ Canola oil
- ❍ Fish oil
- ❍ Flaxseed oil
- ❍ Krill oil
- ❍ Olive oil
- ❍ Vegetable oil
- ❍ Other (fill in option if applicable) _____
- ❍ Not sure

Omega-3 is an umbrella term which includes specific fats: ALA, EPA and DHA. You can find high amounts of ALA in certain vegetable oils, walnuts, flaxseeds and soy products. You can find high amounts of EPA and DHA in fish, seafood, fish oils and some fortified products. You can consume these omega-3 fats from whole foods or from supplements.

Do you think that the average young adult (18-25 years) living in Canada gets enough EPA and DHA omega-3 fat in their diet?

- ❍ Yes
- ❍ No
- ❍ Don't know

You selected "don't know" if the average young adult (18-25 years) living in Canada gets enough EPA and DHA omega-3 fat in their diet. Please explain your answer.

(check all that apply)

- ❑ Would depend on cultural background
- ❑ Would depend on dietary restrictions / preferences
- ❑ Would depend on gender
- ❑ Would depend on income
- ❑ Would depend on knowledge of omega-3 fats
- ❑ Other (fill in option if applicable) _____
- ❑ Not sure

Do you think that other age groups (for example: children, seniors, etc.) in Canada get enough EPA and DHA omega-3 fats in their diet?

- ❍ Yes
- ❍ No
- ❍ Don't know

You selected “don’t know” if other age groups in Canada get enough EPA and DHA omega-3 fats in their diet. Please explain your answer.

(check all that apply)

- ❑ Infants and toddlers (birth - 3 years) would get more
- ❑ School-aged children (4-12 years) would get more
- ❑ Adolescents (13-17 years) would get more
- ❑ Adults (26-44 years) would get more
- ❑ Middle-aged adults (45-65) would get more
- ❑ Older adults (66+ years) would get more
- ❑ More health conscious individuals would get more
- ❑ Would depend on living situation (living with parents, independently, in a nursing home etc.)
- ❑ Would depend on socio-economic status
- ❑ Other (fill in option if applicable) _____
- ❑ Not sure

In your opinion, what are the 3 MOST IMPORTANT factors that influence the consumption of EPA and DHA omega-3 fats in the average young adult (18-25 years) living in Canada?

(please select 3 options from the list below)

- ❑ Access
- ❑ Awareness
- ❑ Convenience
- ❑ Cost
- ❑ Cultural dietary preferences
- ❑ Dietary choices - vegan, vegetarian
- ❑ Education
- ❑ Environmental impact
- ❑ Family health history
- ❑ Geographic location
- ❑ Interest in nutrition and food
- ❑ Lack of time for food preparation
- ❑ Media
- ❑ Overall good health
- ❑ Physical activity
- ❑ Recommendation by health care professional
- ❑ Risk factors
- ❑ Taste preferences
- ❑ Understanding of what omega-3 fats are
- ❑ Upbringing
- ❑ Other (fill in option if applicable) _____

Based on the previous question, please rank your 3 answers with MOST important at the top.

Do you take EPA or DHA omega-3 supplements?

- ❍ Yes
- ❍ No
- ❍ Not currently
- ❍ Irregularly
- ❍ Take a multi-vitamin that contains omega-3 fats
- ❍ Take other supplements / vitamins, but not omega-3 fats

What type of EPA or DHA omega-3 supplement are you taking / have you taken?

- ❍ Algal / Algae oil
- ❍ Fish oil
- ❍ Krill oil
- ❍ Other (can specify if not listed) _____

In the past week, how many days did you take your EPA and DHA omega-3 supplements?

	1	2	3	4	5	6	7
Days in the week							

In the past 4 weeks, on average how many days a week did you take your EPA and DHA omega-3 supplements?

	1	2	3	4	5	6	7
Average number of days supplements were taken							

What factors contribute to your choice to NOT take an EPA and DHA omega-3 supplement?

(check all that apply)

- ❑ Convenience
- ❑ Debate over which supplements are helpful
- ❑ Form supplement is in (pill, liquid, chewable)
- ❑ Supplements can be expensive
- ❑ Wouldn't remember to take it
- ❑ I would need to do some research first
- ❑ Other (fill in option if applicable) _____

Congratulations! You are more than half-way done the survey. Just a reminder: This is not a test! Please answer the questions based on what you currently know and try to avoid guessing the answer. We are interested in what you think and know, not what the "correct" answer is.

Have you heard of any health outcomes (either positive or negative) related to eating high amounts of EPA and DHA omega-3 fats?

- ❍ Yes
- ❍ No
- ❍ Maybe
- ❍ Not sure

Please indicate in the box below what health outcomes (either positive or negative) you have heard of related to eating high amounts of EPA and DHA omega-3 fats.

If you are not sure, please write NA

Have you heard of any health outcomes (either positive or negative) related to eating little or no EPA and DHA omega-3 fats?

- ❍ Yes
- ❍ No
- ❍ Maybe
- ❍ Not sure

Please indicate in the box below what health outcomes (either positive or negative) you have heard of related to eating little or no EPA and DHA omega-3 fats.

If you are not sure please write NA

There has been a lot of research investigating the health outcomes (either positive or negative) related to the consumption of EPA and DHA omega-3 fats. The following questions ask about EPA and DHA omega-3 fats and certain health outcomes.

Heart health can be affected by factors such as levels of cholesterol, triglycerides and plaques in the arteries. Other factors include blood pressure and heart rate. Do you think that increased consumption of EPA and DHA omega-3 fats influence heart health?

- ❍ Yes - good influence
- ❍ Yes - bad influence
- ❍ No influence
- ❍ Don't know

What specific influences on heart health have you heard about related to an increased consumption of EPA and DHA omega-3 fats?

(check all that apply)

- ❑ Improved blood pressure
- ❑ Improved good cholesterol
- ❑ Reduced bad cholesterol
- ❑ Reduced inflammation
- ❑ Reduced triglycerides
- ❑ Reduced risk for coronary heart disease
- ❑ Other (fill in option if applicable) _____
- ❑ Not sure

Obesity, Type-2 Diabetes and Metabolic Syndrome are linked to a variety of factors including body fat as well as lipid, glucose and insulin levels in the blood. Do you think that increased consumption of EPA and DHA omega-3 fats influence diseases or conditions such as Obesity, Type-2 Diabetes or Metabolic Syndrome?

- ❍ Yes - good influence
- ❍ Yes - bad influence
- ❍ No influence
- ❍ Don't know

What specific aspects of Obesity, Type-2 Diabetes or Metabolic Syndrome have you heard about related to an increased consumption of EPA and DHA omega-3 fats?

(check all that apply)

- ❑ Affects body weight
- ❑ Healthy fats help you stay full longer
- ❑ Improved insulin levels
- ❑ Reduced blood lipid levels
- ❑ Reduced fasting glucose levels
- ❑ Other (fill in option if applicable) _____
- ❑ Not sure

Brain growth, development and functioning can be influenced by specific anatomical and physiological changes, resulting in overall impacts on IQ score, cognition and mood.

Do you think that increased consumption of EPA and DHA omega-3 fats influence brain growth, development and/or functioning?

- ❍ Yes - good influence
- ❍ Yes - bad influence
- ❍ No influence
- ❍ Don't know

What specific aspects of brain growth, development and functioning have you heard about related to an increased consumption of EPA and DHA omega-3 fats?

(check all that apply)

- ❑ Affects chemicals in the brain
- ❑ Anatomical / structural role
- ❑ Improved IQ score
- ❑ Improved overall cognition
- ❑ Reduced anxiety
- ❑ Reduced symptoms of depression
- ❑ Other (fill in option if applicable) _____
- ❑ Not sure

The following questions ask about dietary fats and genetics.

Do you think that genes play a role in how the body processes dietary fats?

- ❍ Yes, definitely
- ❍ Yes, but not sure how
- ❍ No
- ❍ Don't know

You selected that "yes, but not sure how" genes play a role in how the body processes dietary fats.

Please explain your answer.

- ❍ Combination of lifestyle and hereditary factors
- ❍ Depends on metabolism
- ❍ Personality and decision making are more important factors
- ❍ Other (fill in option if applicable) _____

Do you think that some people benefit more from eating EPA and DHA omega-3 fats compared to other people - because of their genes?

- ❍ Yes
- ❍ No
- ❍ Don't know

Personalized nutrition can be defined as: Tailoring nutritional needs based on an individual's genetic make-up.

Have you heard of "personalized nutrition" as described by the definition above?

- ❍ Yes
- ❍ Heard of personalized nutrition but with a different definition
- ❍ No
- ❍ Don't know

Have you heard of the terms "nutrigenomics" and/or "nutrigenetics" before this survey?

- ❍ Yes
- ❍ No
- ❍ Don't know

The following questions will be used for statistical purposes.

Slide the bar below to indicate interest in overall health.

0-1: not at all interested in my overall health

9-10: extremely interested in my overall health

- ❍ 0
- ❍ 1
- ❍ 2
- ❍ 3
- ❍ 4
- ❍ 5
- ❍ 6
- ❍ 7
- ❍ 8
- ❍ 9
- ❍ 10

Do you have any dietary restrictions / food allergies?

(check all that apply)

- ❑ Vegetarian
- ❑ Vegan
- ❑ Gluten free
- ❑ Lactose intolerant
- ❑ Allergic to marine products
- ❑ Allergic to shellfish
- ❑ Other (fill in option if applicable) _____
- ❑ No dietary restrictions

Are you currently attending a school, college or university?

- ❍ Yes
- ❍ No

What is the highest degree, certificate or diploma you have obtained?

- ❍ High school diploma
- ❍ Trade certificate or diploma
- ❍ Non-university certificate or diploma
- ❍ Not completed - but currently enrolled in a University undergraduate program
- ❍ Bachelor's degree
- ❍ Master's or Doctorate
- ❍ Professional school (medicine, dentistry, etc.)

What is your current year of study?

- ❍ 1st year undergraduate
- ❍ 2nd year undergraduate
- ❍ 3rd year undergraduate
- ❍ 4th year undergraduate
- ❍ 5th year undergraduate
- ❍ Non-degree student
- ❍ Masters student
- ❍ Doctorate student
- ❍ None of the above

Which one of the fields below best describes your current area of study / work?

- ❍ Agriculture
- ❍ Administrative
- ❍ Computer Science / Mathematics
- ❍ Fine Arts and Music
- ❍ Biological Science
- ❍ Business and Finance
- ❍ Education
- ❍ Engineering
- ❍ Environmental Science
- ❍ Health Care
- ❍ Health Sciences
- ❍ Nutrition
- ❍ Physical Science
- ❍ Psychology
- ❍ Service Industry
- ❍ Social and Applied Human Sciences
- ❍ Other (fill in option if applicable) _____

What gender do you most identify with?

- ❍ Male
- ❍ Female
- ❍ Other

People living in Canada come from many different cultural and racial backgrounds.

Do you identify yourself as:

- ❍ Aboriginal
- ❍ Arabic
- ❍ Asian
- ❍ Black or African Canadian
- ❍ European
- ❍ Hispanic or Latino
- ❍ Middle Eastern
- ❍ White or Caucasian
- ❍ Don't know
- ❍ Not listed (can specify) _____

Last few questions.

Would you be interested in learning more about omega-3 fats and health?

- ❍ Yes
- ❍ No

Would you be interested in learning about your personal genetic information in relation to omega-3 fats and health?

- ❍ Yes
- ❍ No

Would you be interested in participating in a research study where you are able to learn more about your health, your genes and omega-3 fats?

- ❍ Yes
- ❍ No

Thank you for your interest in participating in future research study! Please enter your email address below to receive a follow up email from the study coordinator.

Please use the space provided for any thoughts or comments you may have related to this survey. Your comments would be welcome.

Thank you for completing this survey! Both your responses and time are appreciated. Please enter your email address below to be entered into a draw to win 1 of 13 prizes. The draw will be made on Friday, April 3, 2015. Please note that your responses from this survey will be anonymous and will not be linked to your email address.

Appendix 3: Additional Data Tables

Appendix 3 Table 3.3: Sources of Information used to learn about Omega-3 FAs and Health

Sources of Information[∞]	Full Sample (N=834)	Females (n=655)	Males (n=179)	Biological / Physical Sciences (n=542)	Social Sciences (n=192)	Other Fields (n=100)
Academic/Reputable	656 (78.7%)	529 (80.8%)[a]	127 (70.9%)[b]	457 (84.3%)[a]	139 (72.4%)[b]	60 (60%)[b]
Family/Friends	518 (62.1%)	409 (62.4%)[a]	109 (60.9%)[a]	319 (58.9%)[a]	128 (66.7%)[a]	71 (71%)[a]
Food Derived	558 (66.9%)	434 (66.3%)[a]	124 (69.3%)[a]	362 (66.8%)[a]	122 (63.5%)[a]	74 (74%)[a]
HCPs	215 (25.8%)	174 (26.6%)[a]	41 (22.9%)[a]	161 (29.7%)[a]	35 (18.2%)[b]	19 (19%)[a,b]
Social Media	498 (59.7%)	390 (59.5%)[a]	108 (60.3%)[a]	319 (58.9%)[a]	113 (58.9%)[a]	66 (66%)[a]
Text	372 (69.7%)	303 (46.3%)[a]	69 (38.5%)[a]	243 (44.8%)[a]	77 (40.1%)[a]	52 (52%)[a]
TV Derived	561 (67.3%)	438 (66.9%)[a]	123 (68.7%)[a]	361 (66.6%)[a]	130 (67.7%)[a]	70 (70%)[a]

Answer responses to Appendix 1, Q3. Numbers represent the frequency (n) of respondents who indicated "Yes" to using that source of information to learn about omega-3 FAs and health. Percentages (%) represent the number of respondents who indicated "Yes", out of the total number for that subgroup. There were 3378 total responses (respondents could select more than one answer option). Chi-square (χ^2) analyses assessed differences between *field of study* and *gender*. *Field of study* and *gender* were analyzed separately. Percentage values within a row (Female versus Male; Biological/Physical Sciences versus Social Sciences versus Other Fields) with unlike superscript letters were significantly different in pairwise comparisons by Z test ($P \leq 0.05$), using a Bonferroni adjustment for multiple comparisons.

FAs, fatty acids; HCPs, Health Care Professionals

Appendix 4: Nutrition and Genetics Information

Personalized Nutrition and Genetics Study: FAQs

Do most Canadians get enough omega-3 fats in their diet?

No. Unfortunately, the average Canadian adult does not consume enough omega-3 fats in their diet; 90% of Canadians are not meeting the recommended intake.

What are omega-3 fats?

The information below may, or may not, already be familiar to you.

Macronutrients make up the foods that we eat. There are 3 main categories of macronutrients: fat, protein and carbohydrates. There are different types of fat, including trans, saturated, monounsaturated and polyunsaturated. Omega-3 fats are a specific type of polyunsaturated fat. ALA (alpha-linolenic acid), EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are specific omega-3 fats (**as shown in Figure 1)**.

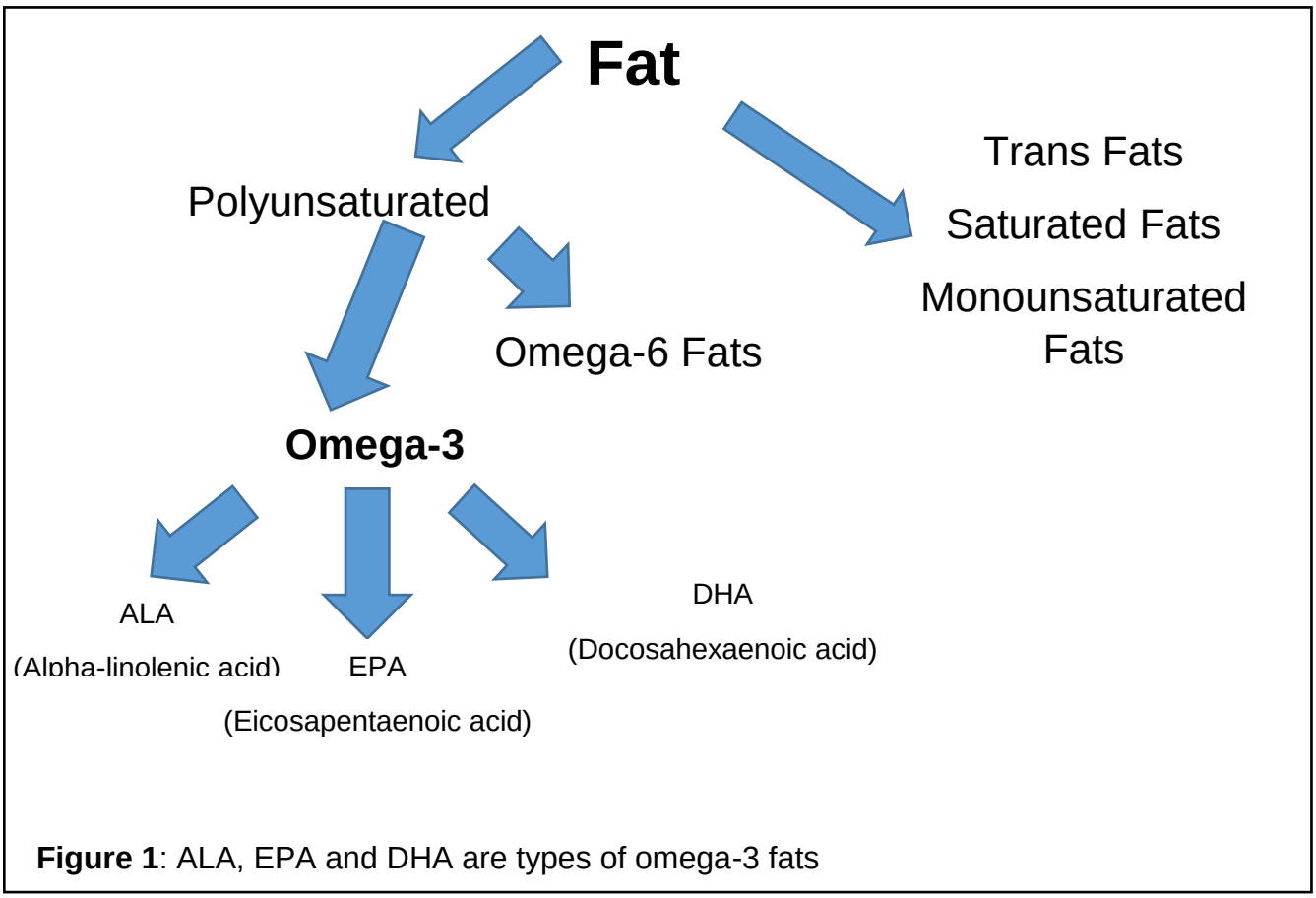


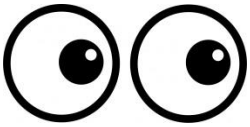
Figure 1: ALA, EPA and DHA are types of omega-3 fats

What are some of the health benefits of omega-3 fats?

There are many known health benefits linked to high consumption of omega-3 fats. There are health benefits which can be noticed within a few weeks of increased omega-3 consumption. There are also health benefits which can help to reduce risk and prevent disease later in life. These health benefits range from helping our brain, heart, eyes and whole body.

Brain: improved cognition, memory, neural connections, reduced risk of depression and mood disorders

Heart: reduced levels of triglycerides, reduced levels of cholesterol, reduced risk of heart disease



Eyes: improved vision, visual acuity and reduction of dry eyes

Whole body: reduced inflammation related to illness (cold, Arthritis) or working out, important component to cells throughout the body,

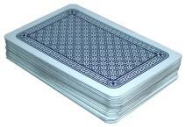
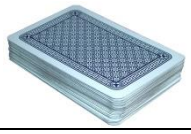
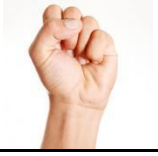
Where can I get omega-3 fats in my diet?

Some, but not all, of the foods we consume in our regular diet contain polyunsaturated fats, and therefore may contain one or all of the omega-3 fats (ALA, EPA, and DHA). There are some foods and supplements that are rich sources of omega-3 fats (**as shown in Figure 2**).

ALA: found in certain vegetable oils, walnuts, flaxseeds, chia seeds and soy products. There are also several fortified omega-3 ALA products, such as granola and crackers.

EPA and DHA: found in some types of fatty fish and seafood, as well as in certain types of eggs and milk. EPA and DHA can also be found in supplements including fish oils, krill oil and algal oil.

How much omega-3 fat do I get in a typical serving size?

Food Source	Approximate Serving Size
Meat and Alternatives	
2 cooked Omega-3 eggs contain approximately 0.06-0.28g of ALA and 0.07g of EPA/DHA.	
3 ounces of cooked carp contains 0.31g ALA and 0.67g EPA/DHA.	 = 
3 ounces of Atlantic salmon raw or cooked contains approximately 0.10-0.13g of ALA and 1.78-1.93g DHA/EPA.	 = 
1 cup of beans (navy, pinto) cooked contains 0.23-0.32g of ALA.	 = 
Nuts and Seeds	
1 tablespoon of flaxseed (ground) contains 2.46g of ALA.	 = 
¼ cup of English walnuts contains 2.3g of ALA.	 = 
Fats and Oils	
1 teaspoon of canola oil contains 0.42g of ALA.	 = 
Milk and Alternatives	
1 cup of soymilk contains 0.19g of ALA.	 = 
1 cup of milk fortified with DHA contains 0.01g of DHA.	 = 

Can my body make EPA and DHA?

Yes. A little bit. Our body converts foods that we eat into useable nutrients within the body. ALA is “essential”, which means our bodies don’t produce it and we must consume this in our diet. Once ALA is ingested, our bodies can convert it into EPA and then to DHA (**as shown in Figure 3**). The conversion in our bodies is not very efficient; this is why health care professionals recommend that we also consume EPA and DHA rich foods in our diet.

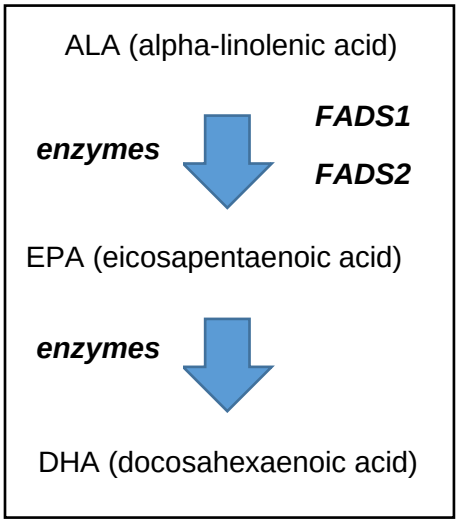


Figure 3: Production of EPA and DHA from ALA

What should I do with my genetic information?

The body makes changes to the nutrients we consume. Specifically, the omega-3 fat ALA can be converted into EPA and DHA. However, depending on an individual's genes, some people are better at converting ALA to EPA and DHA than others. *FADS1* and *FADS2* (fatty acid desaturase 1 and 2) are specifically related to omega-3 fats and can convert ALA into EPA and DHA.

If you have the GG genotype:

Research has shown that your body has an increased ability to convert ALA to EPA and DHA.

This means that you could increase the levels of EPA and DHA in your body in two ways:

1) Consume ALA rich foods and let your body convert the ALA into EPA and DHA
2) Consume EPA and DHA rich foods directly

Overall, you may experience health benefits (i.e. reduced triglycerides, improved mood and cognition) from increasing consumption of food or supplements that have either ALA, or EPA and DHA omega-3 fats. ALA is found in certain vegetable oils, walnuts, flaxseeds and soy products. EPA and DHA are found in foods such as fish (i.e. salmon, herring, tuna), seafood or fortified products such as eggs and milk. Supplements include fish oils, krill oil and algal oil.

If you have the GT or TT genotype:

Research has shown that your body naturally has lower levels of EPA and DHA.

This means that the best way to increase the levels of EPA and DHA in your body is to:

1) Consume EPA and DHA rich foods directly

Overall, you may experience health benefits (i.e. reduced triglycerides, improved mood and cognition) from increased consumption of food or supplements that are rich in EPA and DHA omega-3 fats. These foods can include fish (i.e. salmon, herring, tuna), seafood or fortified products such as eggs and milk. Supplements include fish oils, krill oil and algal oil.

What are the next steps?

As a follow up to this discussion, I will send you an email with material including an omega-3 product list and a some additional nutritional information.

Thank you for taking the time to be part of this study. If you have questions or are looking for more information, I encourage you to contact me at gofish@uoguelph.ca

I love omega-3s. Where I can get more information?

For more information, we recommend that you visit www.alwaysomega3s.com. There is a lot of great information on this website as well as chance to take part in a short quiz to give you an idea of your current omega-3 intake.

http://alwaysomega3s.com/do/are-you-getting-enough-omega-3s

If you go to the main page and scroll to the bottom, you will see the quiz option on the right hand side.

The Dietitians of Canada website provides information about food products high in ALA, EPA and DHA. http://www.dietitians.ca/Your-Health/Nutrition-A-Z/Fat/Food-Sources-of-Omega-3-Fats.aspx

Additionally, www.dhaomega3.org provides detailed scientific information regarding the extensive research conducted to date on omega-3 fats.

Again, if you have questions or are looking for more information, I encourage you to contact me at gofish@uoguelph.ca

Appendix 5: Questions and Answer Options

Participants were provided the consent form and chose whether or not to participate in the study.

The information below was shown to the participants prior to the questions.

We have provided information below to orient you to the topic for this survey.

Macronutrients make up the foods that we eat. There are 3 main categories of macronutrients: fat, protein and carbohydrates. This survey focuses on fat. More specifically, this survey will ask questions about dietary omega-3 fats.

We will be examining your knowledge, awareness, attitudes and beliefs about dietary omega-3 fats and health.

Please answer these survey questions based on your current knowledge, attitudes, awareness, and beliefs. Please do not use the Internet as an aid to answer the questions.

If you are not sure what to select, please select "don't know" when available. Please answer the question based on what you currently know and try to avoid guessing the answer. This is not a test!

Q1: Before beginning this survey, had you heard of the follow fats: *(Asked at Baseline and Final)*

	YES	**NO**
ALA		
EPA		
DHA		
Alpha-linolenic acid		
Eicosapentaenoic acid		
Docosahexaenoic acid		

Q2: The following questions ask your opinion related to selected statements. Please answer these questions honestly. *(Asked at Final)*

1 - Strongly disagree
4 – Neutral
7 - Strongly agree

a. I understood the nutrition information about omega-3 fats provided at the start of the study

b. The recommendations about omega-3 fats that were provided in the document at the start of the study were new to me

c. I enjoyed learning about the dietary recommendations related to omega-3 fats

d. The dietary recommendations were useful when I considered my diet throughout the study

e. When I am I in the grocery store or supplement store, I can confidently determine foods that have been fortified, or have added EPA and DHA omega-3 fats

f. I would like to know more about the dietary recommendations related to omega-3 fats

g. I am interested in the relationship between diet and genetics

Q3: Do you feel that you consciously made changes to your diet throughout this study? *(Asked at Final)*

- o Yes
- o No
- o Sometimes

Q4: Did you choose to consume any foods high in omega-3 fats at any time since starting this study? *(Asked at Final)*

- o Yes
- o No

Q5: Did you choose to consume any omega-3 fortified foods or beverage products at any time since starting this study? *(Asked at Final)*

- o Yes
- o No

Q6: Did you choose to consume any omega-3 supplements (in capsule or liquid form) at any time since starting this study? *(Asked at Final)*

- Yes
- No

Q7: If you did make changes to your diet, what was the MOST important reason for this? *(Asked at Final)*

- I didn't make change to my diet
- Family medical history
- Genetic information I was given at the start of the study
- Improve my health
- Nutritional information I was given at the start of the study
- Resource file online listing products with omega-3 fats
- Took a course that discussed the health benefits of omega-3 fats

Q8: Which factor do you think was the BIGGEST obstacle for you throughout this study? *(Asked at Final)*

- It is difficult for me to get to a grocery store
- I am not involved in the grocery shopping in my home
- I eat the majority of my meals away from home
- Omega-3 foods are expensive
- I have an allergy to an omega-3 containing food:
- I do not buy fortified products
- I do not have time to cook foods high in omega-3s
- I do not like fish
- I do not like taking supplements
- When I get busy I don't take the time to eat healthy foods
- I did not face any obstacles increasing omega-3 intake throughout this study

Q9: Did knowing you are a GG or GT+TT allele carrier influence your decision to consume omega-3 foods? *(Asked at Final)*

- Yes
- No

Q10: Did knowing you are a GG or GT+TT allele carrier influence your decision to consume omega-3 fortified foods or beverage products? *(Asked at Final)*

- Yes
- No

Q11: Did knowing you are a GG or GT+TT allele carrier influence your decision to consume omega-3 supplements? *(Asked at Final)*

- o Yes
- o No

Q12: Did knowing you are a GG or GT+TT allele carrier influence your decision to consume more EPA/DHA? *(Asked at Final)*

- o Yes
- o No

Q13: Did knowing you are a GG or GT+TT allele carrier influence your decision to consume more ALA? *(Asked at Final)*

- o Yes
- o No

<u>The following questions will be used for statistical purposes.</u>

Q14: Slide the bar to indicate your interest in overall health.

0-1: not at all interested in my overall health

9-10: extremely interested in my overall health

Q15: Do you currently attend a school, college or university?

- o Yes
- o No

Q16: What is the highest degree, certificate or diploma you have obtained?

- o High school diploma
- o Trade certificate or diploma
- o Non-university certificate or diploma
- o Not completed – but currently enrolled in a University undergraduate program
- o Bachelor's degree
- o Master's or Doctorate
- o Professional school (medicine, dentistry, etc.)

Q17: What is your current year of study?

- 1st year undergraduate
- 2nd year undergraduate
- 3rd year undergraduate
- 4th year undergraduate
- 5th year undergraduate
- Non-degree student
- Masters student
- Doctorate student
- None of the above

Q18: Which one of the fields below best describes your current area of study / field of work? *(simplified list)*

- Biological/Physical Sciences
- Social Sciences
- Other Fields

Q19: What gender do you most identify with?

- Male
- Female
- Other

Q20: People living in Canada come from many different cultural and racial backgrounds. Do you identify yourself as: *(simplified list)*

- Asian
- European
- Caucasian
- Other Ethnicity