

Fish Oil Supplementation and Cardiovascular Disease  
Risk in Individuals of Blood Type A and Blood Type O

by

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## ABSTRACT

The omega-3 fatty acids in fatty fish and fish oil, eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), have been associated with a reduction in risk for cardiovascular disease. Blood type is a known contributor to risk for cardiovascular events. This study evaluated the effect of fish oil supplements on cardiovascular risk markers in adults with blood types A or O. An 8-week parallel-arm, randomized, double-blind trial was conducted in healthy adult men and women with either blood type A (BTA) or blood type O (BTO). Participants were randomized to receive fish oil supplements (n=10 [3 BTA/7 BTO]; 2 g [containing 1.2 g EPA+DHA]/d) or a coconut oil supplement (n=7 [3 BTA/4 BTO]; 2 g/d). Markers that were examined included total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), high-sensitivity C-reactive protein (hsCRP), and hemoglobin A1C (HbA1C). Results indicated that the percent change in LDL cholesterol was significantly greater in the coconut oil group vs the fish oil group ( $-14.8 \pm 12.2\%$  vs  $+2.8 \pm 18.9\%$  respectively,  $p=0.048$ ). There were no other significant differences between treatment groups, or between blood types A and O, for the other cardiovascular risk markers. Further research with a larger and more diverse sample may yield a more conclusive result.

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## Chapter 1

### INTRODUCTION

In 2010, cardiovascular disease accounted for 31.9% of all deaths in the United States (American Heart Association, 2013). For some people with risk for cardiovascular disease, taking a dietary supplement may help them to reduce their risk of developing this life-threatening ailment. If found to be effective, this treatment method could reduce both the financial costs and the health costs for society and many individuals with risk for cardiovascular disease risk.

Much of the traditional medical nutrition therapy for treating cardiovascular disease has focused on adjusting intake of fat and sodium. These dietary changes, while shown to be highly effective (Aburto et al, 2013; Mozaffarian, Micha, & Wallace, 2010), can be difficult for many individuals to make. It has also been well documented that the omega-3 fatty acids, eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), in fish oil may help to reduce one's risk for cardiovascular disease by promoting a favorable blood lipid profile, stabilizing plaques in arteries, improving blood circulation, and reducing inflammation (Anderson & Ma, 2009; Chan & Cho, 2009; Deckelbaum & Torrejon, 2012; Dessi, Noce, & Di Daniele, 2013; Gazi, Liberopoulos, Saougos, & Elisaf, 2006).

An additional component of this equation is blood type. It has been demonstrated that individuals with blood type A seem to have an increased tendency to form blood clots, when compared to blood type O. This is thought to be due to higher levels of a blood glycoprotein known as von Willebrand factor (de Lorgeril & Salen, 2012). If



combined with other risk factors, this may predispose the blood type A individual to develop ischemia and infarction; potentially fatal if in the form of a myocardial infarction (MI; heart attack) or cerebrovascular accident (CVA; stroke). Because fish oil is known to have an antithrombotic effect, it is then feasible that a fish oil supplement regimen may reduce blood clotting tendency in individuals with blood type A, thereby reducing the risk for a heart attack or stroke.

An extra area of interest is insulin sensitivity and blood glucose levels. Some research suggests that the omega-3 fatty acids in fish oil may increase cellular sensitivity to insulin, thereby reducing glucose levels in the blood (American Diabetes Association, 2010). An abnormally high level of glucose in the blood is damaging to blood vessels and prolonged hyperglycemia is a known risk factor for developing or aggravating atherosclerosis and cardiovascular disease.

If taking a dietary supplement such as fish oil is shown to be effective in reducing one's risk of cardiovascular disease, this may be an easy and simple way to improve health outcomes for many individuals.

### **Purpose of Study**

The current research study intends to explore the association between fish oil supplementation and risk for cardiovascular disease in individuals with blood types A and O. Twenty-four healthy participants, eight males and sixteen females, were recruited from the greater Phoenix metropolitan area. The participants were stratified by blood type and then randomly assigned to one of two treatment groups: fish oil supplementation or

coconut oil supplementation. The primary investigators and the participants were blinded regarding the supplement type.

### **Research Aim and Hypotheses**

In the current study, the fish oil supplement condition involved taking a gel capsule containing 1000 mg of fish oil, with 400 mg EPA and 200 mg DHA, twice a day for eight weeks. The coconut oil supplement condition involved taking a gel capsule containing 1000 mg of coconut oil twice a day for eight weeks. The researchers predicted that an increase in omega-3 fatty acid intake would result in improved blood lipids and reduced inflammation. However, the primary hypothesis of the current study is that the fish oil supplementation will not change lipid profiles and inflammatory markers in healthy adults in Phoenix, Arizona. A secondary hypothesis is that fish oil supplementation will not change glucose control in healthy adults in Phoenix, Arizona. The researchers predicted that supplementation with coconut oil would not change the blood lipid profile, inflammation, or glucose control.

### **Definition of Terms**

EPA: Eicosapentanoic acid is a very long-chain polyunsaturated fatty acid with 20 carbon molecules 5 double bonds along the length of its chain. It is an omega-3 fatty acid due to the presence of a double-bond at the third carbon from the methyl terminus. It is a component of tissues in the body.

DHA: Docosahexanoic acid is a very long-chain polyunsaturated fatty acid with 22 carbon molecules 6 double bonds along the length of its chain. It is an omega-3

fatty acid due to the presence of a double-bond at the third carbon from the methyl terminus. It is major component of the central nervous system and the retina. It is needed optimal brain and eye development and maintenance.

hsCRP: high-sensitivity C-reactive protein biomarker that assesses the presence of inflammation. Less than 1.0 mg/L = Low Risk for CVD; 1.0 – 2.9 mg/L = Intermediate Risk for CVD; greater than 3.0 mg/L = High Risk for CVD.

Inflammatory disease: A condition in which there is an inflammatory response within the body; examples include: infection, rheumatoid arthritis, cardiovascular disease, atherosclerosis, celiac disease, inflammatory bowel disease, inflammatory bowel syndrome, asthma, and more.

### **Delimitations and Limitations**

Participants are healthy male and female adults between the ages of 18 and 50 years. They were recruited in Phoenix, Arizona. They were not taking fish oil supplements when recruited into the study. There was no exclusion for the use of other supplements.

A limitation of this study is that the sample size was relatively small and minimally diverse. Therefore, further study will be needed for enhanced generalizability. Also, the dose and period of time of the supplementation may not have been enough to produce a statistically significant result. Finally, blood samples were not specifically designed to be taken from a fasting state. Whether or not individuals had been fasting at the time of the blood draw is unknown. Therefore, it is uncertain whether serum

triglyceride measurements, as well as LDL cholesterol (LDL-C) measurements, were influenced by recent lipid intake.

## Chapter 2

### REVIEW OF LITERATURE

#### **Cardiovascular Disease**

Cardiovascular disease (CVD) is defined as disease of the heart and vasculature system (Alpert, 2004). It is the leading cause of death in the United States, as well as in many other countries around the world, for both males and females (Alpert, 2004; de Lorgeril & Salen, 2012). The majority (50-60%) of deaths from CVD are a result of sustained ventricular arrhythmias or sudden cardiac death, meaning death within one hour of a myocardial infarction (MI; heart attack) (Gazi et al, 2006). It is also the most common cause of death for individuals with type 1 (T1DM) and type 2 diabetes mellitus (T2DM). Some risk factors for this disease are physical inactivity, poor diet, obesity or overweight, hypertension, aging, genetic factors, smoking, and stress (Alpert, 2004).

#### **Blood Lipid Profile**

The blood lipid profile is commonly measured to assess one's likelihood of developing CVD. The major components that make up the human blood lipid profile include: low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), intermediate-density lipoprotein cholesterol (IDL-C), very-low-density lipoprotein (VLDL-C), and triglycerides (TG). A lipoprotein is a particle in the general blood circulation that is used to transport lipids; cholesterol and triglycerides in particular. The structure of a lipoprotein consists of an outer monolayer of polar phospholipids and free cholesterol. It has a core of nonpolar triglycerides and cholesterol esters. On its surface are integral and peripheral apoproteins that act as identifying and

signaling molecules. The concept of higher or lower density refers to the proportion of lipid to protein, with more protein and less lipid contributing to a higher density (Judge, Phalen, & O'Shea, 2010).

Very-low density lipoprotein (VLDL) is released into the bloodstream by the liver. While in the blood, VLDL matures into intermediate-density lipoprotein (IDL), and then to low-density lipoprotein (LDL). LDL is responsible for transporting cholesterol from the liver to cells. Here, the cholesterol is incorporated into the cell membrane or stored as cholesterol esters. High-density lipoprotein (HDL) is produced by enterocytes and released into the bloodstream. It is responsible for removing any excess cholesterol from cells and bringing it back to the liver (Klop & Cabezas, 2013; Sharma, Farmer, & Garber, 2011; Tonkin & Byrnes, 2014).

According to the Adult Treatment Panel (ATP)-III guidelines from the National Cholesterol Education Program (NCEP), LDL-cholesterol (LDL-C) should be <100 mg/dL, HDL-cholesterol (HDL-C) should be >60mg/dL, and VLDL-cholesterol (VLDL-C) should be 0-29 mg/dL (National Institutes of Health, 2001). The total cholesterol (TC) level in the blood is a sum of the cholesterol within these three lipoproteins. ATP-III guidelines state that total cholesterol (TC) should be <200 mg/dL. The laboratory test for serum triglycerides (TG) is a measure of the total amount of triglycerides present in the LDL, HDL, IDL, and VLDL. A normal serum triglyceride level is considered <150 mg/dL. It is important to recognize that the measurement for serum TGs also detects any TGs absorbed into the bloodstream from recent food intake. Therefore, the measure is most meaningful when the blood sample is from a fasting state.

An aberration from any of the aforementioned values is known as dyslipidemia which indicates an increased risk for developing cardiovascular disease (CVD) (Chan & Cho, 2009; Rudkowska, 2012).

An abnormally high level of triglycerides, or hypertriglyceridemia, is an independent risk factor for developing coronary heart disease (CHD) and ischemic cerebrovascular accident (CVA; stroke) (American Heart Association, 2013). This is largely due to the fact that the highly-atherogenic lipoproteins, VLDL and IDL, are the primary carriers of triglycerides. Thus, a higher triglyceride level is associated with higher levels of these lipoproteins and therefore a higher risk for atherosclerosis. Another mechanism by which high triglycerides are thought to promote CHD is through the competition of triglyceride-rich lipoproteins with HDL, resulting in a decreased production of HDL. This also contributes to an increased density and decreased size of LDL particles, making them more atherogenic (Chan & Cho, 2009; Klop & Cabezas, 2013).

### **Inflammation**

Another indication of CVD risk is inflammation. Inflammation is a process that occurs within the body in response to stress, to the presence of foreign agents such as bacteria, and to trauma. In the case of infection or trauma, inflammation is necessary to assist with destruction of bacteria and promotion of healing. It can be maladaptive, however, in the case of stress. The everyday stresses of modern life can be a major contributor to increased levels of inflammation. The chemical mediators that are released in the process of an inflammatory response can be damaging to body tissues via

oxidation. With stress however, there is no real physiologic benefit from the inflammation to justify this damage. Inflammation from stress also tends to be chronic rather than passing, which can increase risk for such disease processes as hypertension, atherosclerosis, and CVD (Calder, 2013; Dessi, Noce, & Di Daniele, 2013).

The existence and degree of inflammation is commonly assessed using high-sensitivity C-reactive protein (hsCRP) which increases in the presence of inflammation. Normal hs-CRP is any value less than 1 mg/L. The higher the value, the more inflammation is present and the higher the risk for cardiovascular disease (CVD). Unfortunately, this can also indicate acute inflammation as occurs in infection or allergic reactions. Thus, it is not specific enough by itself to necessarily signify the chronic inflammation that relates to chronic diseases such as CVD. There is a laboratory test that is more specific to chronic inflammation called erythrocyte sedimentation rate (ESR), or sed rate, that is very simple and relatively inexpensive to conduct. A normal ESR for males younger than 50 years old is less than 15 mm/hr. For males 50 years old and older, less than 20 mm/hr is considered normal. For females younger than 50 years old a normal value is less than 20 mm/hr, while less than 30 mm/hr is considered normal for females 50 years old and older (National Institutes of Health, 2012).

### **Atherosclerosis**

The progression of CVD often begins with the accumulation of lipids in the vasculature system which then becomes calcified into plaques; a disease process known as atherosclerosis. Atherosclerosis is defined as hardening of the arteries. It is triggered by and exacerbated by a combination of inflammation and elevated levels of cholesterol



in the blood (hypercholesterolemia). Risk factors for developing atherosclerosis include: hypercholesterolemia, hypertriglyceridemia, hypertension, low HDL-C, smoking, and diabetes mellitus (Calder, 2013; Dessi, Noce, & Di Daniele, 2013).

The process of atherosclerosis begins when, for any reason, the endothelial layer of an artery sustains injury. Chemicals called chemo-attractants are subsequently released at the site following the injury. The immune cells, monocytes and leukocytes, migrate to and adhere to the site of injury in response. This process is known as chemotaxis (Calder, 2013). The expression of intracellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) then increases; enhancing the adhesion of monocytes to the endothelial cells. This spurs an inflammatory process in which cytokines are released, attracting the phagocytic cells known as macrophages to the area. These macrophages then engulf circulating particles of LDL and, becoming engorged with lipids, are converted to foam cells (Dessi, Noce, & Di Daniele, 2013). High levels of cholesterol in the blood encourage this step of the process, especially when LDL becomes oxidized due to inflammation. The foam cells infiltrate the damaged endothelium of the artery, forming a fatty streak. Then, smooth muscle cells migrate into the intima layer of the artery and proliferate, forming what is known as a fibrous cap and completing the formation of an arterial plaque (Dessi, Noce, & Di Daniele, 2013).

The more lipid that accumulates in the arteries, the more that they become occluded and the greater the chances are for blockage and ischemia. This is known as arterial stenosis, or thickening of the arteries. The plaques that form are not typically dangerous as long as they are stable. With continuous disturbance via inflammation or

blood pressure changes however, these plaques have the potential to burst. When this happens, the materials that are released can cut off blood supply to muscles and organs. Depending on where the blockage occurs, this can result in thrombosis, skeletal muscle infarction, cerebrovascular accident (CVA; stroke), or myocardial infarction (MI; heart attack). The last two have the potential to become fatal very quickly (Alpert, 2004; Calder, 2013; Dessi, Noce, & Di Daniele, 2013). The goal of nutrition therapy for atherosclerosis is to shrink the size of these plaques and maintain their stability.

Atherosclerotic cardiovascular disease includes coronary heart disease (CHD), peripheral artery disease (PAD), and stroke (cerebrovascular accident; CVA). This disease class represents the number one cause of death around the world. Abnormal levels of blood lipids (dyslipidemia), along with smoking and hypertension, account for as many as 75% of all cases of atherosclerotic cardiovascular disease (Tonkin & Byrnes, 2014).

### **Dietary Fat**

One of the macronutrients that makes up the natural human diet is dietary fat, also known as lipids. Consuming fat from the diet is necessary for the purpose of providing essential fatty acids. It is also needed to sustain lipid storage within adipose tissue. Adipose tissue is beneficial for its contribution to body temperature control, organ protection, and providing for energy needs in certain situations. During carbohydrate deprivation or starvation, fat can be burned for energy in order to spare the protein in muscle. The Acceptable Macronutrient Distribution Range (AMDR) for dietary fat is 25-35% of total kilocalories for healthy individuals. It is important to recognize that it is the

type and quality of the fat consumed that matters, more so than the quantity (Scholl, 2012).

The fat in the food we consume exists in the form of triglycerides. When a fat-containing meal is consumed, the fat is emulsified by bile from the liver to enhance its digestibility (Klop & Cabezas, 2013). Then, these triglycerides are lipolyzed, or broken down, by lipase enzymes from the pancreas into free fatty acids (FFA) and 2-monoacylglycerols (2-MAG). The enterocytes that line the small intestine take up these components via passive diffusion and transporters such as CD36. Within the enterocyte, the FFAs and 2-MAGs are reassembled into triglycerides (Klop & Cabezas, 2013). At this point, any cholesterol that is consumed is also taken up by the enterocyte and converted into cholesterol esters. Then, the triglycerides and cholesterol esters are packaged into chylomicrons with phospholipids and apolipoprotein B48 (apoB48). The chylomicrons are released from the enterocyte into the lymph system where they are transported to blood circulation through the thoracic duct. They then are taken up by the liver where they are used to manufacture very low density lipoprotein (VLDL). VLDL has a similar structure to chylomicrons, though apolipoprotein B100 (apoB100) is involved rather than apoB48. VLDL is then released into the bloodstream by the liver where it is converted to IDL and then to LDL (Klop & Cabezas, 2013).

As mentioned previously, triglycerides have three fatty acids attached to a glycerol backbone. The two major classifications for fatty acids are saturated fatty acids and unsaturated fatty acids. A saturated fatty acid only has single carbon-to-carbon bonds and no double-bonds. They are solid at room temperature. With regards to blood lipid

levels, saturated fatty acids have been shown to elevate levels of cholesterol in LDL and HDL. Common dietary sources of saturated fatty acids are flesh from animals, fat-containing dairy products, and tropical oils. The recommended level of intake for saturated fat is less than 10% of total kilocalories (Skerrett & Willett, 2010).

An unsaturated fatty acid possesses one or more carbon-to-carbon double bonds. They are liquid at room temperature. Generally, these are reported to decrease blood levels of LDL-C (Mozaffarian, Micha, & Wallace, 2010). Within the category of unsaturated fatty acids are monounsaturated fatty acids and polyunsaturated fatty acids. A monounsaturated fatty acid has one carbon-to-carbon double bond at some point along its chain. A polyunsaturated fatty acid is one which possesses two or more carbon-to-carbon double bonds along the length of its chain (Anderson & Ma, 2009; Skerrett & Willett, 2010).

An additional category is *trans* fatty acids. These originate as unsaturated fatty acids but are chemically altered to mimic saturated fatty acids with a process called hydrogenation. Thus, they are solid at room temperature. This is intended to increase stability. These have been shown to not only increase blood levels of LDL-C, but also to decrease HDL-C levels in the blood (Michas, Micha, & Zampelas, 2014). Typically, they do not naturally occur in food and are often added to food during processing to prolong shelf-life. *Trans* fats are to be completely avoided in the diet for optimal health (Michas, Micha, & Zampelas, 2014; Skerrett & Willett, 2010).

Polyunsaturated fatty acids can be further classified as omega-3 or omega-6, depending on the position of the first carbon-to-carbon double bond with respect to the

methyl terminus. Linoleic acid (LA) is the parent fatty acid of the omega-6 subgroup and alpha-linolenic acid (ALA) is the parent fatty acid of the omega-3 subgroup. Both are essential, meaning that they cannot be produced by the human body and must be obtained from the diet (Anderson & Ma, 2009). LA is found in vegetable oils, seeds and nuts and ALA is found in leafy vegetables, walnuts, soybeans, flaxseed, and seed and vegetable oils. LA and ALA can be further converted via elongation and desaturation into long-chain polyunsaturated fatty acids which are physiologically active within the body (Deckelbaum & Torrejon, 2012). LA is metabolized to arachidonic acid (AA), while ALA can be metabolized to eicosapentanoic acid (EPA) and then to docosahexanoic acid (DHA). Unfortunately, these conversions from LA and ALA do not happen to a significant extent within the body and these fatty acids are often used for energy instead. Thus, obtaining the longer-chain derivatives directly from food is typically a much more efficient way of maintaining adequate levels within the body (Deckelbaum & Torrejon, 2012). AA can be obtained from consuming animal fat and dairy fat. EPA and DHA can be obtained from consuming marine products such as fish, mussels, oysters, and seaweed (Anderson & Ma, 2009). A proper balance of omega-3 fatty acids to omega-6 fatty acids is important for optimal health. A ratio between 1:1 and 4:1 has been shown to be beneficial. Furthermore, a ratio of polyunsaturated fatty acids to saturated fatty acids between 1.5:0 and 2:0, or greater, is ideal (Houston, 2014).

Table 1. Adequate Intake (AI) for LA and ALA (Houston, 2014)

Age/Lifestage	LA AI (g/day)	ALA AI (g/day)
0-6 months	4.4	0.5
7-12 months	4.6	0.5
1-3 years	7	0.7
4-8 years	10	0.9
9-13 years	Male: 12, Female: 10	Male: 1.2, Female: 1
14-18 years	Male: 16, Female: 11	Male: 1.6, Female: 1.1
19-50 years	Male: 17, Female: 12	Male: 1.6, Female: 1.1
>50 years	Male: 14, Female: 11	Male: 1.6, Female: 1.1
Pregnancy	13	1.4
Lactation	13	1.3

There is not currently an established AI for EPA or DHA. It is important to note that these AI's are based only on preventing deficiency, not optimal intake. Also, they do not provide a recommended ratio of omega-3 to omega-6 fatty acids (4:1, 6:3, or 1:1; 4:1 is optimal) (Houston, 2014).

### Omega-3 Fatty Acids

The structural characteristic that makes a polyunsaturated fatty acid an omega-3 fatty acid, is the double bond between carbon molecules at the third position from the methyl terminus of the fatty acid chain. The majority of research involving omega-3 fatty acids has focused on three subtypes in particular: alpha-linolenic acid (ALA), eicosapentanoic acid (EPA), and docosahexanoic acid (DHA) (Chan & Cho, 2009).

Humans do not possess the necessary metabolic pathways to intrinsically generate the essential fatty acid known as ALA. ALA can, however, be obtained from the diet and be subsequently converted to EPA and DHA. Even so, this pathway occurs at such a low rate (about 5% to EPA and less than 0.5% to DHA) that it is more effective to obtain EPA and DHA directly from food (Chan & Cho, 2009; Dessi, Noce, & Di Daniele,

2013). EPA and DHA are highly prevalent in fatty fish, which store fat as triglycerides in their flesh rather than in their livers like non-fatty fish (Gazi et al, 2006). Fatty fish typically inhabit cold water (Deckelbaum & Torrejon, 2012). They include but are not limited to: salmon, mackerel, sardines, anchovies, herring, trout, halibut, and tuna. Although they are not technically considered fish, oysters and mussels are also included in this category (Albert, Cameron-Smith, Hofman, & Cutfield, 2013).

EPA is a component of tissues in the body and it may be protective against heart disease by reducing inflammation. DHA is a major component of the central nervous system and the retina. It is therefore necessary for optimal brain and eye development and maintenance (Albert et al, 2013). Adequate intake has been linked to a decreased risk for depression and suicide attempt, as well as with potentially reducing the speed and severity of cognitive decline in older age (Albert et al, 2013; Deckelbaum & Torrejon, 2012).

Research suggests that ALA does not convey the same benefits as its longer-chain metabolites, EPA and DHA. Furthermore, the conversion of ALA to EPA and DHA is not efficient enough to prevent deficiency. Therefore, it appears that it is necessary to consume EPA and DHA directly in order to fully obtain their respective health benefits (Anderson & Ma, 2009; Dessi, Noce, & Di Daniele, 2013).

### **Fish Oil Supplements**

Aside from consuming whole fish, a common and readily available source of the omega-3 fatty acids EPA and DHA is fish oil in the form of a gel capsule supplement. It has been estimated that over one-third of American adults use some form of marine

omega-3 fatty acid supplement (Albert et al, 2013). Though obtaining these fatty acids through eating fatty fish is ideal, it is often not possible to eat the recommended amount on a consistent basis due to factors such as taste preference, location, and cost. A dose of 500 mg/day of EPA plus DHA is equivalent to approximately 2 servings of fish per week. This also fulfills the recommendation regarding fatty fish intake for healthy people from the American Heart Association (AHA). For individuals with coronary artery disease (CAD), the AHA recommends 1000 mg/day of EPA plus DHA. This is equivalent to approximately 4 servings of fish per week. (Chan & Cho, 2009).

The standard concentration of EPA and DHA in a fish oil supplement is about 30%, meaning that a 1000 mg capsule contains a combined total of 300 mg EPA and DHA (Calder, 2013). An EPA to DHA ratio of 3-4 to 2 in fish oil supplements has been shown to be most beneficial (Houston, 2014). For those with serum triglyceride levels greater than 500 mg/dL, the AHA recommends taking an omega-3 acid ethyl esters medication (i.e. Lovaza). This contains 465 mg of EPA and 375 mg of DHA in a 1000 mg capsule and the recommended dosage is two to four times per day. This high concentration (84% EPA and DHA) is not currently available over the counter and must be obtained with a prescription. Effective doses in research are typically between 3000 and 5000 mg per day (Gazi et al, 2006).

Fish oil supplements have been widely recognized for their benefits in cardiovascular health. They have been shown to lower serum triglyceride levels, as well as reduce blood pressure and inflammation. Interestingly, this reduction in inflammation is sometimes but not always represented by a reduced high-sensitivity C-reactive protein



(hs-CRP) level (Chan & Cho, 2009; Dessi, Noce, & Di Daniele, 2013; Swanson, Block, & Mousa, 2012). Rather, the reduction in inflammatory processes is represented by inhibited leukocyte chemotaxis, decreased expression of adhesion molecules, decreased eicosanoid and cytokine production, and reduced T cell reactivity (Anderson & Ma, 2009; Calder, 2013; Chan & Cho, 2009, Gazi et al, 2006). Additionally, some studies suggest a benefit in reducing cardiac arrhythmias from fish oil supplementation (Chan & Cho, 2009; Gazi et al, 2006).

As with all dietary supplements, the use of fish oil can potentially have some negative side effects, particularly when taken in excess. Some individuals may experience gastrointestinal distress. Another common complaint is the fishy-odor that can arise with belching. Many capsules today have an enteric coating that prevents this minor side effect. Another potential side effect is worsened glycemic control in those with diabetes (Gazi et al, 2006; Chan & Cho, 2009). Also, fish oil is known to be an anticoagulant through its action to inhibit the arachidonic acid pathway. As a result, those who are planning to have surgery are often asked to discontinue taking fish oil for a month before the surgery to reduce risk of uncontrollable bleeding (Gazi et al, 2006; Chan & Cho, 2009).

Table 2. Side Effects Associated with Fish Oil Supplementation (Gazi et al, 2006)

Side Effect	Fish Oil Supplement Daily Dose		
	<1000 mg/day	1000-3000 mg/day	>3000 mg/day
Fishy aftertaste	Low risk	Moderate risk	Likely
Gastrointestinal upset	Very low risk	Moderate risk	Moderate risk
Clinical bleeding	Very low risk	Very low risk	Low risk
Poorer glycemc control*	Very low risk	Low risk	Moderate risk
Elevated LDL-C†	Very low risk	Moderate risk	Likely

\*In individuals with impaired glucose tolerance (IGT) or diabetes mellitus (DM)

†In individuals with hypertriglyceridemia

It is important to consider the potential for fish oil supplements, as well as whole fish, to contain methylmercury (MeHg) which is a potentially harmful contaminant. MeHg is considered a neurotoxin, meaning that it is damaging to cells in the nervous system. When exposure occurs during the developmental stage of life, particularly the prenatal period, defects in cognitive development and function have been documented. In adults, sensory disorders and tremors have been reported with MeHg exposure (Ceccatelli, Daré, & Moors, 2010). Choosing a purified supplement and lower-mercury fish may be helpful in reducing risk for consumption of this substance (Gazi et al, 2006). Examples of lower-mercury fish that are also good sources of EPA and DHA include: salmon, mackerel (non-King variety), sardines, anchovies, herring, and trout (Mahaffey, 2008).

Unfortunately, the world's existing population of cold water fatty fish may not be enough to sustain Europeans and North Americans with the recommended levels of EPA and DHA intake (Deckelbaum & Torrejon, 2012; Raatz, Silverstein, Jahns, & Picklo, 2013). Interestingly, like humans, fatty fish are also unable to generate omega-3 fatty

acids on their own. Their stores actually originate from the single cell marine organisms and marine plants that they consume, such as algae, krill, and seaweed (Deckelbaum & Torrejon, 2012; Gazi et al, 2006; Lenihan-Geels, Bishop, & Ferguson, 2013; Raatz, Silverstein, Jahns, & Picklo, 2013). Looking to these alternative and more sustainable sources may be necessary to maintain future needs. At the current time, these sources are significantly more expensive to cultivate than fish. Developing more efficient cultivation methods will be critical to ensuring long-term feasibility and sustainability (Deckelbaum & Torrejon, 2012; Lenihan-Geels, Bishop, & Ferguson, 2013).

The supplemental form of coconut oil has been used in past research studies as a placebo for fish oil. Its use has been justified by the fact that it does not contain polyunsaturated fatty acids and it does not alter the metabolism of omega-3 polyunsaturated fatty acids (Amminger et al, 2013; Karr, Grindstaff, & Alexander, 2012; Mozaffari-Khosravi, Yassini-Ardakani, Karamati, & Shariati-Bafghi, 2013; Trepanowski, Kabir, Alleman, & Bloomer, 2012).

### **Fish Oil Supplementation and Cardiovascular Disease**

Cultures with relatively high fish intake, such as the Inuit populations of northern Canada and Alaska, have lower incidence and prevalence than what would be expected when considering their high overall fat intake. Similarly, the Japanese traditionally consume a diet rich in seafood, as well as in sodium. Nevertheless, the prevalence of CVD in Japan is low when compared with many other countries. The common factor in these two cultures is a high intake of seafood (up to 5000-15,000 mg of EPA and DHA

per day), suggesting that this may be protective against CVD development (Gazi et al, 2006).

### **Fish Oil Supplementation and Blood Lipids**

With regards to medical nutrition therapy, common recommendations for preventing and treating cardiovascular disease have been a reduced intake of omega-6 fatty acids and saturated fatty acids, while increasing intake of polyunsaturated fatty acids. Omega-3 polyunsaturated fatty acids have been thoroughly examined in particular (de Lorgeril & Salen, 2012).

Epidemiological research has shown that Greenland Eskimos, who are known to consume a relatively high amount of omega-3 fatty acids through fish, have lower incidence of cardiovascular disease (Bjerregaard & Johansen, 1987). Since this discovery about three and a half decades ago, the potential cardiovascular health benefits of fish oil have been widely studied and documented (Chan & Cho, 2009).

One mechanism by which EPA and DHA work to lower triglyceride levels has been seen in both animal and human studies. This involves decreasing VLDL synthesis in the liver and increasing its release into the bloodstream (Chan & Cho, 2009; Gazi et al, 2006; Weitz, Weintraub, Fisher, & Schwartzbard, 2010). It has been found that the conversion to IDL and then to LDL occurs more quickly with increased intake of EPA and DHA, explaining the increase in LDL-C that often occurs with fish oil supplementation (Gazi et al, 2006). Another theory is that EPA and DHA increase the activity of lipoprotein lipase which results in increased chylomicron clearance from the bloodstream. Studies have shown that the higher the dose and the higher the baseline

serum triglyceride level, the greater the effect that fish oil appears to have (Chan & Cho, 2009; Dessi, Noce, & Di Daniele, 2013; Judge, Phalen, & O'Shea, 2010; Weitz, Weintraub, Fisher, & Schwartzbard, 2010). This is particularly true with prescription-concentration fish oil (i.e. Lovaza) for very high triglyceride levels (Chan & Cho, 2009). A third mechanism by which EPA and DHA are thought to lower triglyceride levels in the blood is by decreasing triglyceride synthesis, possibly through inhibition of the sterol regulatory element-binding protein (SREBP) pathway. Finally, EPA and DHA appear to increase triglyceride clearance from the blood by the liver (Anderson & Ma, 2009).

A study by Pownall et al. found that in participants with hypertriglyceridemia, those receiving the therapeutic dose of 4000 mg fish oil per day experienced a 38.9% decrease serum triglyceride levels. Those receiving the placebo had a 7.8% decrease. There was no significant effect on HDL-C, but there was a 16.7% increase in LDL-C with fish oil (1999).

A meta-analysis found that with every 1000 mg increase in fish oil dosing, serum triglyceride decreases by about 8 mg/dL. Of those with high baseline triglyceride levels the reduction was even more dramatic, with an average serum triglyceride decrease of 27 mg/dL, an average HDL-C increase of 1.6 mg/dL, and an average LDL-C increase of 6 mg/dL. There was no significant change in total cholesterol levels (Balk et al, 2006).

An analysis of 36 crossover studies found that a fish oil dosing of 3000 to 4000 mg per day decreased serum triglyceride levels by 24% in participants with normolipidemia and 35% in participants with hypertriglyceridemia. A 5-10% increase in

LDL-C and a 1-3% increase in HDL-C was also seen. The association between fish oil and triglycerides appeared to be dose-dependent (Harris, 1997).

A clinical trial examined the use of highly concentrated omega-3 acid ethyl esters in 42 participants who had severe hypertriglyceridemia with serum levels between 500 and 2000 mg/dL. With the dose at 4000 mg per day, there was a 45% decrease in triglycerides, a 15% decrease in total cholesterol, a 32% decrease in VLDL-C, a 13% increase in HDL-C, and a 31% increase in LDL-C (Harris et al, 1997).

Another trial was made up of 30 participants with triglyceride levels between 354 and 2478 mg/dL. A daily dose of 4000 mg/dL of omega-3 fatty acids produced a similar effect on blood lipids and lipoproteins as a 1200 mg daily dose of gemfibrozil.

Interestingly, although LDL-C was increased by both therapies, the LDL particles became larger, more buoyant, and thus less atherogenic as a result (Dessi, Noce, & Di Daniele, 2013; Gazi et al, 2006).

A review of 21 trials found that fish oil consumption resulted in a net change of -21 mg/dL for triglycerides, +1.6 mg/dL for HDL-C, and +6 mg/dL for LDL-C. Larger doses of fish oil were associated with greater effects on serum triglyceride levels. No net effect on total cholesterol was found (Gazi et al, 2006).

### **Pharmacological Therapy vs. Fish Oil Supplementation for Hyperlipidemia**

A commonly utilized pharmaceutical therapy for hyperlipidemia is a medication class known as a statin. Also known as HMG-CoA-reductase inhibitors, these drugs are fairly efficacious in reducing LDL-C, but only have a moderate effect on reducing triglycerides (Chan & Cho, 2009). Fish oil has been demonstrated to lower serum

triglyceride levels and increase LDL-C levels. It is important to note, however, that the amount LDL-C increases is only about 6-10 mg/dL and the size and buoyancy of the LDL particles is also increased, making them less likely to be atherogenic (Chan & Cho, 2009). A randomized, placebo-controlled study demonstrated that in obese men, a combination therapy of 40 mg of atorvastatin and 4000 mg fish oil resulted in a 40% decrease in serum triglycerides (P=0.002). This is compared to atorvastatin alone which had a 26% decrease and fish oil alone which had a 25% decrease. LDL-C levels decreased to a similar degree in both the combination therapy group and the atorvastatin group (Chan, Watts, Nguyen, & Barrett, 2002). The use of fish oil along with a statin may be a more effective therapy for those with both high LDL-C and high serum triglyceride levels than simply a statin alone (Athyros, Tziomalos, & Mikhailidis, 2011; Chan & Cho, 2009).

Another important issue to consider is that the use of statins is associated with adverse side effects in some individuals. The risk for side effects appears to be dose-dependent, though this may vary across individuals due to differences in genetic components and disease states. Drug or drug-nutrient interactions may also increase the potency, and thus the risk of side effects, for statins. Foods that can potentially interact with statins are grapefruit, pomegranate, and the Seville orange. One potential side effect of statins that is especially feared is rhabdomyolysis, a severe form of muscle damage that results in elevated creatinine kinase (CK) and can lead to renal failure and even death. Other adverse side effects that have been reported include: cognitive loss, myositis (muscle inflammation), myalgia (muscle pain), nerve damage (neuropathy), pancreatic

dysfunction, hepatic dysfunction, and sexual dysfunction (Golomb & Evans, 2008). If, because of fear for these side effects, an individual decides to discontinue or never initiate use of statins, having an alternative option may be very important to managing their lipid levels and potentially avoiding life-threatening consequences.

### **Fish Oil Supplementation and Inflammation**

The omega-6 fatty acid arachidonic acid (AA) is essential and contributes to cellular function when it is metabolized into prostaglandins, leukotrienes, and thromboxanes, a group that is collectively called eicosanoids (Anderson & Ma, 2009; Chan & Cho, 2009). Some of these metabolites have pro-inflammatory characteristics, while others can contribute to arrhythmia. It is important to note that EPA is competitive with AA for the same enzymatic pathway. It can also be converted to these eicosanoids. However, the products from EPA's conversion are far less inflammatory than those produced by conversion of AA. Certain products from DHA, specifically resolvins and protectins, are even considered to be anti-inflammatory (Buckley & Howe, 2010; Dessi, Noce, & Di Daniele, 2013; Calder, 2013). As a result of competition with AA, EPA results in decreased production of AA-derived eicosanoids. This then reduces platelet aggregation, vasoconstriction, and leukocyte chemotaxis and adherence. Ultimately, this reduces the potential risk for plaque formation, thrombus formation, and ischemic events (Anderson & Ma, 2009, Chan & Cho, 2009, Gazi et al, 2006).

Beyond EPA's competition with AA, fish oil is associated with a reduction in inflammation through its inhibition of pro-inflammatory factors. EPA can inhibit interleukin-2 (IL-2) production and both EPA and DHA can inhibit interleukin-1B (IL-



1B), tumor necrosis factor alpha (TNF $\alpha$ ), interleukin-6, and interleukin-8 production (Anderson & Ma, 2009).

Although fish oil has long been heavily touted anti-inflammatory properties, there has been limited biochemical evidence to demonstrate this effect in previous studies. A meta-analysis by Balk et al. (2006) examined four studies and found a minimal net change in hs-CRP that ranged from  $-0.15$  to  $+1.7$  mg/L with fish oil doses between 1600 g/day and 5900 g/day. Perhaps looking at sed rate rather than hs-CRP would provide a clearer picture of how fish oil may influence inflammation.

With regards to atherosclerosis, a major mechanism by which EPA and DHA are thought to function has to do with the inflammatory processes that promote and aggravate atherogenesis. A study by Dwyer et al. (2004) showed that high omega-3 fatty acid intake reduces the function of leukotriene-mediated inflammatory pathways. Also, EPA and DHA have been found to inhibit the expression of intracellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells and monocytes. This impedes the atherosclerotic pathway, thereby reducing the risk for myocardial infarction and stroke. Additionally, it has been demonstrated that EPA and DHA reduce the generation of the chemoattractants involved in atherogenesis. These include: leukotriene B<sub>4</sub>, platelet-activating factor (PAF), monocyte chemoattractant protein-1 (MCP-1), platelet-derived growth factor (PDGF), interleukins, and tumor necrosis factor-a (TNF $\alpha$ ). (Gazi et al, 2006).

## **Fish Oil Supplementation and Atherosclerosis**

As has been discussed, fish oil supplementation has been shown to reduce the risk for developing atherosclerosis through its impact on blood lipids and inflammation. There is also evidence to suggest that EPA and DHA from fish oil improve the state of preexisting atherosclerosis. Although fish oil supplementation does not prevent recurrence of stenosis after angioplasty, it may help to stabilize existing arterial plaques. One study found that participants who received 1400 mg of fish oil supplementation before carotid endarterectomy had higher levels of EPA and DHA in their carotid plaques when compared with the control group. This was associated with thicker fibrous caps of the plaques, decreased inflammatory markers, and lower number of arterial wall macrophages. This translates to a reduced risk for atherosclerosis-related complications (Thies et al, 2003).

## **Fish Oil Supplementation and Cardiovascular Function**

There is some evidence to suggest that fish oil may reduce cardiac arrhythmia in those with cardiovascular disease (CVD). This is hypothesized to occur through stabilization of the myocardial membrane (Chan & Cho, 2009; Dessi, Noce, & Di Daniele, 2013). When subjected to ischemia, or restriction of blood supply, myocytes allow potassium ions to escape. This increases the resting membrane potential and lowers the threshold for initiating an action potential through sodium channels, resulting in an increased risk for fatal arrhythmias. Omega-3 fatty acids are thought to inhibit sodium channels by increasing the fluidity of the phospholipid bilayer. This then reduces membrane excitability and the potential for arrhythmia (Chan & Cho,

2009; Dessi, Noce, & Di Daniele, 2013). The studies that have been conducted on this theory have been specific to individuals with an implanted cardioverter-defibrillator (ICD), or pacemaker. While Raitt et al. (2005) found a non-significantly greater incidence of ventricular tachycardia and fibrillation in those receiving 1800 mg fish oil versus a placebo (65% versus 59%;  $p=0.19$ ), Leaf et al. (2005) demonstrated a lower incidence in ventricular arrhythmias with 4000 mg fish oil when compared to the placebo (28% versus 39%;  $p=0.057$ ). Furthermore, Calo et al. (2005) randomized 160 patients, who were 5 days away from undergoing elective coronary artery bypass, into two groups: treatment with 2000 mg fish oil or a placebo. Those in the fish oil group had a significantly lower incidence of arrhythmia after bypass when compared to the placebo group (15.2% versus 33%;  $p=0.013$ ).

Fish oil may have some impact on blood pressure, though its effectiveness as an actual therapy for high blood pressure is minimal. EPA and DHA are incorporated into the membranes of endothelial cells, increasing fluidity and allowing for improved vasodilation through increased nitric oxide production (Chan & Cho, 2009; Dessi, Noce, & Di Daniele, 2013). Some research has demonstrated a decrease in heart rate as a result of this effect in individuals with high baseline heart rate (Dessi, Noce, & Di Daniele, 2013). A meta-analysis found a 2.1 mmHg average reduction in systolic blood pressure and a 1.6 mmHg average reduction in diastolic blood pressure with a median fish oil supplementation of 3700 mg. The greatest reductions were seen in individuals with hypertension and in those over the age of 45 (Geleijnse, Giltay, Grobbee, Donders, & Kok, 2002). A 3.4/2.0 mmHg reduction in blood pressure was found in those with

hypertension with a 5600 mg fish oil dose per day (Morris, M. C., Sacks, F., & Rosner, B. 1993). A 5.5/3.5 mmHg blood pressure reduction was seen in untreated hypertensive individuals with a fish oil dose of  $\geq 3000$  mg per day (Appel, Miller, Seidler, & Whelton, 1993). DHA seems to have a greater effect than EPA in blood pressure reduction and there does not seem to be a reduction in blood pressure with fish oil supplementation for healthy individuals without hypertension (Gazi et al, 2006).

In a review of four trials, a reduced incidence of secondary cardiac events was seen with fish oil supplementation between 1000 and 1800 mg per day or with 1 serving of fish per day (Anderson & Ma, 2009).

The Diet and Reinfarction Trial (DART) focused on secondary prevention of a subsequent myocardial infarction (MI; heart attack) with participants who had already survived one. In male participants who were recommended to increase their fatty fish intake to 7-14 ounces (2.33-4.67 servings) per week and their fish oil intake to 500-800 mg per day, there was a 29% reduction in mortality from any cause over a 2-year period ( $p < 0.05$ ). There was no difference in the incidence of overall CVD events between those who received these recommendations and those who did not. However, there was a 33% reduction in fatal MIs when these recommendations were provided ( $p < 0.01$ ) (Burr et al, 1989).

A randomized placebo-controlled prospective study, known as the Indian Experiment and Infarct Survival-4 study, examined the use of mustard oil (2900 mg ALA) and fish oil (1800 mg EPA and DHA) for secondary prevention in the hospital following a suspected acute myocardial infarction. When compared to the placebo group

one year later, there was a reduction in total cardiac events and non-fatal myocardial infarctions in the mustard oil ( $p < 0.05$ ) and fish oil ( $p > 0.01$ ) groups (Singh et al, 1997).

A meta-analysis of 11 randomized controlled trials found a 27% decrease in fatal myocardial infarctions ( $p < 0.001$ ), a 31% reduction in sudden death ( $p < 0.01$ ), and a 19% decrease in mortality from any cause ( $p < 0.001$ ) with dietary or supplemental EPA and DHA intake. It was noted that there was no difference in the occurrence of non-fatal myocardial infarctions between groups (Bucher, Hengstler, Schindler, & Meier, 2002).

Three mechanisms by which EPA and DHA are thought to decrease CVD risk is by increasing the fluidity of cellular membranes, decreasing the proportion of the membrane occupied by lipid rafts, and modifying the characteristics of these rafts (Deckelbaum & Torrejon, 2012).

### **Cerebrovascular Disease**

Cerebrovascular disease is defined as a disruption in normal brain function related to an abnormality involving the group of blood vessels that supply the brain. Within this category are two specific insults to the brain: transient ischemic attack (TIA) and cerebrovascular accident (CVA; stroke) (Sonni & Thaler, 2013; Sylaja & Hill 2009).

A TIA, often called a “mini stroke”, is defined as a temporary obstruction of blood flow to the brain that typically lasts only a few minutes but has the tendency to recur. A TIA is not usually fatal or permanently damaging on its own. However, it has the likelihood of leading to a potentially fatal CVA, or stroke. This is particularly true when the episode lasts longer than 10 minutes (Sonni & Thaler, 2013; Sylaja & Hill, 2009). Approximately 15-20% of individuals who have had a stroke had previously

experienced a TIA, usually around three months prior. Research suggests that the early window of risk for stroke is 10-15% within ninety days of a TIA (Sylaja & Hill, 2009).

A cerebrovascular accident (CVA), or stroke, occurs when an artery that provides oxygenated blood to the brain is completely obstructed for an extended period of time. This leads to damage of the brain tissue and cells and has the risk of resulting in functional or cognitive impairment (Sonni & Thaler, 2013). Stroke is the third leading cause of death in the United States and is the second leading cause of death worldwide. Additionally, it is the first leading cause of long-term disability around the world. One-third of individuals die within the first year of having a stroke and more than half are unable to recover and regain their independence (Paciaroni & Bogousslavsky, 2010).

As mentioned previously, stroke falls within the disease class of atherosclerotic cardiovascular disease (CVD). Thus, many of the same risk factors for general CVD apply to cerebrovascular disease and stroke as well. These include advanced age, dyslipidemia, smoking, physical inactivity, hypertension, atrial fibrillation, arterial stenosis (specifically of the carotid artery), and diabetes (Paciaroni & Bogousslavsky, 2010; Sonni & Thaler, 2013; Sylaja & Hill, 2009).

### **Fish Oil Supplementation and Cerebrovascular Disease**

In much the same way that fish oil is thought to reduce cardiovascular disease (reduced inflammation, a favorable blood lipid profile, etc.), fish oil is also thought to reduce the risk for cerebrovascular disease. Some epidemiological evidence suggests a decrease in ischemic cerebrovascular accident (CVA), or stroke, risk with increasing fish consumption. It is important to consider, however, that the risk or severity of

hemorrhagic stroke may be increased with excessively high doses of fish oil due to the potential anticoagulative effect (Gazi et al, 2006).

### **Blood Type**

The major blood types in humans are A, B, AB, and O. Of these, A and O are the most common. The distinguishing characteristic that places an individual into one of these categories is the presence or lack of specific components called antigens. These antigens are sugar molecules that are embedded in the cell membrane of erythrocytes, or red blood cells. The A antigen is present in blood type A, B antigen in blood type B, A and B antigens in blood type AB, and neither of the antigens in blood type O (Ketch et al, 2008).

To identify an individual's blood type, blood is drawn by either a syringe or a finger prick. The blood that is collected is placed on a surface in at least two separate locations. One sample is mixed with A antibodies and the other is mixed with B antibodies. Additional antibodies may be tested in separate samples with some tests to further classify blood type. If the blood agglutinates, meaning that the erythrocytes clump together, the blood type is not compatible with the antibodies that were added. For example, if blood agglutinates when B antibodies are added but not when A antibodies are added, the blood type is A. If the blood agglutinates when both A antibodies and B antibodies are added, the blood type is O. Blood type B reacts to A antibodies and AB blood type reacts to neither. This identification process is known as ABO typing (Flegel, 2013; Lee, Park, Yang, Lee, & Kim, 2009).

Although there are numerous ways to classify blood type, the ABO system is the most widely used. It is particularly useful in ensuring the acceptance of foreign tissue; a critical consideration in blood transfusion and organ transplant. Unmatched blood type triggers an immune response, along with agglutination of the blood within the body which can quickly become fatal (Ketch et al, 2008).

### **Blood Type and Cardiovascular Disease Risk**

The blood type of an individual is a strong predictor of hemostasis qualities such as clotting time and risk for embolism formation. This is largely due to differences between blood types in amounts of von Willebrand factor (vWF) in blood plasma, and thus, Factor VIII plasma levels. Both vWF and Factor VIII are considered procoagulant, or clot-promoting, agents and are known to increase risk for embolism formation and thrombosis. Blood type O has been found to have a reduced coagulability when compared to the non-O blood types A, B, and AB (Dentali, Sironi, Ageno, Crestani, & Franchini, 2014; Ketch et al, 2008; Souto et al, 2000). Non-O blood type indicates that levels of these factors are about 25% higher than those associated with the blood type O. It has been established that individuals with non-O blood type are at an increased risk for developing a venous thromboembolism when compared to blood type O. There is also a recently discovered positive association, though it is weak, between non-O blood type and risk for arterial thrombotic events. These events include myocardial infarctions (MI; heart attack), ischemic cerebrovascular accidents (CVA; stroke), and muscle ischemia (Dentali et al, 2014; Ketch et al, 2008).



In individuals who have experienced an MI, the amount of risk estimated to be conferred by non-O blood type is approximately 9.7%. A systematic review by found a consistent relationship between non-O blood type and increased likelihood for an MI (Wu, Bayoumi, Vickers, & Clark, 2008). A large meta-analysis by He et al. (2012) demonstrated that those with a non-O blood type were 11% more likely to develop coronary heart disease (CHD) when compared to O blood type individuals. There was also a significantly greater proportion of non-O blood type in those who had experienced an MI, when compared to blood type O.

The evidence in support of the association between ABO blood type and ischemic CVA, or stroke, is less conclusive. A large case-control study by Hanson et al did not find a link between ABO blood type and ischemic stroke risk. On the other hand, the meta-analysis by Wu, Bayoumi, Vickers, & Clark found a slight but significant association between these variables (2008). Although there appears to be a slightly increased risk for ischemic stroke with non-O blood type, the relationship is not well-supported and thus additional research is needed to confirm it (Dentali et al, 2014).

### **Fish Oil Supplementation and Blood Type**

Fish oil is considered to be an antithrombotic substance, meaning that adequate fish oil intake is associated with reduced tendency for blood clotting and coagulation. This occurs through suppression of platelet cyclooxygenase metabolism, thus reducing thrombin-stimulated platelet aggregation (Keenan, Pedersen, & Newman, 2012). Based on this characteristic, it is expected that fish oil supplementation will mitigate the heightened tendency for blood clotting and thrombosis in non-O blood type.

## **Insulin**

Insulin is an anabolic polypeptide hormone that is produced by the  $\beta$ -cells in the pancreas (Islets of Langerhans). It is released into the blood in response to consumption of a meal containing glucose, the main source of energy within the body (Triplett, 2012). From the pancreas, insulin travels in the blood to cells in need of glucose. Every cell possesses insulin receptors which are embedded within the cell membrane but are accessible outside of the cell. Insulin itself never actually enters the cell; it only binds to these insulin receptors on the outside of the cell. This activates a secondary messenger system which results in the uptake of glucose into the cell via specialized transport proteins in the membrane, such as GLUT-1 and GLUT-4. Plasma glucose concentrations are reduced as a result (Seino, Shibasaki, & Minami, 2011; Triplett, 2012). The glucose transported into the cell is then assembled into chains which create the storage form of glucose known as glycogen (Triplett, 2012). Beyond its function to bring glucose into cells, insulin has additional functions within the body. One other function that it carries out is the inhibition of hepatic glycogen breakdown to glucose. Also, it prevents the breakdown of lipids from adipose tissue, a process called lipolysis (Kalupahana, Claycombe, & Moustaid-Moussa, 2011).

## **Insulin resistance**

Insulin resistance is defined by a lack of response or an inadequate response in cells which are normally quite sensitive to insulin. These cells include hepatocytes, skeletal myocytes, and adipocytes (Kalupahana, Claycombe, & Moustaid-Moussa, 2011). One example of insulin resistance arises when insulin is unable to bind insulin

receptors on the outer membrane of cells. Another scenario in which a person would be considered insulin resistant is when insulin binds the receptor but the transport of glucose into the cell is defective. When cells are resistant to insulin, glucose is unable to enter the cell and it remains in the plasma. As a result, plasma glucose concentrations become elevated and the cells are starved of glucose (Seino, Shibasaki, & Minami, 2011). If this goes on for an extended amount of time, weight loss occurs and the body resorts to another source of energy known as ketones. Weight reduction, exercise, and certain medications can reduce insulin resistance (American Diabetes Association, 2010). To detect insulin resistance, two simple equations are used: the homeostatic model assessment (HOMA-IR) and the QUICKI. HOMA-IR is a direct measure of insulin resistance. An increased HOMA-IR score means that insulin resistance is increased. A score  $>1$  is considered abnormal and a sign of insulin resistance (Matthews et al, 1985; Muniyappa, Lee, Chen, & Quon, 2008). QUICKI, on the other hand, is a measure of insulin sensitivity rather than insulin resistance. Therefore, a decreased QUICKI score would indicate increased insulin resistance. A score  $<0.375$  is abnormal and implies that an individual is insulin resistant (Muniyappa et al, 2008).

### **Characteristics of Diabetes Mellitus**

Diabetes mellitus (DM) is a metabolic disorder that is indicated by high glucose concentration in the blood (hyperglycemia). In type 1 diabetes mellitus (T1DM), the pancreatic  $\beta$ -cells are no longer to produce insulin which leaves the individual completely dependent on exogenous insulin via injections (Bhattacharya, Dey, & Roy, 2007).

The form of diabetes mellitus that this study is interested in, type 2 diabetes mellitus (T2DM), is the most common form with it being 90-95% of cases (Bhattacharya, Dey, & Roy, 2007). Those with T2DM are typically not fully dependent on insulin, though they may need it to maintain adequate glucose control. In T2DM, the pancreatic  $\beta$ -cells still produce insulin. However, the cells in the body tend to be resistant to the insulin that is produced which results in hyperglycemia. In response to this, the pancreatic  $\beta$ -cells produce more insulin to compensate for the insulin resistance. Over time, the pancreas can become fatigued in a sense and its production of insulin may actually decrease. At this point, exogenous insulin with the possible addition of insulin-stimulating or insulin-sensitizing medications may be indicated (American Diabetes Association, 2010; Kalupahana, Claycombe, & Moustaid-Moussa, 2011). This disease process can be a product of genetic predisposition, lifestyle factors, and environmental exposure. This form of diabetes tends to occur more often in those who are obese but it can also occur in normal weight individuals, particularly in those who have excess abdominal adiposity (Triplett, 2012).

Excess glucose in the blood causes oxidative damage to cells and tissues. Damage to the micro- and macrovasculature can occur and existing atherosclerosis can be aggravated by excessive levels of glucose in the blood (Triplett, 2012). The small blood vessels that travel through the kidneys and retina of the eye are often damaged easily by this oxidative effect. Thus, renal failure and impaired vision are common findings in uncontrolled diabetes (American Diabetes Association, 2010; Triplett, 2012). When

insulin cannot bring glucose into cells, those cells must resort to other sources of energy. Most often, lipids are broken down in response to this need (Triplett, 2012).

Classic symptoms of untreated hyperglycemia include increased thirst (polydypsia), increased appetite (polyphagia), increased urination (polyuria), weight loss, blurred vision, and recurrent infections (American Diabetes Association, 2010). These symptoms are often the first sign of the condition and must be present for diagnosis, along with an elevated fasting or casual glucose concentration. The diagnosis of diabetes mellitus is confirmed with an oral glucose tolerance test (OGTT) in which an individual who has been fasting for at least 12 hours drinks a solution with a 75 g glucose load. The diabetic individual has a fasting plasma glucose (FPG) concentration of  $\geq 126$  mg/dL. The concentration at two hours after consuming the solution is  $\geq 200$  mg/dL for the person to be considered diabetic (Roumen, Feskens, Jansen, Saris, & Blaak, 2008). To diagnose diabetes and assess long-term glycemic control (over the past 120 days), a test known as hemoglobin A1C can be run. This is measurement of the amount of glycation on hemoglobin in erythrocytes (red blood cells). A hemoglobin A1C reading of  $\geq 6.5\%$  is the diagnostic cut-off value (American Diabetes Association, 2010).

## **Type 2 Diabetes Mellitus**

T2DM is diagnosed when the classic symptoms of diabetes mellitus are present in addition to having an hemoglobin A1C  $\geq 6.5\%$  or a casual blood glucose concentration of  $\geq 200$  mg/dL (American Diabetes Association, 2010). The diagnosis can then be confirmed with an OGTT if indicated. For those with T2DM, insulin in the blood is

usually normal or it may even be elevated. The absence of antibodies against insulin or pancreatic  $\beta$ -cells will verify that it is not T1DM (American Diabetes Association, 2010).

In many cases of T2DM, weight loss or exercise helps to stabilize glycemic control without the need for further intervention. However, the need for oral glucose-lowering medication is often indicated. Depending on the nature and severity of the disease, an individual with T2DM may or may not be dependent on insulin injections to maintain good glycemic control. Unlike those with T1DM, these individuals produce at least some insulin so that they can survive without exogenous sources if necessary (American Diabetes Association, 2010).

### **Prediabetes**

There is a state of defective glucose metabolism at a level that is not yet able to be diagnosed as diabetes mellitus. This is known as prediabetes, which indicates that an eventual progression to full-blown T2DM may develop if adequate intervention does not occur. The two categories of prediabetes are impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). As in the diagnosis of diabetes mellitus, these conditions are assessed using an OGTT (American Diabetes Association, 2010). In IFG, the individual has a fasting plasma glucose (FPG) above the normal level at  $\geq 100$  g/dL but lower than the level indicating diabetes of  $< 126$  mg/dL. At two hours after consuming a glucose load, those with IGT have a plasma glucose concentration greater than the normal level at  $\geq 140$  mg/dL but less than the level indicating diabetes of  $< 200$  mg/dL. The diagnostic hemoglobin A1C level for prediabetes is  $\geq 6.0\%$  and  $\leq 6.5\%$ . Like diabetes mellitus, prediabetes is often connected to obesity and abdominal adiposity (American Diabetes

Association, 2010). It is important to recognize that, at this point, any disruptions of normal metabolism are still considered largely reversible (Liu et al, 2009). Therefore, early detection and effective intervention are imperative. The same general treatment methods for managing diabetes apply to reversing prediabetes as well. Weight loss, increased physical activity, and certain medications can help to prevent or at least postpone the progression of prediabetes into diabetes mellitus (American Diabetes Association, 2010; Mensink et al, 2003).

### **Diabetes Mellitus and Cardiovascular Disease Risk**

Due to the damaging effect of high blood glucose levels, individuals with diabetes mellitus are at an increased risk for developing cardiovascular disease (CVD) when compared to individuals without diabetes. Thus, CVD is a common cause of morbidity and mortality in those with diabetes mellitus. The degree of severity also tends to be higher when diabetes mellitus is involved (Polidori, 2003; Voulgari, Papadogiannis, & Tentolouris, 2010). Both T1DM and T2DM present an increased risk for developing CVD. Uncontrolled and prolonged hyperglycemia leads to oxidative damage of the vasculature system, resulting in the complications known to be associated with diabetes mellitus. These include aggravated atherosclerosis, kidney damage (nephropathy), nerve damage (neuropathy), and retinal damage (retinopathy). With regards to cardiovascular disease risk, atherosclerosis is of particular concern. Monitoring and controlling blood glucose levels, with or without medication and insulin, is the most effective way to prevent such complications (American Diabetes Association, 2010; Polidori, 2003; Triplett, 2012).

An increased risk for cardiovascular disease translates to an increased risk for transient ischemic attack (TIA) and cerebrovascular accident (CVA; stroke). One population-based study with a 20-year follow up revealed that 27% of individuals who had T2DM had experienced a stroke. Interestingly, stroke tends to be more severe in those with diabetes than in those without diabetes. Tight control of blood glucose levels appears to be critical to preventing stroke in this population (Samson, 2010).

### **Fish Oil Supplementation and Diabetes Mellitus**

Some research suggests that fish oil supplementation may have an insulin sensitizing effect. The mechanism by which this is thought to occur is related to the incorporation of EPA and DHA into the cellular membrane, thereby increasing its fluidity. This then increases the number of insulin receptors that can be incorporated into the membrane, allowing increased opportunity for insulin to bind and act on a cell. This, in turn, has the potential to decrease insulin resistance (Anderson & Ma, 2009).

Another potential benefit from fish oil with regards to diabetes is related to the cardiovascular aspect of diabetes management. When the endothelial cells of the vasculature are subjected to hyperglycemia, a transcription factor known as nuclear factor kappa B (NF-kb) is upregulated. It appears that EPA and DHA act to downregulate this factor and, as a result, may reduce the vascular damage from the chronically elevated levels of blood glucose that are often found in those with diabetes (Anderson & Ma, 2009).

An additional component of insulin resistance and diabetes in which fish oil supplementation may have an effect is proliferator-activated receptor gamma (PPAR $\gamma$ ).



PPAR $\gamma$  is responsible for increasing the expression of GLUT-1 and GLUT-4 which increases glucose uptake by adipocytes and myocytes. EPA and DHA act as ligands for PPAR $\gamma$  to promote its function and decrease levels of glucose in the blood. This activation of PPAR $\gamma$  has the secondary effect of inhibiting the expression of insulin resistance-promoting cytokines. This then increases plasma levels of adiponectin, a hormone that promotes insulin sensitivity and reduces hepatic glucose release (Anderson & Ma, 2009; Kalupahana, Claycombe, & Moustaid-Moussa, 2011). Additionally, adiponectin acts to increase the breakdown of lipids. This may reduce the inflammatory consequences that can arise with excess adipose tissue (Kalupahana, Claycombe, & Moustaid-Moussa, 2011). By these pathways, EPA and DHA appear to have an anti-diabetic influence (Anderson & Ma, 2009; Kalupahana, Claycombe, & Moustaid-Moussa, 2011).

Despite the evidence of these numerous mechanisms, there is limited research regarding the effect of fish oil supplementation on insulin sensitivity and the findings are not consistent. In one study, healthy participants were asked to eliminate 6000 mg of visible fat from their diet and add 6000 mg of fish oil. This resulted in decreased body fat mass and increased lipid oxidation within the body. Another study by Browning et al. (2007) demonstrated that supplementation with fish oil increased insulin sensitivity in overweight women, though it is not clear whether reduced inflammation was a mediating factor. Yet another study did not find any beneficial effect for insulin sensitivity with fish oil supplementation (Kusunoki et al, 2007). It appears that the benefit of increased insulin sensitivity may be limited to healthy non-diabetic individuals. Based on this information,

the use of fish oil supplementation could potentially provide some benefit by preventing the development or delaying the onset of prediabetes and T2DM (Anderson & Ma, 2009).

### **Fish Oil Supplementation and Obesity**

Conventional treatment for obesity involves encouraging a healthy, reduced-energy diet and regular participation in physical activity. For many individuals however, these lifestyle modifications can be very difficult to initiate and maintain. Research suggests that EPA and DHA, the long-chain omega-3 fatty acids found in fish oil supplementation, may be associated with reduced risk for obesity (Buckley & Howe, 2010).

The reduction in risk for obesity is thought to be due to the function of EPA and DHA in suppressing appetite, encouraging the development of lean tissue, increasing oxidation of lipids, and reducing deposition of lipids into adipose tissue. These metabolic changes have been shown to occur in rodents and humans, though there is limited evidence to demonstrate an actual reduction in obesity for humans. It has been hypothesized that the increase in lean tissue accumulation and maintenance is a result of enhanced blood circulation. This is thought to contribute to improved distribution of nutrients to skeletal muscle tissue, allowing the tissue to receive the substrates it needs to maintain function. There is also some evidence to suggest that EPA reduces the activity of the proteolytic ubiquitin-proteasome pathway, thus suppressing the catabolism (breakdown) of muscle tissue (Whitehouse & Tisdale, 2001; Wing & Goldberg, 1993; Wyke & Tisdale, 2005). Additionally, muscle anabolism (building) may be enhanced through EPA's upregulation of the kinases mTOR and S6K which are involved in muscle

protein synthesis (Gingras et al, 2007). The enhancement of lipid oxidation has been linked to an upregulation of mitochondrial carnitine palmitoyl transferase I (CPT-I). This enzyme is involved in the transport of fatty acids into the mitochondria for oxidation. The reduction of lipid deposition into adipose tissue is thought to be a result of decreased levels of serum triglycerides (Buckley & Howe, 2010).

Research with rodents has explored the addition of EPA and DHA to a high-fat, obesogenic diet. There is some evidence showing a reduction lipid deposition with this feeding method. Unfortunately, the results of studies on this topic have not been fully consistent. Several studies with rodents who had diabetes actually demonstrated an increase in body weight when fed the high-fat diet with supplementation of EPA and DHA (Baillie, Takada, Nakamura, Clarke, & 1999; Belzung, Raclot, & Groscolas, 1993; Cunnane, McAdoo, & Horrobin, 1986; Hainault, Carlotti, Hajdich, Guichard, & Lavau, 1993; Ruzickova, 2004). Nevertheless, this was not seen in rodents without diabetes (Todoric et al, 2006). Since body fat was not measured in these studies, however, it is uncertain whether the differences in weight were in fact due to changes in body fat accumulation (Buckley & Howe, 2010).

Perhaps, EPA and DHA may be more efficient in preventing obesity in normal weight individuals and reducing further accumulation of adipose stores in those who are already obese (Buckley & Howe, 2010). It is also worth recognizing that EPA and DHA have been shown to reduce levels of inflammation that are associated with high amounts of adipose stores (Kalupahana, Claycombe, & Moustaid-Moussa, 2011). Additionally, research suggests that fish oil may encourage a more favorable distribution of body fat.

Visceral (epididymal) fat, also referred to as abdominal adiposity, is associated with greater levels of inflammation, insulin resistance, and dyslipidemia (Athysos, Tziomalos, & Mikhailidis, 2011; Klop & Cabezas, 2013). Evidence from rodent studies has shown a reduction in visceral fat stores with fish oil supplementation. DHA appears to have a more profound impact than EPA in this particular phenomenon (Buckley & Howe, 2010). Some evidence suggests an enhanced improvement in body tissue composition may occur when EPA and DHA are added to a reduced-energy diet, exercise regimen, or combination of the two. Interestingly, the changes seen in body weight and body composition appear to occur independently of energy intake (Buckley & Howe, 2010). Though a reduction in actual amount of adipose tissue may not occur, these changes may potentially reduce the risk for comorbidities associated with obesity such as cardiovascular disease (CVD) and diabetes (Buckley & Howe, 2010; Kalupahana, Claycombe, & Moustaid-Moussa, 2011).

### **Participant Recruitment**

This 8-week study was a randomized, double-blinded, and placebo-controlled trial. Male and female adults, 18 to 50 years of age and generally healthy, were recruited for the present study. Participants were recruited starting in November 2013 using an advertising email sent out via a listserv which contained a link to a web-based questionnaire. After this initial screening process twenty-four participants, eight male and sixteen females, were enrolled on the basis of the following criteria: in good general health, 18-50 years old, no current or recent use of fish oil supplements (within the past 2 months), no heavy use of cigarettes (>10 cigarettes daily), no excessive physical activity, non-vegetarian diet, not lactating or pregnant, and not allergic to fish, nuts, or soy products. Six females and one male belonged to blood type A. Seven males and ten females belonged to blood type O. Approximately 26% of the individuals who responded to the initial advertisement fulfilled these criteria and volunteered to participate in the study (24 of 92 total respondents).

Exclusion was based on the following criteria: 1) blood type other than A or O, 2) current or past pharmaceutical treatment for cardiovascular disease, 3) regular consumption of more than two 3.5 ounce servings of fish per week, 4) underweight (BMI<18.5 kg/m<sup>2</sup>) or obesity (BMI >35 kg/m<sup>2</sup>), and 5) use of prescription medication that may influence body weight (thyroid medication, corticosteroids, or NSAIDS).

Participants were instructed maintain their diet and exercise habits during the study period. They were also asked to report any new medical diagnoses or medication use.

Participants came in for 3 visits over the span of about 2 months. The study was completed January 2014. Informed consent was obtained in writing after the purpose, nature, and potential risks were explained to the participants. The experimental protocol was approved by the Institutional Review Board at Arizona State University.

### **Prestudy Qualification**

Following the initial screening using the answers from the online questionnaire, eligible participants were asked to come to the university laboratory for further assessment of eligibility (Visit 1). After obtaining informed consent, all participants were asked to fill out a health history questionnaire which addressed demographic characteristics, smoking habits, alcohol intake, eating and exercise habits, and current medication use. Height, weight, waist circumference, and body fat (Tanita bioelectrical impedance scale) were measured and recorded. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Participants also completed a food frequency questionnaire that has been validated for approximating an individual's typical omega-3 fatty acid intake (Ritter-Gooder, Lewis, Heidal, & Eskridge, 2006). If found to be ineligible based on the exclusion criteria, participants were released from the study. Using a finger prick, a small sample of blood was used to assess blood type (EldonCard™ 2511-1 ABO-Rh Home Blood Typing Test Kit). If participants were a blood type other than A or O, they were released from the study. If the participants were blood type A or O, they were entered into the study and scheduled for visit #2.

At visit #2, venous blood sample was taken from an arm vein (<2 Tbsp). Blood was processed and used for immediate analyses or frozen (-80°) for future analyses. The markers of interest were hemoglobin A1C (HbA1C), high sensitivity C-reactive protein (hsCRP), and serum lipids. Blood clotting times were also assessed for a companion study. Participants were stratified by age, BMI, weight, blood type, fish consumption and gender and then randomized to one of two groups: fish oil supplementation (FO; n=13) or coconut oil supplementation (CO; n=11). In the fish oil supplementation group, 9 were female (3 blood type A, 6 blood type O) and 4 were male (1 blood type A, 3 blood type O). In the coconut oil supplementation group, 7 were female (3 blood type A, 4 blood type O) and 4 were male (4 blood type O, 0 blood type A). Participants were instructed to ingest 2 capsules daily (fish oil, 1000 mg with an EPA:DHA ratio of 4:2, or coconut oil, 1000 mg) for the course of the 8 week trial.

Table 3. Fatty Acids in Two 1000 mg EnergyFirst OmegaEnergy Fish Oil Supplements

Fatty acid	Content of Two 1000 mg capsules
Eicosapentanoic acid	800 mg
Docosahexanoic acid	400 mg

Table 4. Fatty Acids in Two 1000 mg Puritan's Pride Organic Coconut Oil Supplements

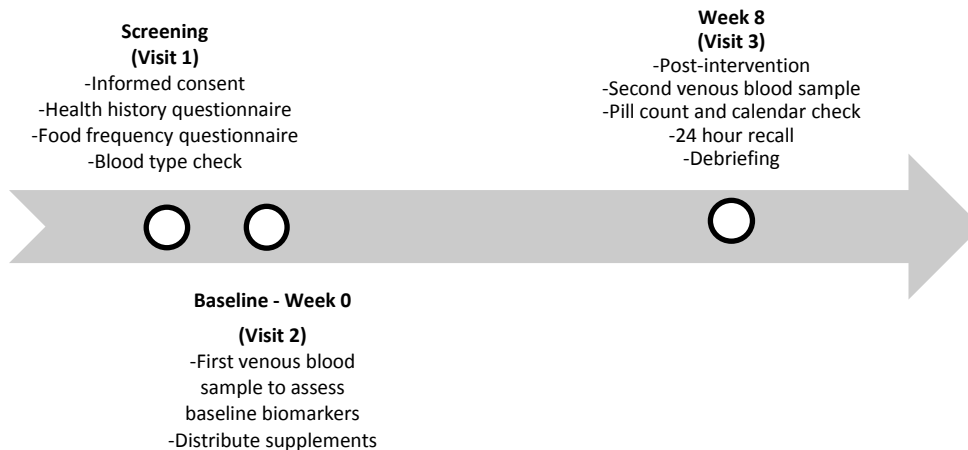
Fatty acid	Content of Two 1000 mg capsules
Lauric acid	1760 mg
Myristic acid	520 mg
Caprylic acid	212 mg
Palmitic acid	300 mg
Capric acid	180 mg
Oleic acid	200 mg
Stearic acid	40 mg
Linoleic acid	40 mg

For additional Supplement Facts details, please reference page 88.

Participants were given enough capsules of their assigned supplements to take one each day until the next scheduled visit. Calendars were provided with instruction to note days that supplements were taken.

Eight weeks later, participants came in for the final visit (Visit 3). This involved a second venous blood draw from an arm vein (<2 Tbsp). The blood was again processed and used for immediate analyses or frozen (-80°) for future analyses. The same anthropometric measurements were taken as in Visit 1. Twenty-four hour recalls were collected from participants at the final visit for the companion study. The same markers were assessed as in the previous blood draw. Supplement adherence was determined using the days marked off on participants' calendars.

### Study timeline





## Laboratory Analyses

At baseline (week 0) (Visit 2) and week 8 (Visit 3), all participants returned to the study laboratory and venous blood (2 Tbsp) was collected. The blood samples were assessed for cardiovascular biomarkers and long-term glucose control. The samples were casual, meaning that they were not specifically taken in a fasting state. The measurement of cholesterol (TC, HDL-C, LDL-C, and VLDL-C) does not require fasting blood for accuracy. The serum TG measurement, however, is most meaningful when fasting blood is used. Blood lipids were assessed using Piccolo. Piccolo directly measures total TC, TG, and HDL-C. The machine calculated VLDL-C is by dividing TG by 5 and LDL-C was calculated by the machine using the Friedewald equation ( $LDL-C = TC - (HDL-C + TG/5)$ ). Therefore, in this case, LDL-C and VLDL-C measurements were impacted by the state of fasting. Hemoglobin A1C was assessed using DCA Vantage Analyzer (Model 2000) and hsCRP was assessed using COBAS methodologies (model).

## Statistical Methods

Statistical analyses were performed using the SPSS Statistical Analysis system 22.0. Before statistical analysis was conducted, normal distribution and homogeneity of the variances were statistically tested. A 2-way ANOVA was run using univariate analyses to determine the effects of treatment and blood type on the outcome measures. Data are expressed as mean  $\pm$  standard deviation. Significant difference within- or between-groups was defined as  $P < 0.05$ . A large effect size was defined as  $\geq 0.2$ .

## Chapter 4

### RESULTS

Eight men and 16 women were enrolled in the study and randomized to treatment groups ( $26.1 \pm 8.6$  y;  $25.2 \pm 3.6$  kg/m<sup>2</sup>). Two participants never started the study, and an additional 4 participants did not complete the 8-week trial. Two of these individuals withdrew for medical reasons that were unrelated to the study protocol. The remainder of the participants was lost to attrition (reason unknown). A blood sample could not be collected from one of the completing participants; hence, blood data are only available for 17 participants. There were no significant differences by gender, age, BMI, or blood type for those that did and those that did not complete the study (data not shown). The data presented below are for only the 17 participants completing the study for which blood data were available.

The fish oil supplementation group had 10 participants. Of these, 7 were female (2 blood type A, 5 blood type O) and 3 were male (1 blood type A, 2 blood type O). There were 7 participants in the coconut oil supplementation group. Of these, 4 were female (3 blood type A, 1 blood type O) and 3 were male (0 blood type A, 3 blood type O).

Table 5. Baseline Descriptives

	Group (Blood Type Supplement Type)				Statistical Significance (P<0.05)	
	A FO	A CO	O FO	O CO	P (blood type)	P (group) (interaction)
Number (M/F)	3 (1/2)	3 (0/3)	7 (2/5)	4 (1/3)	---	---
Age, y	25.3±3.1	31.3±13.6	28.3±9.3	24.3±11.2	0.692	0.850
Weight, pounds	165.1±25.7.5	144.6±20.3	179.7±40.9	170.2±28.7	0.266	0.401
BMI (kg/m <sup>2</sup> )	23.7±3.8	23.3±3.5	25.6±4.4	23.9±3.4	0.551	0.619
PA, METS	62.3±35.6	91.0±35.2	54.1±21.3	61.3±17.3	0.176	0.200
n-3 intake (g/day)	1.25±1.04	0.75±0.82	0.66±0.56	0.58±0.54	0.308	0.438
TC (mg/dL)	164.0±40	220.3±40.5	166.9±54.9	155.8±42.3	0.233	0.376
LDL-C (mg/dL)	75.3±31.5	131.0±43.9	84.5±41.9	83.3±32.3	0.354	0.199
HDL-C (mg/dL)	61.3±14.6	66.7±5.7	62.0±13.2	41.5±14.7	0.094	0.282
TG (mg/dL)	136.3±128.6	112.0±2.7	145.0±142.1	153.3±102.7	0.692	0.898
HbA1C (%)	5.1±0.4	5.2±0.1	5.2±0.2	5.1±0.2	0.771	0.882
CRP (mg/L)	3.2±3.6	0.7±0.7	1.5±1.5	1.0±1.2	0.498	0.136

Statistical analyses were performed using the SPSS Statistical Analysis system 22.0. Before statistical analysis was conducted, normal distribution and homogeneity of the variances were statistically tested. A 2-way ANOVA was run using univariate analyses to determine the effects of treatment and blood type on the outcome measures. Data are expressed as mean ± standard deviation. Significant difference within- or between-groups was defined as  $P < 0.05$ . A large effect size was defined as  $\geq 0.2$ .

As shown in Table 5, the age of the participants ranged from 22 to 47 years old for individuals with blood type A and 18 to 44 years old for those with blood type O. Baseline weight for blood type A individuals ranged from 132 to 192 pounds and 131 to 250 pounds for blood type O individuals. Height for blood type A 167 to 179 centimeters blood type O ranged from 168 to 192 centimeters. Baseline body mass index (BMI) for blood type A ranged from 20 to 28 kg/m<sup>2</sup> and for blood type O, BMI ranged between 21 and 31 kg/m<sup>2</sup>. Physical activity (PA) at baseline ranged from 31 to 128 metabolic equivalents (METs) and from 19 to 83 metabolic equivalents (METs) for blood type A and blood type O individuals respectively. Omega-3 (n-3) fatty acid intake at baseline was 0.16 to 2.24 grams in those with blood type A and 0.03 to 1.74 grams per day in those with blood type O. Baseline total cholesterol (TC) ranged from 124 to 266 mg/dL in those with blood type A and 95 to 282 mg/dL in those with blood type O. Baseline low-density lipoprotein cholesterol (LDL-C) was 39 to 179 mg/dL with blood type A and 43 to 166 mg/dL with blood type O. Baseline high-density lipoprotein (HDL-C) was 51 to 78 mg/dL in blood type A and 28 to 80 mg/dL in blood type O. Baseline triglyceride (TG) ranged from 35 to 281 mg/dL in blood type A individuals and 46 to 434 mg/dL in blood type O individuals. Baseline hemoglobin A1C (HbA1C) was 4.8 to 5.6 percent with blood type A and 4.9 to 5.6 percent with blood type O. Baseline C-reactive protein (CRP) for blood type A was 0.15 to 7.11 and 0.17 to 3.96 for blood type O.

As shown in Table 5, there was no association or interaction between blood type and group for age, weight, body mass index (BMI), physical activity (PA), omega-3 (n-3) fatty acid intake, TC, LDL-C, HDL-C, serum TG, HbA1C, or hsCRP.

Table 6. Percent Change Data (Week 8 - Week 0)

Percent Change Data (Week 8 - Week 0)

	Group (Blood Type Supplement Type)				Statistical Significance			
	A FO	A CO	O FO	O CO	P (blood type)	P (group)	P (interaction)	Effect size*
Number (M/F)	3 (1/2)	3 (0/3)	7 (2/5)	4 (3/1)	---	---	---	---
Adherence (%)	94±3	97±3	94±6	94±3	0.613	0.572	0.522	0.032
Weight	0.8±2.5%	-1.8±3.0%	-0.02±4.4%	-1.1±2.3%	0.961	0.318	0.679	0.014
Body fat	1.7±2.4%	-0.1±5.2%	5.2±14.8%	-9.6±7.9%	0.607	0.166	0.274	0.091
TC	-1.3±6.8%	-9.1±11.2%	-3.3±13.2%	-7.1±18.9%	0.996	0.429	0.778	0.006
LDL-C	10.1±19.8%	-15.9±16.7%	-0.8±19.1%	-14.0±10.5%	0.622	0.048	0.484	0.042
HDL-C	-7.9±8.5%	2.1±7.5%	-2.5±12.2%	1.4±27.3%	0.792	0.434	0.726	0.011
TG	14.5±54.3%	-8.2±7.9%	-8.6±28.8%	7.0±47.7%	0.838	0.855	0.333	0.003
HbA1C	-4.2%±5.7%	0.02%±1.9%	0.86%±5.2%	0.59%±5.9%	0.300	0.460	0.403	0.043
hsCRP	24.0%±119.0%	63.6%±55.1%	87.3%±172.0%	49.3%±119.0%	0.739	0.991	0.599	0.000

\*Effect size for interaction

For the fish oil supplementation group, the percent change in weight for blood type A individuals ranged from -1% to +4% and -9% to +5% for blood type O individuals. Percent change in percent body fat ranged from -1% to +1% for blood type A and -14% to +8% for blood type O. Percent change in TC ranged from -9% to +3% for individuals with blood type A and -21% to +15% for individuals with blood type O. Percent change in LDL-C for blood type A ranged from -13% to +23% and O ranged from -25% to +15%. Percent change in HDL-C for blood type A ranged from -13% to +2% and for blood type O, from -17% to +16%. Percent change in serum TG ranged from -19% to +77% for those with blood type A and -41% to +29% for those with blood type O. Percent change in HbA1C for blood type A ranged from -11% to -2% and for blood type O, from -6% to +9%. Percent change in hsCRP ranged from -90% to +147% for individuals with blood type A and -53% to +443% for individuals with blood type O.

For the coconut oil supplementation group, the percent change in weight for blood type A individuals ranged from -5% to 0% and -3% to +2% for blood type O individuals. Percent change in TC ranged from -19% to +3% for individuals with blood type A and -26% to +19% for individuals with blood type O. Percent change in LDL-C for blood type A ranged from -32% to +1% and for blood type O, from -25% to -5%. Percent change in HDL-C for blood type A ranged from -3% to +11% and for blood type O, from -34% to +32%. Percent change in serum TG for blood type A individuals ranged from -16% to 0% and -17% to +64% for those with blood type O. HbA1C ranged from -2% to +2% for those with blood type A and -8% to +6% for those with blood type O. Percent change in

high-sensitivity hsCRP ranged from +25% to +127% in those with blood type A and -81% to +197% in those with blood type O.

As shown in Table 6, there was a significant difference in percent change in LDL-C between treatment groups ( $p=0.048$ ). There were no significant associations or interactions for blood type and group and percent change in weight, body fat, TC, HDL-C, TG, HbA1C, or hsCRP.

## Chapter 5

### DISCUSSION

This study represents a randomized, double-blind comparison of four groups of healthy individuals: one with blood type A that consumed fish oil supplements, one with blood type A that consumed coconut oil supplements, one with blood type O that consumed fish oil supplements, and one with blood type O that consumed coconut oil supplements. The impact of group and/or blood type for cardiovascular disease markers was examined. The biomarkers examined were total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), serum triglycerides (TG), hemoglobin A1C (HbA1C), or high-sensitivity C-reactive protein (hsCRP). All are well established as indicators of cardiovascular disease risk.

The data show that after 8 weeks of coconut oil supplementation, a significant reduction in LDL-C was observed compared to the fish oil supplement treatment but this difference was not impacted by blood type. Conversely, there was a significant increase in LDL-C for blood type A after 8 weeks of fish oil supplementation. There was no significant change in weight, body fat, HDL-C, TC, serum TG, HbA1C, hsCRP in either blood type A or O with use of fish oil supplements.

The supplemental form of coconut oil has been used in several past studies that have also examined fish oil supplementation. Dosing was similar, if not identical, to that used in the current study (2000 mg/day). At this time, there has not been a documented change in the blood lipid profile when coconut oil is used in this manner. Its use in research has been quite limited, however, and many of the studies in which it has been



used have not actually measured blood lipids (Amminger et al, 2013; Karr, Grindstaff, & Alexander, 2012; Mozaffari-Khosravi, Yassini-Ardakani, Karamati, & Shariati-Bafghi, 2013; Trepanowski, Kabir, Alleman, & Bloomer, 2012). Aside from the supplemental form, the dietary form of coconut oil has been studied for its impact on health. This tropical oil is known to be quite high in saturated fatty acids (90%) (Palazhy et al, 2012). However, the majority of these are medium-chain length (65%), meaning that they are absorbed directly into the bloodstream and transported to the liver. Medium-chain saturated fatty acids are metabolized by the liver to provide energy, while other fatty acids would be used for lipoprotein synthesis (DebMandal & Mandal, 2011). Because of this, it has been suggested that coconut oil should have a neutral impact on blood lipids. It has been proposed that a beneficial effect may be seen when coconut oil replaces other forms of saturated fat, along with *trans* fats. Some past studies that have demonstrated an adverse effect of coconut oil on lipid profiles have been critiqued for using a hydrogenated form of the oil (Kintanar, 1988). Studies that have used a non-hydrogenated supplemental form have shown modest favorable changes in blood lipids. Animal studies have shown a decrease in total cholesterol and lipoproteins with coconut oil supplementation (Feranil, Duazo, Kuzawa, & Adair, 2011). Nevertheless, much of the research on coconut oil has focused on its use as part of the diet rather than as a nutritional supplement. Many studies have been non-experimental and have merely assessed the impact of individuals' current dietary habits (i.e. cooking with coconut oil versus cooking with another oil) (Feranil, Duazo, Kuzawa, & Adair, 2011; Palazhy et al, 2012). Also, these studies have involved the use of coconut oil in relatively large

amounts; some as high as 20% of total kilocalories (Nevin, 2008). Participants in the current study were only asked to take a daily dose of 2000 mg coconut oil and this was not intended to replace other fat in the diet. It is likely that the observed decrease in LDL-C was due to extraneous factors, such as sample size and lifestyle changes. Further exploration of this potential association is warranted.

Previous literature has shown that fish oil supplementation increases blood levels of LDL-C. While this would typically be considered an undesirable effect, it has been shown that the LDL particles become larger and more buoyant with fish oil use. These qualities make the LDL particles less atherogenic, or less likely to contribute to atherosclerosis (Chan & Cho, 2009; Dessi, Noce, & Di Daniele, 2013; Gazi et al, 2006). A significant increase in LDL-C was demonstrated in the current study, as expected based on this literature.

The EPA and DHA content of the fish oil supplements taken by participants was 600 mg per capsule. The capsules were to be taken twice a day to provide a total daily dose of 1200 mg EPA and DHA. According the United States Department of Agriculture (USDA), high omega-3 fish contain approximately 407 mg of EPA and DHA per ounce (2004). Using this information, it can be determined that the participants in this study consumed the equivalent of 20.6 ounces of high omega-3 fish per week. That is equal to five 4-ounce servings per week, or seven 3-oz servings per week. Based on previous literature, this dosing is adequate to produce a modest observable change in blood lipids.

The current study was limited by a small and relatively uniform sample. The majority of the participants (22 of 24; 92%) in the study identified themselves as

Caucasian. The others (2 of 24; 8%) identified themselves as Hispanic. Because the Hispanic individuals resigned from the study before blood could be drawn, it is unclear whether ethnicity could have influenced the results of this study. This resulted in an ethnically homogenous sample (100% Caucasian). When considering those who completed the study, it is important to note that females were overrepresented in the sample (65%; 11 of 17), along with those who had blood type O (65%; 11 of 17). Age may have been a factor as well, given that the majority (82%; 14 of 17) of the participants was between the ages of 18 and 36 years old. Also, the length of time that the supplements were taken was relatively short. It may take several months for significant changes in blood lipids and hemoglobin A1C to occur. Another consideration is that past literature has demonstrated a more dramatic change in triglycerides when they are abnormally high. The current study's participants did not have very high serum triglyceride levels (<500 mg/dL). Additionally, the blood draws were not specifically taken in a state of fasting. The number of participants who were and were not fasting is unknown, Although it has been shown that fasting blood is not necessary for measuring total cholesterol and lipoproteins, it is necessary for serum triglycerides which can vary depending on recent lipid intake. Further research studies with larger and more diverse samples may yield statistical significance, whereas the current study only shows trends for change in many parameters.

## Chapter 6

### CONCLUSION AND RECOMMENDATIONS

#### **Conclusion**

Aside from aforementioned increase in low-density lipoprotein (LDL) for blood type A, the results of the current study did not demonstrate a significant difference in cardiovascular disease markers between blood types A and O after consuming fish oil supplements relative to coconut oil supplements. However, the effect of fish oil supplementation on markers of cardiovascular disease, without consideration for blood type, has been thoroughly researched and documented in past studies. It is then feasible that a larger and more diverse sample may be enough to show a more definitive difference.

#### **Recommendation**

Additional research that further explores the topic at hand is recommended to improve understanding of fish oil's influence on cardiovascular disease risk for blood types A and O. Recruiting a larger and more diverse sample will be necessary to draw a stronger conclusion that can be generalized. It is recommended that blood draws are specifically conducted in a state of fasting so that serum triglycerides (TG) and LDL cholesterol (LDL-C) levels are more meaningful. Also, the fatty acid content of the coconut oil supplement is considerably greater when compared to that of the fish oil supplement. This may have influenced the difference in the change observed with the two supplements. Based on effective doses in previous research, it is recommended that a larger fish oil dose of 3000 to 5000 mg per day be used in future studies. Finally,

conducting a study over a longer period of time may be necessary to see more significant results.

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APPENDIX A

PRE-STUDY DOCUMENTS





APPROVAL: MODIFICATION

Carol Johnston  
SNHP - Nutrition  
602/827-2265  
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 12/16/2013 the ASU IRB reviewed the following protocol:

Type of Review:	Modification
Title:	
Investigator:	Carol Johnston
IRB ID:	1201007333
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	None

The IRB approved the modification.

When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:

**CONSENT FORM**  
**Dietary Supplementation and Health**

**INTRODUCTION**

The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

**RESEARCHERS**

Dr. Carol Johnston, Associate Director of the Nutrition Program at Arizona State University, as well as ASU Honor student, Ashley Ryder, have invited your participation in a research study.

**STUDY PURPOSE**

The purpose of the research is to examine the effect of a dietary supplement in adult men and women, 18-50 years old with either type A or O blood, on blood markers associated with health.

**DESCRIPTION OF RESEARCH STUDY**

If you decide to participate, then as a study participant you will join a trial to evaluate the effect of ingestion of a supplement daily for 8 weeks on health markers in individuals with type A or O blood. You will be instructed to consume the supplement daily. If you are interested in joining the study, you will be asked to come to an initial screening where your blood type will be determined. In addition, your height and weight will be measured and you will complete a health history and dietary questionnaire. If you are eligible for the study, you and the other participants will be randomly assigned to either the control (placebo) or experimental (dietary supplement) group. Subjects will be asked to visit the research site on 2 additional occasions separated by 8 weeks. At these visits you will be weighed, you will be asked to record what you ate the day before, and a blood sample will be drawn from a vein in your arm. At each blood sampling approximately 2 tablespoons of blood will be collected.

If you say YES, then your participation will last about 9 weeks at the Downtown Phoenix campus at Arizona State University. Approximately 50 subjects will be participating in this study.

**RISKS**

All gel capsules used in this study have been purchased from retail vendors. There may be a slight chance of gastrointestinal distress (including an aftertaste, burping, and reflux) when taking the supplement on an empty stomach. This risk is reduced if you ingest the capsule with a meal and consume plenty of water. Blood draws may cause light-headedness, headaches, and/or temporary bruising. A research nurse will be performing the blood draws.

**BENEFITS**

Although there may be no direct benefits to you, the possible benefit of your participation is that you will be able to experience what it is like to be a part of a research study that may provide new evidence to support the health of adults.

**NEW INFORMATION**

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

**CONFIDENTIALITY**

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. Your name will not be associated with any data related to the study. In order to maintain confidentiality of your records, you will be assigned to a subject number, which will be used throughout the course of the study to identify you. Only the investigators will have access to subject names and their corresponding codes.

**WITHDRAWAL PRIVILEGE**

It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

**COSTS AND PAYMENTS**

The researchers want your decision about participating in the study to be absolutely voluntary, yet they recognize that your participation may pose some costs such as inconvenience and a small time commitment. In order to help defray your costs, you will receive a \$15 Target gift card at the final visit of the study.

**COMPENSATION FOR ILLNESS AND INJURY**

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

**VOLUNTARY CONSENT**

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, Principal Investigator and Professor of Nutrition at ASU (602-827-2265)

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given (offered) to you.

Your signature below indicates that you consent to participate in the above study.

\_\_\_\_\_  
Subject's Signature

\_\_\_\_\_  
Printed Name

\_\_\_\_\_  
Date

Preferred contact: phone and/or email:  
  
\_\_\_\_\_

**INVESTIGATOR'S STATEMENT**

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided (offered) the subject/participant a copy of this signed consent document."

Signature of Investigator \_\_\_\_\_

Date \_\_\_\_\_

## **Volunteers Needed for Supplement and Blood Type Study**

The Nutrition Program is recruiting healthy young men and women to consume a dietary supplement for 8 weeks. The study will investigate whether a commonly consumed supplement affects health parameters in adults with type A or O blood. Participation in this study is voluntary. Participants will receive the supplement and \$15 in gift cards during the study.

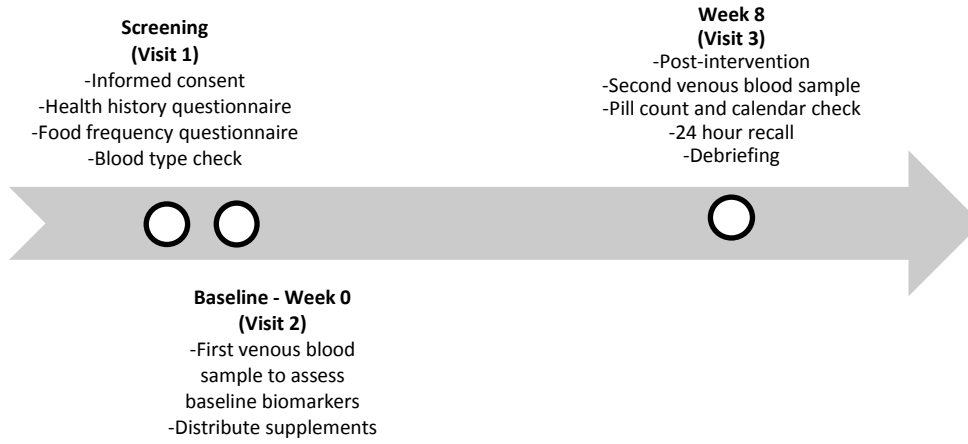
Individuals who are healthy, 18-50 years of age, willing to consume the commercially purchased gel capsule supplements daily for eight weeks, and are willing to have blood taken on two occasions may be eligible to participate.

For more information or to apply for the study, please visit our recruitment site:

<https://www.surveymonkey.com/s/ASUBloodTypeStudy>

## APPENDIX B

### METHODOLOGY DOCUMENTS



### Puritan's Pride Organic Coconut Oil 1000 mg Supplement Facts

Serving Size 2 Softgels

**Servings Per Container 60**

Amount Per Serving	% Daily Value
Calories 25	
Calories from Fat 25	
Total Fat	2.5 g 4%*
Saturated Fat	2.5 g 12%*
Protein	<1 g *
Organic Coconut Oil (Cocos nucifera) (fruit)	2,000 mg (2 g) **
which typically contains:	
Lauric Acid	880 mg **
Myristic Acid	260 mg **
Caprylic Acid	106 mg **
Palmitic Acid	150 mg **
Capric Acid	90 mg **
Oleic Acid	100 mg **
Stearic Acid	20 mg **
Linoleic Acid	20 mg **

\*Percent Daily Values are based on a 2,000 calorie diet.

\*\*Daily Value not established.

### EnergyFirst OmegaEnergy Fish Oil 1000 mg Supplement Facts

Serving Size 1 Softgel

**Servings Per Container 60**

Amount Per Serving	% Daily Value
Calories 10	
Calories from Fat 10	
Total Fat	1 g 2%**
Polyunsaturated Fat	0.5 g*
Vitamin E	2 IU 7%
Fish Oil	1000 mg*
EPA (Eicosapentaenoic Acid)	400 mg
DHA (Docosahexaenoic Acid)	200 mg

\*\*Percent Daily Values are based on a 2000 calorie diet.

\*Daily Value not established.

### n-3 FFQ Calculation Template

Ritter-Gooder PK, Lewis NM, Heidal K, et al. Validity and Reliability of a Quantitative Food Frequency Ques

Possible values		0, 0.5, 1, 1.5** 1, 3, 6, 14, 22, 30, 60***			F
Food Item	n-3 Fatty Acid Content in Grams/ Medium Serving*	Portion Size	Frequency		Total value
Tuna	0.230	0	0		0
Salmon	1.270	0	0		0
Whitefish	1.570	0	0		0
Herring	1.180	0	0		0
Walleye	0.400	0	0		0
Lake trout	1.710	0	0		0
Rainbow trout	0.510	0	0		0
Sablefish	1.290	0	0		0
Mackerel	2.230	0	0		0
Catfish	0.260	0	0		0
Flounder	0.170	0	0		0
Perch	0.380	0	0		0
Atlantic cod	0.430	0	0		0
Atlantic bluefish	1.030	0	0		0
Atlantic sturgeon	1.290				
		0	0		0
Greenland halibut	0.770	0	0		0
Surimi	0.000	0	0		0
Swordfish	0.900	0	0		0
Mussels	0.700	0	0		0
Scallops	0.660	0	0		0
Oysters	1.220	0	0		0
Shrimp	0.280	0	0		0
Sardines	1.370	0	0		0
Anchovy	1.200	0	0		0
Blue Crabs	0.310	0	0		0
Northern lobster	0.170	0	0		0
Turkey	0.080	0	0		0
Chicken	0.090	0	0		0
Beef	0.170	0	0		0
Pork	0.090	0	0		0
Regular egg	0.040	0	0		0
UNL n-3 enriched egg	0.350	0	0		0
Eggland Best n-3 egg	0.100	0	0		0
2% milk	0.070	0	0		0
1% milk	0.040	0	0		0
Skim milk	0.000	0	0		0



Cheddar cheese	0.100	0	0	0
Swiss Cheese	0.100	0	0	0
Mozzarella	0.060			
Cheese		0	0	0
2% fat cottage	0.020			
cheese		0	0	0
1% fat cottage	0.010			
cheese		0	0	0
Sweet potato	0.010	0	0	0
Potato	0.040	0	0	0
Green peas	0.030	0	0	0
Spinach	0.050	0	0	0
Cauliflower	0.040	0	0	0
Carrots	0.010	0	0	0
Kale	0.070	0	0	0
Red radish	0.020	0	0	0
Leeks	0.020	0	0	0
Broccoli	0.040	0	0	0
Eggplant	0.010	0	0	0
Snap Green	0.040			
Beans		0	0	0
Romaine lettuce	0.020			
		0	0	0
Iceberg lettuce	0.020	0	0	0
Looseleaf	0.130			
lettuce		0	0	0
Cabbage	0.040	0	0	0
Zucchini squash	0.120			
		0	0	0
Cucumber	0.020	0	0	0
Tomatoes	0.010	0	0	0
Bell Peppers-	0.020			
any color		0	0	0
Avocado	0.110	0	0	0
Banana	0.040	0	0	0
Cherries	0.100	0	0	0
Raspberries	0.060	0	0	0
Strawberries	0.060	0	0	0
Cranberries	0.040	0	0	0
Cranberry Juice	0.050	0	0	0
Apple	0.030	0	0	0
Apple Juice	0.020	0	0	0
Orange	0.010	0	0	0
Orange Juice	0.030	0	0	0
Red/green	0.030			
grapes		0	0	0
Grape Juice	0.010	0	0	0
Pears	0.020	0	0	0
Mango	0.040	0	0	0
Kiwi fruit	0.030	0	0	0

Papaya	0.080	0	0	0
Cantaloupe/muskmelon	0.070	0	0	0
Honeydew Melon	0.020	0	0	0
Blackberries	0.070	0	0	0
Blueberries	0.060	0	0	0
Pineapple Juice	0.030	0	0	0
English Walnuts	2.570	0	0	0
Pumpkin seeds	0.020	0	0	0
Ground Flaxseeds	2.000	0	0	0
Butternuts	2.470	0	0	0
Chia seeds	1.110	0	0	0
Hickory nuts	0.280	0	0	0
Beechnuts	0.480	0	0	0
Almonds	0.110	0	0	0
Pistachio nuts	0.070	0	0	0
Pine Nuts	0.180	0	0	0
Pecans	0.280	0	0	0
Brazilnuts	0.020	0	0	0
Sunflower seeds	0.020	0	0	0
Sesame Seeds	0.040	0	0	0
Poppy seeds	0.030	0	0	0
Banana bread	0.090	0	0	0
Pumpkin bread	0.480	0	0	0
Whole wheat bread	0.010	0	0	0
White bread	0.130	0	0	0
Oatmeal	0.020	0	0	0
Uncle Sams Cereal	1.200	0	0	0
All-bran	0.040	0	0	0
Special K	0.010	0	0	0
Cream of Wheat	0.010	0	0	0
Wheat Germ	0.800	0	0	0
Barley bran	0.340	0	0	0
Miracle whip	0.290	0	0	0
Margarine, hard	0.010	0	0	0
Soybean oil	0.120	0	0	0
Wheat germ oil	1.950	0	0	0
Flax oil	2.510	0	0	0
Canola oil	0.420	0	0	0
Olive Oil	0.030	0	0	0
Walnut oil	0.470	0	0	0
Red currant seed oil	1.370	0	0	0

Black currant seed oil	0.570	0	0	0
Gooseberry seed oil	0.900	0	0	0
Soybeans	1.450	0	0	0
Garbanzo beans	0.040	0	0	0
Navy beans	0.180	0	0	0
Lentils	0.040	0	0	0
Tofu	0.300	0	0	0
Soy milk	0.240	0	0	0
Pinto beans	0.340	0	0	0
Baked beans	0.160	0	0	0
Baked beans with pork	0.040	0	0	0
Refried Beans/frijoles	0.080	0	0	0
Red Kidney beans	0.170	0	0	0
Blackeyed peas	0.020	0	0	0
Great Northern beans	0.010	0	0	0
Lima beans	0.020	0	0	0
Fresh Spearmint	0.010	0	0	0
Fresh Peppermint	0.010	0	0	0
Garlic cloves	0.010	0	0	0
Black Pepper	0.040	0	0	0
Cayenne Pepper	0.020	0	0	0
Ground Ginger	0.040	0	0	0
Dried Rosemary	0.010	0	0	0
Fresh Rosemary	0.030	0	0	0
Dried Thyme	0.010	0	0	0
Fresh Thyme	0.060	0	0	0
Dried Basil	0.030	0	0	0
Fresh Basil	0.060	0	0	0
Paprika	0.010	0	0	0
Cumin	0.010	0	0	0
Monthly total				0
daily total				0

\* Food  
Processor/Version  
8.1, 2003,  
ESHA Research

\*\* 0.5= 1/2  
portion  
1.0= 1portion  
1.5= 1 1/2  
portion  
\*\*\* 1= once a  
month  
3= less than  
once a week  
6= 1-2  
times/week  
14=3-4  
times/week  
22= 5-6  
times/week  
30= daily  
60= more than  
once/day

## APPENDIX C

### QUESTIONNAIRE DOCUMENTS

Dietary Supplement and Blood Study

**\*1. Please provide your email address**

**\*2. Please select your gender**

- Male  
 Female

**\*3. Please enter your height and weight**

Please enter your  
height and weight  
Height (inches)

Weight (pounds)

**\*4. Do you know your blood type?**

- Yes  
 No

If yes, please specify:

**\*5. Are you a vegetarian and exclude all meat, fish, and poultry from your diet?**

- Yes  
 No

**\*6. Do you consume any of the following foods more than once per week? (Please check yes or no):**

	Yes	No
Eggs	<input type="checkbox"/>	<input type="checkbox"/>
Dairy	<input type="checkbox"/>	<input type="checkbox"/>
Fish	<input type="checkbox"/>	<input type="checkbox"/>
Beef	<input type="checkbox"/>	<input type="checkbox"/>
Flax seed or flax oils	<input type="checkbox"/>	<input type="checkbox"/>
Soy products (e.g., milk, tofu, etc.)	<input type="checkbox"/>	<input type="checkbox"/>

**\*7. Do you take any dietary supplements?**

- Yes  
 No

If yes please specify:

**8. Are you allergic to soy, eggs, fish, and/or nuts?**

- Yes  
 No

If you are allergic to other foods, please specify:

**\*9. For females: Are you pregnant, lactating, or do you anticipate becoming pregnant?**

- Not Applicable  
 Yes  
 No

**\*10. If you smoke, please select how many cigarettes you smoke per day**

- 0
- 1-5
- 6-10
- >10

**\*11. Do you participate in vigorous, highly intense exercise more than 5 times per week?**

- Yes
- No

**\*12. Do you take any prescription medications, e.g. beta-blockers, ACE inhibitors, diphenhydramine or cyproheptadine (allergy medications), lithium carbonate, corticosteroids, insulin, thiazolidinediones (Actos, Avandia, or Avandamet), sulfonylureas, incretins, sodium valproate, anticoagulants (such as Warfarin or heparin), or thyroid replacement therapy?**

- Yes
- No

**\*13. Will you be able to maintain your current diet and physical activity for a consecutive 8 weeks?**

- Yes
- No

**\*14. Are you willing and able to travel to the ASU Downtown Campus to meet with research investigators on three (3) separate occasions?**

- Yes
- No



**\*15. Are you willing to consume a gel capsule twice daily?**

- Yes
- No

Done

Powered by [SurveyMonkey](#)  
Check out our [sample surveys](#) and create your own now!

HEALTH /HISTORY QUESTIONNAIRE

ID# \_\_\_\_\_

1. (To be completed by researchers): Height \_\_\_\_\_ Weight \_\_\_\_\_  
Percent body fat \_\_\_\_\_ BMI \_\_\_\_\_
2. Age: \_\_\_\_\_
3. Have you lost or gained **more than** 5 lbs in the last 12 months? Yes No  
If yes, how much lost or gained? \_\_\_\_\_ How long ago? \_\_\_\_\_
4. College Status (please circle) Fresh. Soph. Jr. Sr. Grad.
5. Ethnicity: (please circle) Native American African-American Caucasian Hispanic Asian Other
6. Do you smoke? No, never \_\_\_\_\_  
Yes \_\_\_\_\_ # Cigarettes per day = \_\_\_\_\_  
I used to, but I quit \_\_\_\_\_ months/years (circle) ago
7. Do you take any medications regularly? Yes No *If yes, list type and frequency:*  

<u>Medication</u>	<u>Dosage</u>	<u>Frequency</u>
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
8. Do you currently take supplements (vitamins, minerals, herbs, etc.)? Yes No *If yes, list type and frequency:*  

<u>Supplement</u>	<u>Dosage</u>	<u>Frequency</u>
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
9. Have you ever been hospitalized? \_\_\_\_\_ If yes, for what? \_\_\_\_\_

OVER →

10. Please ANSWER (YES/NO) if **you** currently have or if **you** have **ever** been diagnosed with any of the following diseases or symptoms:

	YES	NO		YES	NO
Coronary Heart Disease			Chest Pain		
High Blood Pressure			Shortness of Breath		
Heart Murmur			Heart Palpitations		
Rheumatic Fever			Any Heart Problems		
Irregular Heart Beat			Coughing of Blood		
Varicose Veins			Feeling Faint or Dizzy		
Stroke			Lung Disease		
Diabetes			Liver Disease		
Low Blood Sugar			Kidney Disease		
Bronchial Asthma			Thyroid Disease		
Hay Fever			Anemia		
Leg or Ankle Swelling			Hormone Imbalances		
Eating Disorders			Emotional Problems		

Please elaborate on any condition listed above. \_\_\_\_\_  
\_\_\_\_\_

11. How would you rate your lifestyle?  
 Not active \_\_\_\_\_ Active \_\_\_\_\_  
 Somewhat active \_\_\_\_\_ Very Active \_\_\_\_\_

12. Please circle the total time you spend in each category for an average week.

**Light activities** such as:

Slow walking, golf, slow cycling, doubles tennis, easy swimming, gardening  
 Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

**Moderate activities** such as:

Mod. Walking, mod. cycling, singles tennis, mod. swimming, mod. weight lifting  
 Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

**Vigorous activities** such as:

Fast walking/jogging, fast cycling, court sports, fast swimming, heavy/intense weight lifting  
 Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

13. How much alcohol do you drink? (average drinks per day) \_\_\_\_\_

14. Do you have any food allergies? Yes No If yes, explain: \_\_\_\_\_

15. Do you follow a special diet? (weight gain/loss, vegetarian, low-fat, etc.) Yes No  
 If yes, explain: \_\_\_\_\_

**Food Frequency Questionnaire**

This form asks about your usual dietary intake over the past month. Read each food item. If you have not eaten this food in the past month, mark "none" and move onto the next food item. Indicate whether you think your usual serving size is small (S), medium (M), or large (L) by marking the correct serving size box. Think over the past month. How often do you usually eat each of the following food items? Answer each question as best you can; estimate if you are not sure. **NOTE: A small (S) serving is equal to half (½) the usual serving. A medium (M) is equal to the medium servings listed on the form. A large (L) is equal to one and a half (1 ½) times as much or more of the medium serving.**

Seafood & Fish	Medium Serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Tuna	3 ounces											
Salmon	3 ounces											
Whitefish	3 ounces											
Herring	3 ounces											
Walleye	3 ounces											
Lake trout	3 ounces											
Rainbow trout	3 ounces											
Sablefish	3 ounces											
Mackerel	3 ounces											
Catfish	3 ounces											
Flounder	3 ounces											
Perch	3 ounces											
Atlantic cod	3 ounces											
Atlantic bluefish	3 ounces											
Atlantic sturgeon	3 ounces											
Halibut	3 ounces											
Swordfish	3 ounces											
Mussels	3 ounces											
Scallops	3 ounces											
Oysters	3 ounces											
Shrimp	3 ounces											
Sardines	3 ounces											
Anchovy	3 ounces											
Blue crab	3 ounces											
Northern lobster	3 ounces											

3 ounces is about the size of a deck of cards.

Meat	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Turkey	3 ounces											
Chicken	3 ounces											
Beef	3 ounces											
Pork	3 ounces											

\* 3 ounces is about the size of a deck of cards.

Eggs	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Regular egg	1 egg											
Omega-3 enriched egg	1 egg											
Eggland's Best egg	1 egg											

Dairy products	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
2% milk	1 cup											
1% milk	1 cup											
Skim milk	1 cup											
Cheddar cheese	¼ cup											
Swiss Cheese	1 ounce											
Mozzarella Cheese	1 ounce											
2% fat cottage cheese	½ cup											
1% fat cottage cheese	½ cup											
Feta cheese	1 ounce											
Yogurt- no fat or low fat	8 ounces											

Nuts/seeds	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Walnuts	1 ounce											
Pumpkin seeds	1 ounce											
Flaxseeds	1 ounce											
Butternuts	1 ounce											
Cashews	1 ounce											
Hickory nuts	1 ounce											
Beechnuts	1 ounce											
Almonds	1 ounce											
Pistachios	1 ounce											
Pine nuts	1 ounce											
Pecans	1 ounce											
Brazilnuts	1 ounce											
Sunflower seeds	1 ounce											
Sesame seeds	1 Tbsp											
Poppy seeds	1 Tbsp											

Fats and oils	Medium Serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Miracle whip	1 tsp											
Margarine	1 tsp											
Soybean oil	1 tsp											
Sunflower oil	1 tsp											
Flax oil	1 tsp											
Canola oil	1 tsp											
Olive Oil	1 tsp											
Walnut oil	1 tsp											

APPENDIX D  
PROTOCOL DOCUMENTS

#### Protocol for Blood Lipid Analysis

1. Turn on the Piccolo Blood Chemistry Analyzer.
2. Remove all samples of frozen plasma from the freezer and place on the rocker table to thaw.
3. Once the plasma is completely thawed, remove one Piccolo® Lipid Panel Reagent Disc from the refrigerator and place on the lab bench.
4. Using pipette, draw up 100 µL of plasma from one sample.
5. Slowly pipette plasma into Piccolo® Lipid Panel Reagent Disc, stopping when the triangular window at the end of the filling area is full and free of visible air or bubbles.
6. Open disc drawer on Piccolo Blood Chemistry Analyzer and place filled disc into drawer.
7. Close drawer and program the machine to begin analysis.
8. Once analysis is complete, insert a Piccolo-specific paper strip into the machine to print results.
9. After results have been printed, open disc drawer and remove used disc.
10. Discard used disc and remaining plasma in designated biohazard container.
11. Repeat for remaining samples.

#### Protocol for Hemoglobin A1C Analysis

1. Turn on the DCA 2000 Vantage Analyzer.
2. Remove chilled whole blood from refrigerator and place on rocker table to mix and warm. Allow blood to rock for 10 minutes or until approximately room temperature.
3. While the blood is mixing and warming, remove the DCA Hemoglobin A1C Reagent Cartridges and DCA Hemoglobin A1c Capillary Tubes from refrigerator and place on lab bench. Allow to sit for 10 minutes or until approximately room temperature.
4. After 10 minutes has passed, open the packages for one DCA Hemoglobin A1C Reagent Cartridge and one DCA Hemoglobin A1c capillary tube.
5. Open one sample of whole blood and tilt until blood is at the edge of the vial.
6. Touch the end of the capillary tube to the blood until the tube is filled with blood.
7. Insert the capillary tube into the DCA Hemoglobin A1C Reagent Cartridge until it clicks into place.
8. Swipe the barcode of the cartridge through scanning strip of the DCA 2000 Vantage Analyzer. A beep will be heard.
9. Open the cartridge door on the DCA 2000 Vantage Analyzer and insert cartridge until it clicks into place. Pull tab from cartridge. Close the cartridge door.
10. Program the machine to begin analysis.
11. Place vial of remaining whole blood into centrifuge and balance as appropriate. Spin blood at 3000 rpm for 10 minutes.
12. Once spinning is complete, remove vial of spun blood from centrifuge, being careful not to agitate or mix the blood.
13. Pipette approximately 100 µL of plasma from centrifuged blood and pipette into vial.
14. Freeze vial for future analysis needs.
15. Once hemoglobin A1C analysis is complete, program machine to print results.
16. After results have been printed, open cartridge door and remove used cartridge.
17. Discard used cartridge in designated biohazard container.
18. Discard remaining blood in designated biohazard sharps container.
19. Repeat for remaining samples.



## Calculations

$$\text{BMI} = \text{weight (kg)} / \text{height (m}^2\text{)}$$

$$\text{Friedewald Equation: LDL-C} = \text{TC} - (\text{HDL-C} + \text{TG}/5)$$