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Influence of food on the bioavailability of a docosahexaenoic acid supplement

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by

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DEDICATION

Dedicated to my Lord and Savior, Jesus Christ

I can do all things through Christ who strengthens me.

Philippians 4:13

TABLE OF CONTENTS

ABSTRACT

The purpose of this project was to observe whether consuming a DHA supplement with or without a meal influenced the bioavailability of the DHA supplement. Healthy males (n = 109) aged 18-45 years were randomized to 4 study groups that included 200 mg/d DHA consumed with a meal, 200 mg/d DHA consumed fasted, 1000 mg/d DHA consumed with a meal or 1000 mg/d DHA consumed in a fasted state over 8 weeks. Fasted venous blood samples were collected pre- and post-DHA supplementation for analysis of plasma phospholipid (PL) fatty acid analysis. Plasma PL DHA increased significantly in all groups ($P < 0.0001$). The increase in DHA was significantly different between all dose groups (200 mg versus 1000 mg; $P < 0.0001$). There were no differences between the meal or fasted groups. Thus, consuming a DHA supplement with a meal did not increase the bioavailability of the DHA supplement.

CHAPTER 1: INTRODUCTION

The essential fatty acids, linoleic acid (LA), an omega-6 (n-6) fatty acid, and α-linolenic acid (ALA), an omega-3 (n-3) fatty acid, have been the subject of much study. Docosahexaenoic acid (DHA) is an omega-3 (n-3), long-chain polyunsaturated fatty acid (LCPUFA). DHA is synthesized from ALA, and it can interconvert with another n-3 fatty acid, eicosapentaenoic acid (EPA). The conversion of ALA to DHA is inefficient (1), thus resulting in recommendations to include DHA specifically in the diet. DHA has multiple biological functions including roles in early development, reduction of inflammation, and promotion of heart health (2-4).

DHA is involved in fetal and infant brain and visual development (2). This role of DHA is primarily a result of its incorporation into membranes in the central nervous system, specifically the brain and retina (5-6). Innis and Friesen (6) found that the infants of mothers who consumed DHA supplements during pregnancy (400 mg/d) had higher scores for visual acuity. While DHA plays an important role in development of the nervous system, including vision, there is evidence of continued beneficial effect on brain health from DHA beyond fetal development. In a study that examined influences of DHA on memory and learning throughout the lifespan, DHA was more strongly associated with cognitive function than was either EPA or ALA (7). In addition to development, DHA also plays a role in heart health. EPA and DHA together reduce risk for heart disease and lower elevated serum triacylglycerols. Additionally, in adults DHA alone (1000 mg/d for 8 weeks) also has been shown to decrease triacylglycerols (21.8%) similarly to the decrease in triacylglycerols (18.3%) via DHA and EPA combined (4). There also has been research exploring the potential benefit of DHA consumption to assist with the management of diabetes as indicated by two systematic reviews from earlier this year (3, 8). One review suggests that, while n-3 fatty acids lower triacylglycerols and thus provide a cardioprotective benefit, there

is no additional benefit in type 2 diabetes mellitus (T2DM) in humans in terms of lowering serum glucose or insulin (9). Another review sought to clarify potential mechanisms and concluded n-3 reduced insulin resistance, thus indirectly assisting with plasma glucose control (10). Overall, although there were slight indications of some benefit from n-3 FAs in diabetes, there does not seem to be strong associations between n-3 FAs or DHA specifically and diabetes.

DHA is found in many food sources including fish, especially fatty fish, and to a lesser extent in eggs and meats. In addition to naturally occurring in some foods, DHA also has been added to foods that do not otherwise contain it. The list of such fortified foods is lengthy and includes breads, juices, milk, and margarine just to name a few (11).

Supplements also provide DHA either singly or in combination with EPA and other fatty acids. These supplements can be from a variety of sources including oils originating from fish body, cod liver, or additionally as concentrated fish oil which decreases the number of capsules needed to reach the same dose with the other oils. Concentrated fish oil is synthesized via trans-esterification and re-esterification and usually consists of free fatty acids, more accurately non-esterified fatty acids (NEFA), LCPUFA ethyl esters or re-esterified triacylglycerols. In addition to fish oils, algal oil also provides a rich source of DHA and is much higher concentration of DHA relative to other fatty acids normally found in n-3 supplements. Algal oil supplements are also an acceptable option for individuals who are vegan and cannot consume fish oil or the marine foods that are high in DHA. Another source of DHA is via ethyl esters of LCPUFA as found in some of the concentrated fish oils and in prescription pharmaceutical capsules. DHA ethyl esters allow a higher concentration of DHA than is found in standard n-3 supplements.

DHA can be found in different structural forms depending on whether it is consumed as food, fortified food, fish oil, concentrated fish oil, algal oil, or prescription. These forms

include natural triacylglycerol, phospholipid, ethyl esters, and re-esterified triacylglycerol. In a review by Dyerberg et al. (12), various studies explored the role of structure on the bioavailability of n-3 fatty acid supplements, however, to our knowledge no work has been done to evaluate directly whether a DHA supplement consumed with a meal can influence the absorption of DHA when consumed with food versus consumed in a fasted state. This question was the primary objective of this study.

The goal of this study was to determine if consuming a DHA supplement with food influences the absorption of DHA compared with consuming a DHA supplement in a fasted state. We hypothesize that consuming a DHA supplement with food will influence the bioavailability of an algal-based DHA supplement.

CHAPTER 2: REVIEW OF LITERATURE

Docosahexaenoic Acid and Omega-3 Fatty Acids

Specific omega-3 (n-3) and omega-6 (n-6) fatty acids are both needed by the human body for optimal function. Essential nutrients are those the body cannot synthesize itself, and are thus need obtained via the diet. There are two essential fatty acids, linoleic acid (LA), an n-6 fatty acid, and α-linolenic acid (ALA), an n-3 fatty acid. From these two fatty acids, the body can derive various other n-6 and n-3 fatty acids that are required for normal metabolic function; thus, LA and ALA serve as precursors. LA only yields n-6 fatty acids and ALA only leads to n-3 fatty acids. The n-6 and n-3 fatty acids follow parallel pathways from precursor to later products. These processes share enzymes for the conversion from one fatty acid to the next in the sequence as can be seen in Figure 1. The sharing of enzymes in these pathways is what has contributed to the interest in the ratio between n-6 and n-3 fatty acids.

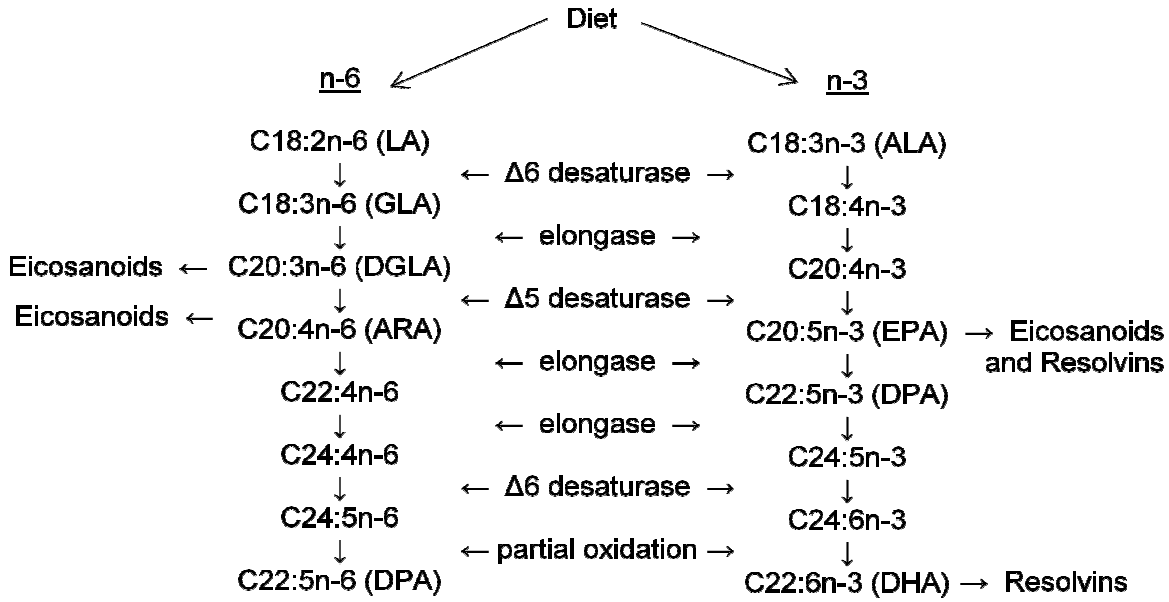


Figure 1. Parallel synthesis pathways for omega-6 and omega-3 fatty acids. Linoleic acid (LA), α-linolenic acid (ALA), gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DGLA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA). Figure was adapted from others (1, 13.)

Arachidonic acid (ARA) is a n-6 long-chain polyunsaturated fatty acid (PUFA) synthesized from LA and gives rise to a class of signaling molecules called eicosanoids. Parallel to ARA formation from LA in the n-6 class of fatty acids, ALA is used to synthesize other n-3 fatty acids. Some of the better known n-3 fatty acids include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA is the precursor to eicosanoids in a comparable manner to ARA. DHA also can be used to form biologically active compounds similar to the eicosanoids, called resolvins (14).

DHA is a long-chain polyunsaturated fatty acid (LCPUFA) containing 22 carbons and six double bonds and belonging to the n-3 class of FAs. DHA and other LCPUFAs are found in the PL plasma membranes, and also located in sphingolipids and plasmalogens

(1). Because of its presence in cell membranes, DHA can be found anywhere in the human body. Although DHA is incorporated into lipids of various tissues in the body including brain, retina, heart, and other organs, it is known to be in especially high concentration in the brain and retina (1). The conversion of ALA to DHA proceeds via EPA and DPA. The first steps of this conversion process take place on the endoplasmic reticulum; however, translocation to the peroxisome is necessary for the final conversion from C24:6n3 to DHA via oxidation (1, 13). The conversion of dietary ALA to DHA is inefficient in humans, with estimates ranging from <0.05% up to 4% at the highest (15).

In addition to increasing plasma DHA concentration, DHA supplementation also increases EPA plasma concentration. The increase in plasma EPA concentration resulting from DHA intake occurs in a linear fashion with each 1 g/d increase in DHA dose yielding an approximately 0.4 g/100g increase in EPA concentration that is thought to be a result of retroconversion from DHA to EPA (1). Conquer and Holub (16) evaluated the retroconversion of DHA to EPA in 20 healthy adults including both vegetarians (12 subjects, 6 male and 6 female) and omnivores (8 participants, 4 each male and female) by supplementing with algal DHA at a dose of 1.62 g/d for 6 weeks and estimated a retroconversion rate from DHA to EPA of 9.4% without significant difference between the groups. However, a review evaluating the biological effects of DPA suggests that there is more evidence for the retroconversion of DPA to EPA than there is for retroconversion of DHA to EPA (17). Although there is little evidence for retroconversion of DHA to form EPA, there is evidence that DHA supplementation results in an increase in EPA (1). In addition to causing an increase in plasma EPA concentration, DHA intake also leads to a decrease in plasma ARA concentration although not in linear manner (1). A recent review examining differential effects of DHA and EPA states that the conversion of ALA to form DHA does not

take place beyond infancy (18). Average plasma PL DHA in unsupplemented humans is approximately 2.5%-3.5% (1).

Accurate and non-invasive methods are needed to accurately estimate LCPUFA status in the tissues of the human body. Plasma PL and erythrocyte (RBC) PL fatty acids can both be used as an estimate of tissue fatty acid status (1, 19-20). The normal human plasma PL response to DHA supplementation is a dose-response relationship occurring between 2 weeks (21) and 1 month (1). DHA concentration reaches a steady state thereafter, although a low dose (e.g. 0.2 g/d) of DHA will result in a slower response than a high dose (e.g. 6 g/d) of DHA (1). Additionally, the plasma PL DHA response approaches saturation with a dose of approximately 2 g/d or more DHA (1, 20). In contrast to plasma PL DHA response, RBC DHA increases more slowly in response to supplementation with DHA. The DHA concentration in RBCs increases more gradually, and it can require as long as 4 and up to 6 months to reach steady state in RBC DHA with supplemented DHA (1). While RBCs provide an acceptable biomarker for measuring FA status, study design must provide adequate duration of supplementation to observe a response to supplementation in RBCs.

In a supplementation study by Cao et al. (19), provided either fish oil containing 1296 mg/d EPA and 864 mg/d DHA or flaxseed oil containing 3,510 mg/d ALA and 900 mg/d LA, to 20 healthy adults over 8 weeks, they found both RBCs and plasma PL to be effective biomarkers of EPA and DHA supplementation. Although subjects consumed the supplements for 8 weeks, blood samples were collected throughout the 24-week study period allowing RBC EPA and DHA amounts to be observed post-supplementation. Fish oil supplementation led to increases in DHA (42%, $P < 0.001$), EPA (300%, $P < 0.0001$), and DPA (39%, $P < 0.01$), with no change in ALA concentration and a 23% decrease in ARA ($P < 0.001$). Supplementation of flaxseed increased EPA (33%, $P < 0.05$), ALA (100%, $P <$

0.001), and n-3 DPA (20%, P < 0.01), decreased ARA (9%, P < 0.01), but had no significant effect on DHA (19). Because plasma PL responded more quickly both to n-3 LCPUFA supplementation and also its withdrawal, compared with the slower response of RBCs, the authors suggest that plasma PL would likely be a better measure for short-term (e.g. 4 weeks) intervention supplementation and an indicator of compliance (19).

In addition to plasma PL and RBC DHA, other biomarkers that also have been used to assess DHA status include DHA from total plasma lipids, PL, TG, cholesteryl esters (CE), and NEFA as well as RBC PL DHA, and platelet DHA. Plasma PL DHA was found to be an effective biomarker without regard to supplementation dose or DHA status prior to supplementation whereas as other biomarkers were influenced by supplementation dose or DHA status prior to supplementation (20). There was more evidence for plasma PL as a biomarker because more studies use plasma PL to assess fatty acid status. Additionally, plasma PL EPA has been shown to be an effective measure for EPA. Thus, plasma PL provides a useful tool to estimate both DHA and EPA following supplementation with these FAs. Plasma PL is commonly utilized to assess fatty acid status of tissues because the PL fraction of plasma lipids represents tissue DHA concentration and exhibits a rapid response time in a dose-dependent manner. Separating the PL from the other lipid components in whole blood (e.g., TG, CE) avoids the normal inter-individual variance in plasma TG concentrations. A 14-week supplementation study was conducted using fish (670 mg/d DHA, 380 mg/d EPA, and 80 mg/d DPA), fish oil capsules (119 mg/d DHA, 166 mg/d EPA, and 19 mg/d DPA) and algal oil DHA capsules (210 mg/d DHA, no EPA or DPA) in 59 healthy male students aged 19-32 years old (22). Diet records indicated that approximately 14 g/d of fish (variety of types of fish including rainbow trout, Balting herring, and vendace) were consumed by the fish diet group. In addition to the fish diet and fish oil groups, there was a control group that was simply asked to maintain normal diet and activity. Fatty acids

were analyzed in plasma lipids (PL, TG and CE), RBCs, and platelets. There were no changes in fatty acids for the control group. Plasma PL EPA and DHA were related to EPA and DHA in RBCs and platelets. Results indicated DHA is primarily incorporated into PL and TG, but only minimally in CE whereas EPA was observed in PL and CE. Interestingly, they noted an increase in EPA plasma lipids, RBCs and platelets in the DHA supplementation group, suggesting retroconversion and also an increase in LA in RBCs and platelets but not in plasma lipids (22). Collectively, these studies suggest that a DHA supplementation study needs to last at least four weeks if measuring plasma PL DHA and eight weeks or longer for analysis of RBC DHA.

Health Benefits of Docosahexaenoic Acid

DHA has many biological functions including fetal and infant development (5). In a study evaluating the influence of maternal DHA intake on infant development, 227 participating breastfeeding mothers were randomly assigned to consume either an algal oil DHA capsule (200 mg/d DHA) or a placebo containing soy and corn oil for the first 4 months post partum (2). Plasma PL FAs were analyzed as well as milk fatty acids. Outcomes for the infants included Bayley Scales of Infant Development at 30 months of age and assessment of infant visual ability at age 4 and 8 months via Teller Acuity Card procedure. DHA in milk samples from mothers in the DHA group increased significantly compared with the control group ($P < 0.0001$), and there was a decrease in ARA ($P < 0.001$). At 4 months, infant plasma PL FA composition was similar to that of milk FA. By 8 months of age, there were no significant differences in plasma PL FAs between infants of mothers who received DHA versus infants of mothers who consumed the placebo. The only significant difference between groups on infant developmental indices was the Bayley Psychomotor Development Index that was significantly higher at 30 months of age for the group whose mothers had consumed DHA (2). Another study examined influence of maternal intake of DHA in a

snack bar during pregnancy on infant visual acuity (23). This study was a randomized controlled trial of 30 pregnant women more than 20 weeks gestation and 18-35 years of age. The mothers consumed a snack bar containing either 300 mg of DHA from a fish oil with low EPA (EPA:DHA of 1.8:1) or the placebo corn oil. Additionally, some of the women from each group consumed three, five, or seven bars per week starting at week 24 of gestation and until delivery. The bars in the DHA group yielded a DHA dose of approximately 214 mg/d DHA; there was no difference between DHA and placebo bars in macronutrient composition. Blood draws and dietary recalls were conducted prior to supplementation and three other times during the study period. The infants received testing on visual acuity at ages 4 and 6 months using the Teller Visual Acuity cards. There were no differences between groups regarding infant feeding method: whether breastfed, or fed infant formula with DHA, infant formula without DHA, or combinations of these. At 4 months of age, the infants in the DHA group had a significantly higher visual acuity score versus the placebo group, but there was no difference between groups in visual acuity score at 6 months of age (23). This research group also conducted a study looking at maternal intake of DHA from bars as in the previous study but looking at infant problem-solving ability as outcome for this study (24). This study enrolled 29 healthy pregnant women from 18-35 years old and who were at least 20 weeks gestation. DHA dose from the bars was the same (~214 mg/d DHA from five bars per week), and placebo bars were also the same. Infants were given a problem-solving test at 9 months of age to assess their ability to follow more than one step in a process and a test of memory, the Fagan Test of Infant Intelligence. Infant feeding method was not significantly different between groups. The infants of the mothers who had consumed the DHA bars had a significantly higher ($P = 0.017$) total average score for the intention component of the problem-solving test and significantly higher in all three scores for the intentional solution aspect of the problem-solving test ($P =$

0.008, P = 0.004, P = 0.011) versus the infants of the mother how had consumed placebo bars. However, there were no differences in memory between the groups (24).

DHA decreases risk of heart disease and lowers elevated plasma TG (4). DHA along with EPA found in fish oil, is capable of decreasing human deaths resulting from cardiac causes (25). The benefits of combined EPA and DHA are well established, and it is recommended to supplement them both (4). It has been well established that combined DHA and EPA cause a decrease in plasma TG. DHA alone also has been found to lower plasma TG. In a study using 1000 mg/d DHA from algal oil for 8 weeks, it was found that DHA alone contributes to lowered risk of cardiovascular disease by decreasing fasting TG (4, 26). This study provided either 1000 mg/d DHA from algal DHA or 1252 mg/d DHA and EPA, of which 1000 mg/d was DHA to 116 adult patients who had coronary artery disease (CAD) and fasting TG over 200 mg/dL. Results indicated significant decreases in fasting TG in both groups and they observed a greater proportion of the DHA-only group who lowered fasting TG below the goal of 150 mg/dL (26). The actual dose of DHA used in studies varies greatly with one systematic review observing DHA dosages in different studies ranging from 83 mg/d up to 4900 mg/d DHA (20). Another study used a dose as low as 50 mg/d DHA (27). DHA provides a variety of health benefits including promotion of heart health and contributing to optimal fetal and infant development.

Docosahexaenoic Acid and Lipid Structure

A TG is a structure composed of three fatty acids (FA) esterified to a glycerol backbone. There are three hydroxyl positions available for fatty acids. These positions are referred to as sn-1, sn-2, and sn-3. The middle position is sn-2, whereas the ends of the glycerol are sn-1 and sn-3. PUFAs are typically in the sn-2 position as a result of selectivity of enzyme function. Another lipid structure includes PL (Figure 2), which compose membranes and are a class of compounds with a phosphate group in sn-3 between the

glycerol and a functional group. The functional group can be a variety of compounds. For example, if choline is the functional group, then phosphatidylcholine (PC) results (also commonly called lecithin). Likewise, there is phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI). DHA usually is located in the sn-2 position on PE. The reason for this specificity is explained by lipid digestion. Normal lipid digestion utilizes lipases to hydrolyze lipids into their component parts based on the specialized function of the enzyme. Gastric lipase starts the catabolic process in the stomach and targets the sn-3 location. Of note, gastric lipase does not catalyze hydrolysis of PL, thus digestion of these lipids does not occur until the small intestine. As digestion progresses, pancreatic lipase further assists in hydrolysis of consumed lipids with action on either the sn-1 or the sn-3 position of TG. Once in the small intestine, another pancreatic enzyme, phospholipase A_2, finally initiates break down of phospholipids via preferential hydrolysis at the sn-2 position. Phospholipase A_2 is secreted by the pancreas in a pro-enzyme form and not activated until it reaches the small intestine. The action of this enzyme allows it to free LCPUFAs in sn-2 of phospholipids. Figure 2 shows examples of both TG and PL.

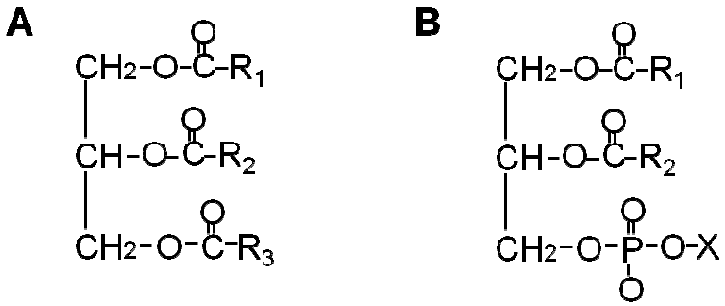


Figure 2. Structures of triacylglycerol, A, and phospholipid, B. Any fatty acid can be esterified in place of the "R" groups. In A, R_1 corresponds to sn-1, R_2 to sn-2 and R_3 to sn-3. In B, a variety of functional groups can be in the place of "X" such as choline, ethanolamine and others.

In addition to PL and TG, DHA and other FAs also can be found in a variety of lipid forms including NEFA, fatty acid methyl esters (FAME) and ethyl esters (EE) (See Figure 3). Dietary lipids are primarily in TG and PL forms. Fish oils, made from the fish body, cod liver oil, and algal oil are in TG form. Concentrated fish oils have had the lipids trans-esterified to EE to selectively increase the concentration of EPA and DHA, and, in some fish oil concentrates, the EE have undergone re-esterification back to TG, (rTG) (12). Algal oil can be from more than one type of algae, and, although sometimes different species are used for different FAs, more than one species can yield the same FA. DHA is one example of a FA that can be synthesized by more than one type of algae. Algal oil DHA is the source used to fortify infant formulas, in the prenatal supplement, Expecta®, and for other DHA-only supplements (11). This source of DHA has been used in many different fortified food products (11).

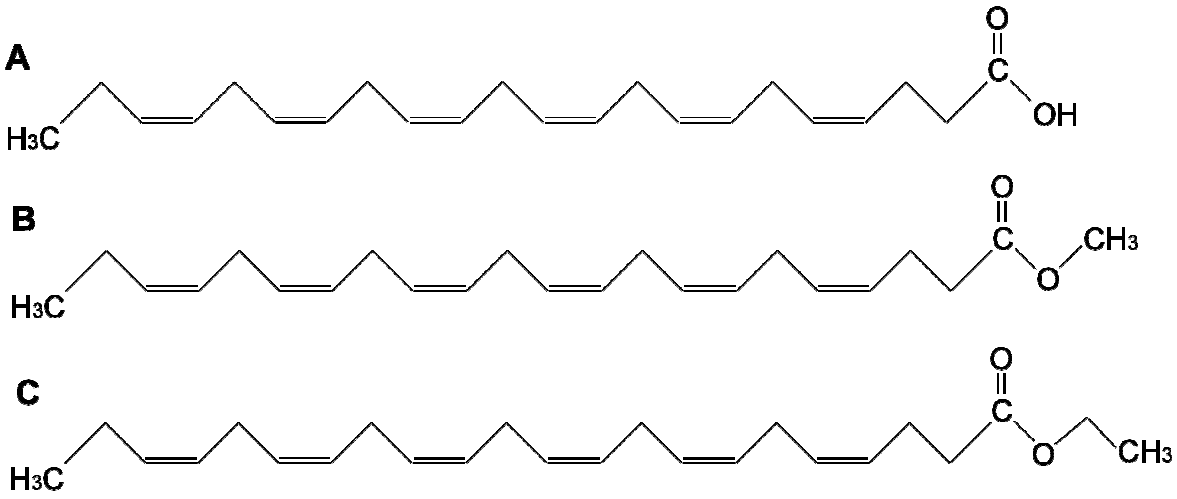


Figure 3. Structures illustrating DHA as different lipid forms. A. DHA as non-esterified fatty acid (NEFA), B. DHA as fatty acid methyl ester (FAME), C. DHA as fatty acid ethyl ester.

DHA can be incorporated into TG and PL, or found as NEFA or EE. An example of EE is found in LOVAZA ®, a prescription omega-3 containing pharmaceutical. The DHA in fish is both as PL and TG, DHA is also in TG in most fish oils, algal oil supplements, and

fortified foods (Figure 3). One exception to DHA being found in TG form in fish oil is krill oil, which contains DHA as PL.

Bioavailability of DHA

Bioavailability is the amount and rate a substance is absorbed and biologically available to be utilized at the intended tissue site, and various factors can affect how well a substance is absorbed and used (28). Bioavailability and bioequivalence are not synonymous. Rather, bioequivalence compares the bioavailability of two or more different, though usually similar, compounds. The present study focuses on bioavailability and not bioequivalence.

There are many factors that can influence bioavailability, and these vary depending upon the nature of compound being examined. Biological variables influencing bioavailability include physiological status, presence of disease, and gender (28). There are various structural components that can influence bioavailability including structure of the compound itself, or the composition of other foods consumed with it. A review comparing across studies the bioavailability and food microstructure of a variety of different nutrients, discussed the many effects of food structures that can influence absorption and utilization of nutrients (29). An interesting example given illustrates the ability of one type of food structure to increase absorption of one type of nutrient but decrease the absorption of a different nutrient. The example given included the observations that fiber in food could lower absorption of carotenoids, but the absorption of ferulic acid, a phytonutrient, was increased by fiber-rice bran. This observation regarding the different effects of fiber added to a food on bioavailability of a specific nutrient is one illustration of how the addition of a component to a food matrix may either decrease or increase absorption (29). The observation that the bioavailability of fat-soluble vitamins (A, E, D, K) and other lipid

nutrients are generally more bioavailable when consumed with other fats is potentially relevant when considering bioavailability of a lipid, such as algal oil DHA

In addition to various factors influencing bioavailability, there are also multiple challenges to consider when assessing and determining bioavailability. There are different ways to assess bioavailability including both in vitro and in vivo approaches. A large obstacle with in vivo studies is the large variation between individuals participating in a study (29). One key aspect of assessing bioavailability of any nutrient is the influence of individual variation on absorption, utilization, or incorporation of a nutrient and the variable response to the dose administered (30). In a review examining some of the complexities in determining bioavailability and bioequivalence the need for standardization in the process was emphasized to enable easier comparisons among research findings (30). Included in the lack of standardization of nutrient bioavailability are varying definitions, different methods of testing nutrient composition, and different interpretations of observations as a result of non-standard definitions.

Several studies have examined various aspects of bioavailability of DHA supplements. In one study, 86 non-pregnant, healthy women who were 20-45 year-old completed 4 weeks of DHA supplementation in which they were given different DHA supplement capsules containing tuna fish oil, algal oil as either DHA alone or DHA with ARA, or placebo (microcrystalline fructose). The actual DHA doses were as follows: low fish oil--0.266 g/d DHA, high fish oil--0.532 g/d DHA, low algal oil--0.285 g/d DHA, high algal oil--0.570 g/d DHA, and high algal oil DHA and ARA--0.570 g/d DHA and 0.259 g/d ARA. As expected, there was a dose-dependent increase in DHA concentration in plasma PL with an observed equilibrium between 2 and 3 weeks. RBC PL DHA also increased with the four weeks of supplementation albeit more slowly and there was no change in either plasma or RBC PL DHA for the control group (31). All forms of DHA supplementation resulted in

significantly ($P < 0.0001$ for all groups except the low algal oil DHA group with $P < 0.001$) increased plasma PL DHA.

The bioequivalence of algal oil DHA from different species of algae have been compared. A study examined algal oil DHA supplements from two different species of microalgae, *Crypthecodinium cohnii* (DHASCO-T) and *Schizochytrium* (DHASCO-S) along with a snack bar fortified with DHASCO-S (21). This study was a randomized, parallel arm, clinical trial and outcome measures included plasma PL and RBC DHA. There were 96 healthy men and women between 18 to 71 years of age randomized to eight different treatment groups for the 28 days of supplementation. The groups included each of the following doses from both DHASCO-T and DHASCO-S, 200 mg/d DHA, 600 mg/d, and 1000 mg/d. Additionally, there was one group that consumed a snack bar containing 465 mg/d DHASCO-S and finally a control group consuming placebo capsules (corn and soy oil). Plasma PL and RBC DHA were assessed at baseline, 2 weeks, and 4 weeks of the study. They found that all forms of DHA were equally bioavailable as evidenced by no differences in plasma PL DHA between DHASCO type for a given dose, and all results followed dose-response relationships in that a higher dose of DHA supplementation contributed to a greater increase in plasma PL and RBC DHA across supplementation. DHASCO-T and DHASCO-S plasma PL DHA was ~4 g/100 g FA after 4 weeks of 200 mg/d DHA, ~5.5 g/100 g FA after 600 mg/d DHA for 4 weeks, and ~7 g/100 g FA following 1000 mg/d DHA. Interestingly, the 465 mg DHASCO-S in the snack bar yielded plasma PL DHA of ~5.5 g/100g FA similar to the 600 mg/d DHA capsule groups (21). The same plasma PL DHA response from 600 mg/d DHA from capsules and 465 mg/d DHA from snack bars would suggest that consuming a DHA supplement with food increases the bioavailability of the DHA.

In another study, the bioequivalence of an algal DHA supplement versus a high-DHA fish, salmon was explored (32). Healthy males and females, 20-65 years of age, consumed 600 mg/d of algal DHA (n = 16) or ate approximately 2 ounces of salmon per day providing 600 mg/d DHA (n = 16) for 14 days. The salmon used in the study was analyzed and DHA content quantified prior to providing fish to participants and again after the study ended. The DHA content of the salmon remained constant over the duration of the study. Plasma PL and RBC DHA were both analyzed for FAs. Plasma PL DHA was not different between capsule and fish groups after supplementation. RBC DHA did show a significant difference, with capsules resulting in higher DHA (34.42 μg/mL for algal DHA versus 31.90 μg/mL for salmon). The DHA for the two groups were concluded to be bioequivalent (32). Although dietary DHA intake, assessed at baseline, was similar between groups, DHA from diet was not evaluated at any other time during the study. The results from the previous study would suggest that DHA from salmon would yield a greater increase in DHA status compared with the DHA supplement. However, this study showed equal plasma PL DHA response from DHA from both salmon and a supplement.

It is interesting that when examining the bioavailability of DHA, little focus seems to have been placed on any potential influence of food. Most studies evaluating the benefits of DHA do not make note of instructing participants to consume the supplement with or without food. In the study by Arterburn et al. (21), no mention was made of how participants consumed the supplements other than whether it was as a capsule or in a snack bar as a fortified food. Interestingly, the group consuming the snack bars, which contained 465 mg DHA, had similar plasma PL DHA concentration with DHA supplementation to the group taking the 600 mg DHA capsule supplement. The similar plasma PL DHA response to both 600 mg DHA capsule and 465 mg DHA snack bar suggests that the presence of food may enhance DHA absorption. On the other hand, in the study comparing 2 ounces of salmon

(600 mg/d DHA) and an algal DHA supplement (600 mg/d DHA), Arterburn et al. (32), showed the algal oil DHA supplement to be as effective at increasing DHA in plasma PL as DHA from salmon as evidenced by increase in plasma PL DHA from baseline to 2 weeks of DHA supplementation of 4.21 g/100g total fatty acids to 6.56 g/100g with DHASCO-T and 4.37 g/100g to 7.85 g/100g for salmon group, $P < 0.05$ for differences between baseline and end of supplementation and no significance difference between increase in plasma PL DHA between fish and algal DHA groups (32). These findings may actually suggest no influence of food on absorption if DHA from capsules was absorbed in a bioequivalent manner to DHA from food. Again, it was not specified how the supplements were consumed, whether with or without food. Another study provided 50 or 100 mg/d algal DHA in orange juice to healthy children aged 4 to 12 years old (27). Blood samples were collected at baseline and after the 6 weeks of DHA supplementation. Both DHA dose groups had significantly increased DHA (mole percent) from baseline ($P < 0.05$). A significant difference in plasma PL DHA was observed between the two groups with the 100 mg/d DHA dose group (plasma PL DHA 4.61% as mole percent) being significantly higher than the low dose group (DHA 3.80%, $P < 0.05$) (27). Here again, it seems that potentially food, in this case juice, may aid the absorption of DHA when it is consumed as a supplement. Two of these studies suggest a potential influence of food on the bioavailability of DHA supplements, but a third does not show evidence for an effect from food on DHA bioavailability.

An additional factor that affects absorption of DHA is hormones. Giltay et al. (33), found that estrogen seems to play a significant role in DHA status. The influence of natural hormone differences between healthy men and women was examined as well as the effects of taking exogenous hormones, such as oral contraceptives for one example. Higher estrogen was related to an increased concentration of DHA as evidenced by highest DHA seen in women ingesting oral contraceptives (n = 32, DHA 0.58% by wt) followed by women

not consuming oral contraceptives (n = 71, DHA 0.53%) and men (n = 72, DHA 0.48%) having the lowest concentration of DHA in plasma CE. The differences in DHA concentrations were not significant between the two groups of women, but the women not consuming oral contraceptives had significantly higher DHA concentration than did the men ($P < 0.0005$) (33). Two reviews have noted an effect on DHA absorption by estrogen as well (1, 34). Additionally, there is increased circulating maternal DHA during gestation that is thought to be a result of higher estrogen during pregnancy (33). The increase in circulating DHA starts very early in pregnancy (31). Collectively, these studies indicate an influence of estrogen on absorption of DHA. To avoid any potential confounding of our results by variations in estrogen, we chose to enroll only males in the study.

Summary

While different questions regarding bioavailability and bioequivalence of DHA supplements have been researched, the specific question of how food influences the absorption of DHA has not been studied yet. The primary objective of this study is to determine if consuming a DHA supplement with or without food can influence plasma PL DHA. The hypothesis is that food from a meal will influence the bioavailability of an algal oil DHA supplement. Findings from this study are important because they will provide information helpful in the design of future DHA supplementation research studies as well as potentially contributing to future guidelines regarding DHA supplementation. Thus, the information will be relevant in both research and clinical settings.

CHAPTER 3: METHODS AND PROCEDURES

Study Participants

One hundred nine healthy males aged 18 to 45 years enrolled into the DHA Supplement Study conducted at Iowa State University. These participants were recruited campus-wide and by posting flyers posted on the Iowa State University campus and surrounding communities. Over the course of the study, 10 participants withdrew or were excluded as a result of time constraints (n=5), failure to comply with study protocol (n=1), complaints regarding supplement side effects (n=1), or illness or family reasons (n=3). Thus, 99 participants successfully completed the study.

Recruitment took place between March and October 2010, although data collection was not complete until January 2011. Participants qualified for inclusion if they were male, between the ages of 18 to 45 years, and consumed less than 100 mg DHA per day. Other exclusion criteria included current smoking, gastrointestinal or lipid disorders, chronic diseases, or consuming n-3 supplements. Additionally, potential participants were excluded if they donated plasma, platelets, or other blood products because of the potential interference with measuring blood biomarkers. There is evidence that estrogen may increase the absorption of DHA (33-34); therefore, to avoid any possible influence of fluctuations in or higher levels of estrogen, only males were included. The study was approved by the Iowa State University Institutional Review Board. Participants provided written informed consent prior to taking part in the study.

Study Design

The DHA Supplement Study was a parallel arm, randomized study (Figure 4). The experimental design was a 2 x 2 factorial. The two factors included food, whether the supplements were consumed with or without food, and the dosage of the DHA supplements which was either 200 mg per day or 1000 mg per day. Randomization was done using the

random number function in Excel. Participant codes were assigned to treatment groups entirely at random prior to commencement of enrollment. Participants were assigned a code number based on the order of entry into the study; thus, allocation of participants to treatment groups was not influenced in any way. Participants were asked to come to the Nutrition and Wellness Research Center (NWRC) campus location for each study session. The first visit for all who responded to recruiting efforts included signing the consent form, having height and weight measured and completing a DHA screening questionnaire (see Appendix I). Once a participant passed this initial screening verifying they met inclusion criteria (<100 mg/d DHA), there were four additional time-points corresponding to weeks 0, 2, 6, and 10 of the study. The first 2 weeks of the study were a run-in period; all participants consumed a control supplement (corn and soy oils) daily.

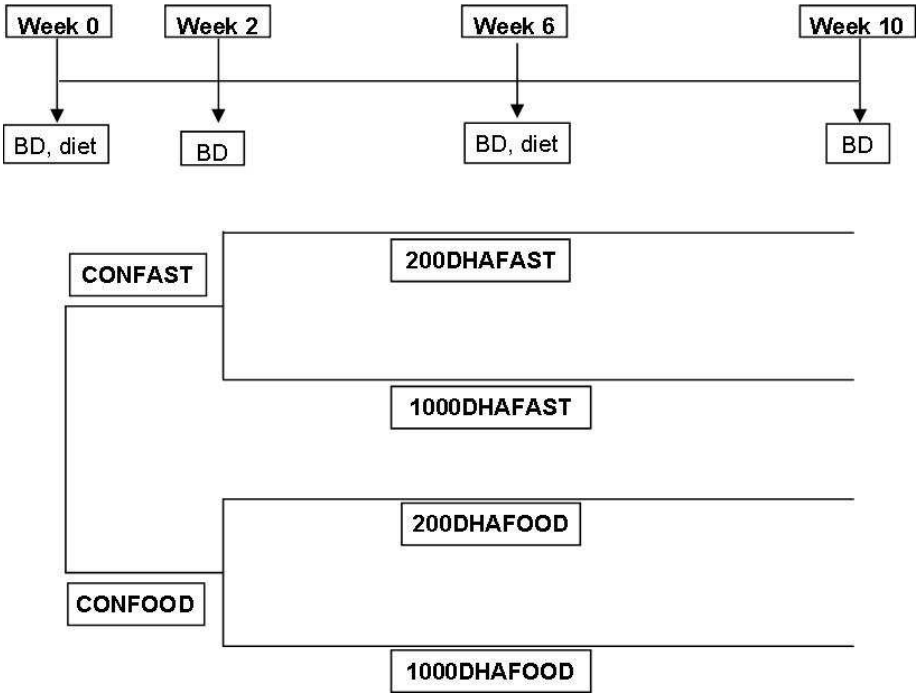


Figure 4. Study Design for the DHA Supplement Study. Blood draw (BD), control supplement consumed in fasted state (CONFAST), control supplement consumed with food (CONFOOD), 200 mg DHA consumed daily while fasted (200DHAFAST), 1000 mg DHA

consumed fasted (1000DHAFAST), 200 mg DHA consumed with food (200DHAFOOD), 1000 mg DHA consumed with food (1000DHAFOOD).

Data Collection

Data collection began in May 2010 and concluded in late January 2011. There were various items collected throughout the study including information on health history (screening), diet (screening, week 0 and week 6), as well as information to help assess compliance (throughout) to the study protocol. Figure 5 shows the timeline for the study and lists what data items were collected at each time-point.

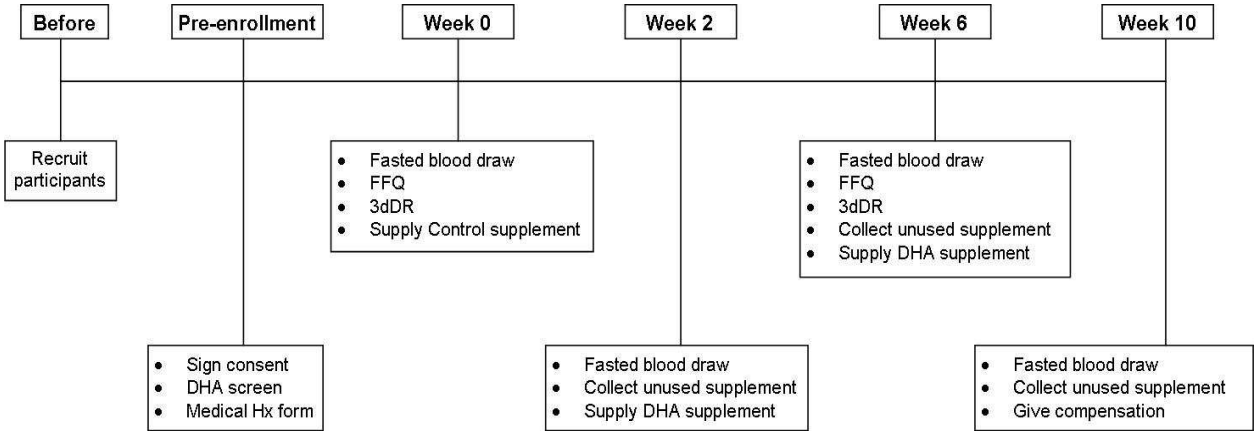


Figure 5. Timeline for data collection for the DHA Supplement Study. Medical history questionnaire (Medical Hx form), food frequency questionnaire (FFQ), 3-day weighed diet record (3dDR).

At screening, all participants completed a DHA screener consisting of 10 food categories (e.g., tuna, fatty fish, lean fish, eggs, and n-3 fortified foods) to estimate DHA intake over the previous month. In addition, all participants also were instructed on how to complete a three-day weighed diet record (3dDR) that was completed at weeks 0 and 6. The instructions and diet record sheets can be found in Appendix I along with the other materials used in the study. Participants were provided with all equipment and data sheets needed to weigh and record all foods consumed for 3 days. They were further instructed to

record their entire dietary intake for 2 weekdays and 1 weekend day with the goal of capturing usual intake. Trained staff administered a semi-quantitative food frequency questionnaire (FFQ) to each subject at weeks 0 and 6 of the study. The FFQ had 19 major categories (e.g., dairy, vegetables, and meats) with many subcategories (e.g., milk, cheese, and yogurt). Responses were assisted with the use of food models to illustrate various amounts of food items. All diet data were analyzed by using Nutritionist Pro™ software version 4.4.0 (Axxya Systems, Stafford, TX). Food items not found within the software program were added or existing items modified to match actual composition of the actual foods that the participants consumed. With a few exceptions (instances where staff moved elsewhere prior to completion of data collection), the same staff member interviewed a specific participant over the duration of the study to limit potential for apparent variation because of slight differences in staff style of collecting data. Similarly, all data for a given participant were entered by the same staff member again to minimize variability. All staff members were trained in a similar manner to promote consistency to the data collection process.

Assessment of Supplement Compliance

Participants were given 2-weeks supply of the control supplement at week 0 while at weeks 2 and 6 they were provided with sufficient DHA supplement to last a month. DHA was supplied in excess of amount needed to get to the next time point and participants were asked to return any unused supplements when they returned for their next blood draw. To assess participant supplement compliance, the supplements supplied to participants and the remaining supplements returned from participants were each quantified and recorded.

Supplement Instructions

Participants received detailed verbal and written instruction regarding how to consume the supplement (Appendix I). This instruction was specific for the treatment group

to which the participant was randomly assigned (e.g., with or without food and 200 mg/d or 1000 mg/d DHA). For control supplements, all participants took one control capsule each day. However, directions regarding how to consume the supplement differed between those consuming the supplement fasted versus those consuming it with food. The participants consuming the supplement capsules with food were instructed to ingest the supplement with their main meal of the day. Those participants assigned to the fasted group were instructed to consume the supplement with water each day upon waking and then to refrain from eating or drinking anything other than water for 90 minutes. Once participants started DHA supplements, they consumed the capsules daily for four weeks. Because all of the DHA supplements contained 200 mg DHA, the number of capsules differed between the two dosage amounts of DHA. The participants consuming 200 mg DHA per day consumed one capsule while the participants consuming 1,000 mg per day of DHA needed to consume five capsules. Those participants consuming the supplements with food were still instructed to ingest the capsules with the main meal of the day while the participants in the fasted group were still asked to consume the capsules with water first thing after waking and then refrain from eating or drinking anything other than water for 90 minutes. Additionally, for the fasted group, it was specified that participants needed to fast 8 hours; hence the direction to consume the capsules in the morning after fasting over night. All supplements used in this study were provided by Martek Biosciences Corporation (Columbia, MD). Copies of the instructions given to the participants regarding how to consume the supplements appropriate for whichever group to which they were randomly assigned are in Appendix I.

Supplement Composition

The control, or placebo, supplement was a combination of soy and corn oils and the DHA supplement contained vegetarian, algal oil DHA (Martek's DHASCO-S product using algae species, *Schizochytrium*). The DHASCO name stands for docosahexaenoic acid

single cell oil. Notably, there was no DHA in the control supplement. Fatty acid composition of the capsules is listed in Table 1. The specific analysis details regarding quality and characteristics of both the control and DHA supplements can be found in Appendix II.

Table 1. Fatty acid composition in Martek DHA and placebo (control) supplements.

Fatty Acids	DHASCO-S	Placebo
C8:0	0.12	
C9:0	< 0.1	
C10:0	0.12	0.82
C12:0	0.28	<0.1
C11:0	< 0.1	
C14:0	7.93	<0.1
C14:1	< 0.1	
C15:1	0.13	
C16:0	22.47	10.67
C16:1	0.3	0.1
C17:0	< 0.1	
C18:0	0.62	3.06
C18:1n-9	0.26	26.53
C18:1n-7	0.27	1.08
C18:2n-6t	< 0.1	
C18:2n-6c	0.28	50.52
C18:3n-3	< 0.1	2.95
C18:3n-6	0.29	
C20:0	0.18	0.43
C21:0	< 0.1	
C20:1n-9	< 0.1	
C20:2n-6	< 0.1	
C20:3n-6	0.46	<0.1
C20:4n-6	0.9	<0.1
C20:5n-3	2.53	<0.1
C22:0	0.4	
C22:5n-3	0.4	<0.1
C22:5n-6	16.62	<0.1
C22:6n-3	40.5	<0.1
C24:0	0.24	
Others	4.7	

Values are relative weight percentage of total fatty acids.

Fasted Blood Samples

A fasted venous blood sample was collected from each participant at the 4 time points, corresponding to weeks 0, 2, 6, and 10 of the study. Participants were instructed to fast over night (for a minimum of 8 hours), and then come to campus for the blood draw. All samples were collected by trained phlebotomists. Immediately after collection, blood

samples were put on ice; then serum, plasma, and RBCs were separated via centrifugation. One vacutainer of whole blood and two vacutainers of separated serum were sent to LabCorp ® (Kansas City, MO) for analysis of clinical values. One vacutainer each of whole blood and serum were retained for future analysis of fatty acids. Following separation, serum and plasma were placed into microcentrifuge tubes in 500 µL aliquots, labeled and frozen at -80℃ until further analysis. The RBCs w ere washed prior to also being frozen (-80℃) in 500 µL aliquots.

Fatty Acid Analysis

Plasma fatty acid analysis was conducted at Colorado State University by Dr. Mary Harris's laboratory. Samples from two time points for every subject who completed the 10-week study as well as 2 additional participants who finished 6 weeks of the study protocol were shipped to Dr. Harris's laboratory. Samples from week 2 and week 10 and were selected based on study design and information on plasma PL response to DHA supplementation. Week 2 samples provided information prior to DHA supplementation but after all participants had completed the two weeks with control supplements. Week 10 was the final time point, thus representing post-supplementation following eight weeks of DHA supplementation. For the 2 individuals who only completed 6 weeks, the week 6 sample was used in place of week 10. Because plasma PL fatty acid status responds to DHA supplementation within 4 weeks (1), this ensures ample duration of supplementation.

The fatty acid analysis was conducted at Colorado State University by Professor Mary Harris's group using a procedure from Glaser et al. (35). This is a relatively recent method for analyzing human plasma phospholipid fatty acids.

Glaser et al. (35) developed and validated the new procedure against one of the more standard protocols that had been more commonly used for analysis of plasma PL FAs from human samples. This reference method included the Folch method of lipid extraction

(36), separation of lipid fractions via thin-layer chromatography (TLC), and the fatty acid methyl esters (FAME) were synthesized using acid catalyzed trans esterification using methanolic hydrochloric acid (35). The new method did not yield PL FAs, but instead resulted in glycerophospholipid (GP) fatty acids, which were claimed to be a superior representation of tissue fatty acid status than PL fatty acids although the results were still nearly identical (35). A recent systematic review evaluating various methods used to assess n-3 FA status in humans, concluded that the method validated by Glaser in 2010 was a satisfactory means to accurately assess n-3 FA status in humans more efficiently (37).

The Harris laboratory is a lipid laboratory that regularly conducts analysis of lipid samples from their own research as well as samples from other laboratories, including the samples I sent. This laboratory adopted the newer lipid analysis method of Glaser et al., (35) because of how much less time is required, and the ability to eliminate the TLC step that is the most time-consuming part of the older method. Prior to altering methods, results from the new method were compared with results from the old method. Rigorous testing was done to verify results were accurate and consistent. Controls were run on samples, with greater than 99% accuracy, and coefficients of variation (CV) were less than 5%, providing evidence that the new (Glaser) method yielded accurate results. Supplies utilized included HPLC-grade solvents and disposable glass tubes with Teflon lined caps.

Analysis of fatty acids from the plasma samples was conducted by the Harris laboratory using the newer, more efficient Glaser method. Lipids were extracted from the plasma samples using methanol to precipitate the plasma proteins, followed by separation via centrifugation. Plasma PL, or more precisely GP, were not further separated, but rather selectively converted to methyl esters (and not other lipid classes) utilizing sodium methoxide (35) and allowed to incubate at room temperature for three minutes. The FAME were extracted using hexane and were analyzed via gas chromatograph using a 30 m

capillary column (250 um diameter, 0.25 µm thickness) and detected via flame ionization detector (FID). Initial temperature was 120 ℃ and increased at a rate of 10 ℃ per minute to a final temperature of 200 ℃. After a 6-minute hold time, the temperature was again increased to 215 ℃. The temperature for both the injector and the detector was 300 ℃. A split ratio of 15:1 was used. The results were presented as relative weight percentage of total fatty acids.

Statistical Analysis

The number of participants needed to detect the desired difference of 1% DHA per total fatty acids was determined via power analysis. Using PS: Power and Sample Size Calculation program, version 3.0, 2009 (Vanderbilt University Department of Biostatistics, Nashville, TN) and the restrictions pertinent to the study, including power of 0.80, estimated average standard deviation of 0.9, and the desired ability to detect a difference of 1% DHA per total fatty acids, the minimal number of 24 participants per group was calculated. Additional information used for the power calculations included a ratio between groups of one and an adjusted alpha of 0.005 to allow for multiple pair wise comparisons (up to ten). To allow for a 10% drop or loss of participants from the study, 27 participants per group were recruited. With 4 treatment groups, this meant a minimum of 108 participants had to be enrolled.

All results were analyzed using SAS® software version 9.2 (SAS Institute Inc., Cary, NC). To determine if there were differences between groups, one-way analysis of variance (ANOVA) was conducted. For significant findings from ANOVA, pair-wise comparisons were utilized to identify the specific groups differences. To determine if any changes from pre- to post-supplementation were significant, Student's t-tests for each group were utilized. The analyses for absolute and relative changes in DHA in response to supplementation were

done by using the mixed procedure for unequal variance in SAS. Significance was set at P < 0.05 for all analyses.

CHAPTER 4: RESULTS

Study Participants

Of the 633 responses to recruiting efforts, 109 individuals met eligibility requirements and were enrolled in the study. Of those who enrolled in the study, 10 of these participants withdrew prior to completing all study requirements, leaving 99 participants who completed the 10-week study. Baseline participant characteristics are similar between groups (Table 2). Mean DHA intake at baseline was below the established cutoff point of 100 mg/d for all groups.

Table 2. Characteristics at baseline of healthy young men participating in DHA bioavailability trial.

	200DHAfood	1000DHAfood	200DHAfast	1000DHAfast
n	25	27	25	24
Age (yr)	25.9 ± 8.1	24 ± 7.1	26 ± 6.9	26.1 ± 7.5
Weight (kg)	80.5 ± 14.3	78.4 ± 10.6	79.7 ± 12.8	76.4 ± 12.4
Height (cm)	177.2 ± 7.5	179.7 ± 7.4	178.9 ± 6.4	178.5 ± 8.4
BMI (kg/m^2)	25.5 ± 3.1	24.3 ± 2.8	24.9 ± 3.9	23.9 ± 3.4
DHA Intake (mg/d)	66 ± 72	46 ± 47	76 ± 111	46 ± 36

Values are means ± standard deviation. Treatment groups are labeled as 200 mg/d DHA with food (200DHAfood), 1000 mg/d DHA with food (1000DHAfood), 200 mg/d DHA in fasted state (200DHAfast), 1000 mg/d d DHA fasting (1000DHAfast). DHA intake was estimated using a semi-quantitative food-frequency questionnaire.

Study Outcomes

Results are shown for dietary intake both prior to and following supplementation with DHA (Table 3). DHA and EPA intakes were estimated using the food frequency questionnaire (FFQ) to capture episodic consumption associated with the foods high in these FAs. All other macro and micronutrients represent data collected from the 3-day

weighed diet record (3dDR). Two participants had not completed the final 3dDR; thus, the values from their FFQs were used instead.

Table 3. Pre- and post-supplementation dietary intake for each group of participants.

	200DHAfood		1000DHAfood		200DHAfast		1000DHAfast	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Energy (Kcals/day)	2646 ± 1020	2364 ± 839	2720 ± 1091	2261 ± 858	2390 ± 836	2178 ± 565	2582 ± 862	2197 ± 721
Carbohydrate (% of energy)	49.8 ± 9.8	51.2 ± 11.0	53.8 ± 9.4	50.1 ± 7.3	53.0 ± 11.6	49.9 ± 10.4	52.0 ± 9.8	53.0 ± 8.4
Protein (% of energy)	18.4 ± 4.9	18.0 ± 4.3	15.2 ± 3.6	17.1 ± 4.2	16.3 ± 4.6	17.4 ± 5.9	18.1 ± 5.4	16.9 ± 4.8
Fat (% of energy)	32.6 ± 7.3	31.6 ± 7.6	31.6 ± 6.8	33.6 ± 5.9	30.6 ± 7.6	33.8 ± 6.9	29.5 ± 7.1	30.6 ± 7.0
SFA (% of energy)	11.2 ± 3.7	10.7 ± 3.9	10.1 ± 3.2	10.7 ± 2.7	10.2 ± 2.9	10.7 ± 2.3	9.1 ± 3.0	10.5 ± 3.0
MUFA (% of energy)	12 ± 3.0	11.6 ± 3.2	11.6 ± 3.4	12.6 ± 3	11.2 ± 3.3	12.3 ± 3.1	11.4 ± 3.1	11.2 ± 3.1
PUFA (% of energy)	6.3 ± 2.3	6.1 ± 1.8	7 ± 2.2	7.1 ± 2.7	6.4 ± 2.1	7.9 ± 3.4	6.2 ± 2.0	6.2 ± 2.4
EPA (mg/day)	24 ± 31	20 ± 38	15 ± 17	15 ± 25	36 ± 63	13 ± 14	16 ± 16	10 ± 12
DHA (mg/day)	66 ± 72	40 ± 40	46 ± 47	37 ± 33	76 ± 111	46 ± 46	46 ± 36	46 ± 55

Data are presented as means ± standard deviation. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA). Treatment groups are labeled as 200 mg/d DHA with food (200DHAfood), 1000 mg/d DHA with food (1000DHAfood), 200 mg/d DHA in fasted state (200DHAfast), 1000 mg/d d DHA fasting (1000DHAfast). Percent of energy (% of energy) is the average daily kilocalories (Kcals) from a given nutrient as a percent of total average daily kilocalories.

Study Compliance

Compliance to study protocol was evaluated by assessing the number of supplements consumed, adherence to maintaining usual intake and keeping body weight stable during the study period. Supplement compliance was estimated by examining the number of supplements consumed as a percentage of the expected number needed for the duration of supplementation. Average compliance for supplement consumption was 90.5% (with a standard deviation of 13.8%) for all participants, and there were no significant differences between study groups for supplement compliance. Other measures used to explore compliance to study protocol included changes in dietary intake, specifically EPA and DHA, or change in body weight from baseline to completion of the study because we had asked participants to maintain their normal diet and lifestyle. Average change in body weight was minimal at less than 0.5 kg (less than 1 pound) for each group. Overall, there were no major changes in dietary intake from baseline through the study completion (Table 3.)

Fatty Acid Analysis

Data from two time points was used in these analyses; blood samples from week 2 and week 10 samples were used as pre- and post-supplementation, representing 8 weeks of DHA supplementation. Two participants withdrew from the study prior to week 10, but had completed week 6; thus week 6 plasma was used in place of plasma from week 10. The week 6 plasma samples would reflect 4 weeks of DHA supplementation which has been shown to be a sufficient amount of time to evaluate a response from DHA supplementation in plasma PL fatty acids (1). The fatty acid analysis of plasma PL FAs included 99 samples from the participants who completed all 10 weeks of the study as well as the 2 participants who had completed week 6 blood samples, but excluding 2 samples that had an insufficient amount of plasma. There was nearly equal representation between groups with number of

participants per group ranging from 23 to 27. The average values for each individual plasma PL FA pre- and post-DHA supplementation are shown in Table 4.

DHA supplementation resulted in several significant changes in individual PL fatty acids in plasma PL as well as significant differences between groups for these changes; however, no differences between groups for food versus no food were found. All differences observed between groups were a result of the dose of DHA supplementation (200 mg/d or 1,000 mg/d). Changes in individual fatty acids were examined both as an absolute change (week 10 – week 2) and also as a relative change ([week 10 – week 2]/week 2*100).

DHA showed significant ($P < 0.0001$) increases from baseline in plasma PL for all four groups. Several plasma PL FAs decreased as a result of the increase in plasma PL DHA. Some of these decreases were significant. These FAs include 18:1n-9 with 1000DHAfood ($P < 0.01$) and 1000DHAfast ($P < 0.05$), 18:1n-7 (1000DHAfood, $P < 0.05$ and 1000DHAfast, $P = 0.0001$), 20:3n-6 (1000DHA food, $P < 0.01$, 1000DHAfast, $P < 0.01$) and 20:4n-6 (ARA, 1000 mg/d with food, $P < 0.0001$, 1000 mg/d DHA fasted, $P < 0.0001$). DPA (22:5n-3) decreased significantly for all four treatment groups, 200DHAfood ($P < 0.05$) and $P < 0.0001$ for the other three groups. The only FA other than DHA that increased following DHA supplementation, was EPA (20:5n-3) which increased significantly for three groups, 200DHAfood ($P < 0.01$), 1000DHAfood ($P < 0.0001$), and 1000DHAfast ($P < 0.01$), but not for 200DHAfast.

One-way analysis of variance (ANOVA) showed differences between groups for several FAs, and pair-wise comparisons indicated which groups were significantly different from each other. Absolute change in DHA was significantly different within food groups (200 mg versus 1000 mg), $P < 0.0001$, fasted (200 mg and 1000 mg), $P < 0.0001$, between 200DHAfood and 1000DHAfast ($P < 0.0001$) and between 1000DHA food and 200DHAfast ($P < 0.0001$). DPA also showed differences for the same groups as seen for DHA,

(200DHAfood and 1000DHAfood, $P < 0.0001$, 200DHAfast and 1000DHAfast, $P < 0.01$, 200DHAfood and 1000DHAfast, $P < 0.0001$, and 1000DHAfood and 200DHAfast, $P < 0.0001$). EPA only differed between the 1000DHAfood and 200DHAfast groups ($P < 0.05$). 18:1n-9 also only differed between two groups, but it was between 200DHAfood and 1000DHAfast ($P < 0.05$), although ANOVA yielded a P -value of 0.0501. ARA differed between groups 200DHAfood and 1000DHAfast ($P < 0.001$), groups 1000DHAfood and 200DHAfast ($P < 0.05$), and groups 200DHAfast and 1000DHAfast ($P < 0.001$). Table 5 shows the average absolute change for each fatty acid.

Table 4. Plasma phospholipid fatty acids pre- and post-DHA supplementation consumed with or without food for 8 weeks.

	200DHAfood		1000DHAfood		200DHAfast		1000DHAfast	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
16:0	25.17 ± 2.14	25.31 ± 2.49	25.42 ± 2.15	25.52 ± 1.93	25.37 ± 2.04	25.37 ± 2.36	26.73 ± 1.99	26.39 ± 2.25
18:0	14.23 ± 1.37	14.55 ± 1.32	14.69 ± 1.39	14.97 ± 1.53	14.75 ± 1.76	14.86 ± 1.70	14.66 ± 1.48	14.92 ± 1.08
18:1n-9	11.38 ± 2.67	11.32 ± 1.88	11.13 ± 2.34	9.98 ± 2.03*	11.67 ± 3.00	11.12 ± 3.12	11.01 ± 2.41	10.12 ± 1.79*
18:1n-7	1.59 ± 0.21	1.58 ± 0.25	1.64 ± 0.20	1.54 ± 0.20^{*ab}	1.61 ± 0.24	1.56 ± 0.23^{ab}	1.67 ± 0.18	1.50 ± 0.18^{*b}
18:2n-6	28.97 ± 2.56	27.92 ± 2.98	27.08 ± 3.31	26.16 ± 3.82	26.79 ± 2.93	26.51 ± 3.26	25.61 ± 2.93	26.08 ± 3.61
18:3n-3	0.39 ± 0.23	0.38 ± 0.21	0.37 ± 0.21	0.38 ± 0.27	0.35 ± 0.16	0.35 ± 0.16	0.32 ± 0.11	0.37 ± 0.28
20:3n-6	3.36 ± 0.99	3.21 ± 0.85	3.28 ± 0.58	2.87 ± 0.64*	3.43 ± 0.98	3.26 ± 0.84	3.15 ± 0.88	2.62 ± 0.72*
20:4n-6	11.16 ± 1.81	10.87 ± 1.82^{ab}	12.47 ± 2.46	11.04 ± 2.10^{*bc}	11.86 ± 2.54	11.62 ± 2.70^{a}	12.84 ± 1.52	10.80 ± 1.79^{*c}
20:5n-3	0.46 ± 0.16	0.59 ± 0.22^{*ab}	0.53 ± 0.21	0.71 ± 0.22^{*a}	0.59 ± 0.19	0.60 ± 0.23^{b}	0.52 ± 0.17	0.65 ± 0.13^{*ab}
22:5n-3	0.87 ± 0.21	0.79 ± 0.13^{*a}	1.01 ± 0.22	0.62 ± 0.19^{*b}	1.02 ± 0.21	0.84 ± 0.15^{*a}	0.99 ± 0.18	0.59 ± 0.15^{*b}
22:6n-3	2.40 ± 0.73	3.50 ± 0.98^{*a}	2.35 ± 0.62	6.21 ± 1.81^{*b}	2.56 ± 0.71	3.91 ± 1.01^{*a}	2.49 ± 0.71	5.93 ± 2.13^{*b}

Units are relative weight percentage of total fatty acids; values are means ± standard deviation. Treatment groups are labeled as 200 mg/d DHA with food (200DHAfood), 1000 mg/d DHA with food (1000DHAfood), 200 mg/d DHA in fasted state (200DHAfast), 1000 mg/d d DHA fasting (1000DHAfast). *P < 0.05, for differences from baseline. Groups with different letters are significantly different from each other.

Table 5. Raw change scores from pre- to post-DHA supplementation.

	200DHAfood	1000DHAfood	200DHAfast	1000DHAfast
16:0	0.19 ± 1.39	0.11 ± 1.23	0.00 ± 1.29	-0.40 ± 1.84
18:0	0.31 ± 0.84	0.28 ± 1.12	0.11 ± 1.74	0.34 ± 1.15
18:1n-9	-0.06 ± 1.65	-1.15 ± 1.54*	-0.55 ± 2.6	-0.94 ± 2.42*
18:1n-7	-0.02 ± 0.18^{a}	-0.1 ± 0.18*^{ab}	-0.05 ± 0.21^{ab}	-0.18 ± 0.26*^{b}
18:2n-6	-0.93 ± 2.12	-0.92 ± 3.37	-0.28 ± 2.75	0.52 ± 2.60
18:3n-3	-0.02 ± 0.24	0.02 ± 0.24	0.01 ± 0.13	0.05 ± 0.28
20:3n-6	-0.19 ± 0.64	-0.42 ± 0.67*	-0.17 ± 0.83	-0.48 ± 0.76*
20:4n-6	-0.40 ± 0.99^{ab}	-1.43 ± 1.46*^{bc}	-0.24 ± 1.59^{a}	-2.11 ± 1.73*^{c}
20:5n-3	0.12 ± 0.18*^{ab}	0.18 ± 0.23*^{a}	0.01 ± 0.22^{b}	0.14 ± 0.20*^{ab}
22:5n-3	-0.10 ± 0.16*^{a}	-0.39 ± 0.23*^{b}	-0.19 ± 0.20*^{a}	-0.40 ± 0.17*^{b}
22:6n-3	1.12 ± 0.67*^{a}	3.86 ± 1.85*^{b}	1.35 ± 0.60*^{a}	3.44 ± 1.88*^{b}

Units are relative weight percentage of total fatty acids; values are means ± standard deviation. Treatment groups are labeled as 200 mg/d DHA with food (200DHAfood), 1000 mg/d DHA with food (1000DHAfood), 200 mg/d DHA in fasted state (200DHAfast), 1000 mg/d d DHA fasting (1000DHAfast). *P < 0.05, for difference from baseline. Groups with different letters are significantly different from each other.

The relative changes in plasma PL FAs following 8 weeks of DHA supplementation are shown in Table 6. DHA (22:6n-3) increased significantly with $P < 0.0001$ for all 4 groups and the 1000 mg/d DHA groups increasing by more than 150%. Plasma PL FAs that decreased significantly from pre- through post-supplementation include 18:1n-9 for the 1000DHA food group only ($P < 0.01$), 20:3n-6 for both the 1000 mg/d DHA dose groups ($P < 0.01$ for with food, $P < 0.01$ for fasted), 20:4n-6 (ARA) 1000 mg/d DHA with food ($P < 0.0001$), 1000 mg/d fasted ($P < 0.0001$) and 22:5n-3 (DPA) for all groups: 200 mg/d with food ($P < 0.01$), 1000 mg/d with food ($P < 0.0001$), 200 mg/d fasted ($P < 0.0001$), and 1000 mg/d DHA without food ($P < 0.0001$). As with the absolute changes, EPA (20:5n-3) was the only FA other than DHA that increased significantly from baseline to completion of DHA supplementation. EPA increased significantly for both food groups, 200 mg/d ($P < 0.01$), 1000 mg/d ($P < 0.0001$) and also for 1000 mg/d fasted ($P < 0.001$) but not for 200 mg/d fasted.

One-way ANOVA and pair-wise comparisons revealed differences between groups for several of the plasma PL FAs. All differences were related to dose effect and none were associated with any effect of food. ARA (20:4n-6) had differences between 200DHAfood and 1000DHAfast ($P < 0.01$), 200DHA fast and 1000DHAfood ($P < 0.05$), and 200DHAfast and 1000DHAfast ($P < 0.001$). EPA (20:5n-3) only exhibited differences between 200DHAfast and 1000DHAfood and ($P < 0.01$), n-3 DPA (22:5n-3) had group differences for both food groups ($P < 0.0001$), both fasted groups ($P < 0.0001$), between 200DHAfast and 1000DHAfood ($P < 0.0001$) and 200DHAfood with 1000DHAfast ($P < 0.0001$). Significant differences ($P < 0.0001$) were found for several group comparisons for DHA (22:6n-3): between doses for food, between doses for fasted, 200DHAfood versus 1000DHAfast, and 200DHAfast compared to 1000DHAfood.

Table 6. Change scores from pre- to post-DHA supplementation as a percentage of pre-supplementation values.

	200DHAfood	1,000DHAfood	200DHAfast	1,000DHAfast
16:0	0.78 ± 5.48	0.59 ± 4.83	-0.01 ± 4.95	-1.35 ± 6.98
18:0	2.46 ± 6.29	2.06 ± 7.87	1.58 ± 12.84	2.98 ± 9.20
18:1n-9	1.72 ± 13.98	-9.22 ± 14.52*	-3.40 ± 19.35	-5.90 ± 20.33
18:1n-7	-0.73 ± 12.35	-5.70 ± 10.79*	-1.82 ± 13.58	-9.61 ± 15.03*
18:2n-6	-3.09 ± 7.74	-2.76 ± 13.90	-0.64 ± 10.32	2.26 ± 10.18
18:3n-3	14.57 ± 75.12	16.31 ± 70.17	12.59 ± 49.03	18.96 ± 79.48
20:3n-6	-2.86 ± 19.67	-11.66 ± 18.78*	-1.23 ± 26.72	-13.35 ± 20.80*
20:4n-6	-3.42 ± 9.29^{ab}	-10.69 ± 10.49*^{bc}	-1.68 ± 13.40^{a}	-15.91 ± 13.46*^{c}
20:5n-3	34.05 ± 50.26*^{ab}	51.39 ± 70.80*^{a}	5.30 ± 31.13^{b}	40.11 ± 48.02*^{ab}
22:5n-3	-8.82 ± 16.82*^{a}	-37.56 ± 17.65*^{b}	-16.50 ± 16.30*^{a}	-39.99 ± 13.46*^{b}
22:6n-3	50.89 ± 33.69*^{a}	180.8 ± 101.6*^{b}	55.06 ± 26.61*^{a}	145.6 ± 81. 7*^{b}

Units are percentage change relative to pre-supplementation with DHA; values are means ± standard deviation. Treatment groups are labeled as 200 mg/d DHA with food (200DHAfood), 1000 mg/d DHA with food (1000DHAfood), 200 mg/d DHA in fasted state (200DHAfast), 1000 mg/d d DHA fasting (1000DHAfast). *P < 0.05, for differences from baseline. Groups with different letters are significantly different from each other.

There were indications that some of the clinical laboratory values changed in response to DHA supplementation. Clinical test results included total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglycerides (TG), and fasting plasma glucose (Glu). Values for the clinical results are shown pre- and post-supplementation with DHA (Table 7). Following DHA supplementation total cholesterol increased for the 1000DHAfood group ($P < 0.0001$), 200DHAfast ($p < 0.05$) and 1000DHAfast ($P < 0.05$), and LDL increased for the 1000 mg/d dose with food only ($P < 0.05$) and HDL for the 1000 mg/d doses only (food, $P < 0.01$), fasted ($P < 0.001$). No changes in serum TG were observed following DHA supplementation for either dose or condition. There were differences between groups for the changes in clinical values. There was a difference between the food groups (200 vs. 1000 mg/d DHA) for total cholesterol ($P < 0.01$) and LDL ($P < 0.05$), but no differences between groups for any other lab values (see Table 7).

Table 7. Clinical laboratory results pre- and post-DHA supplementation consumed with or without food for 8 weeks.

	200DHAfood		1,000DHAfood		200DHAfast		1,000DHAfast	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Total cholesterol	159 ± 28	156 ± 29[b]	156 ± 28	170 ± 35*[a]	157 ± 29	165 ± 26*[ab]	156 ± 29	164 ± 37*[ab]
LDL-cholesterol	91 ± 27	86 ± 29[b]	86 ± 22	95 ± 29*[a]	89 ± 25	94 ± 24[ab]	87 ± 26	94 ± 33[ab]
HDL-cholesterol	49 ± 12	50 ± 10	49 ± 13	53 ± 16*	45 ± 10	48 ± 11	48 ± 15	54 ± 18*
Triglycerides	96 ± 39	102 ± 50	108 ± 51	112 ± 66	117 ± 53	114 ± 62	105 ± 78	83 ± 36
Glucose	89 ± 11	88 ± 8	92 ± 8	92 ± 8	91 ± 7	92 ± 6	89 ± 8	90 ± 6

Values are means ± standard deviation; units are mg/dL. Treatment groups are labeled as 200 mg/d DHA with food (200DHAfood), 1000 mg/d DHA with food (1000DHAfood), 200 mg/d DHA in fasted state (200DHAfast), 1000 mg/d d DHA fasting (1000DHAfast). *P < 0.05, for differences from baseline. Groups with different letters are significantly different from each other.

The residuals for all response items were examined and all fell within expected ranges with the exception of the 22:6n-3 (DHA) results for both absolute and relative differences. The 1000 mg/d DHA dose groups had several times greater variance than did the 200 mg/d dose. Plots showing the skewed residuals for the change scores for DHA can be found in Figure 6. The analyses for absolute and relative changes in DHA in response to supplementation were done using the mixed procedure for unequal variance in SAS.

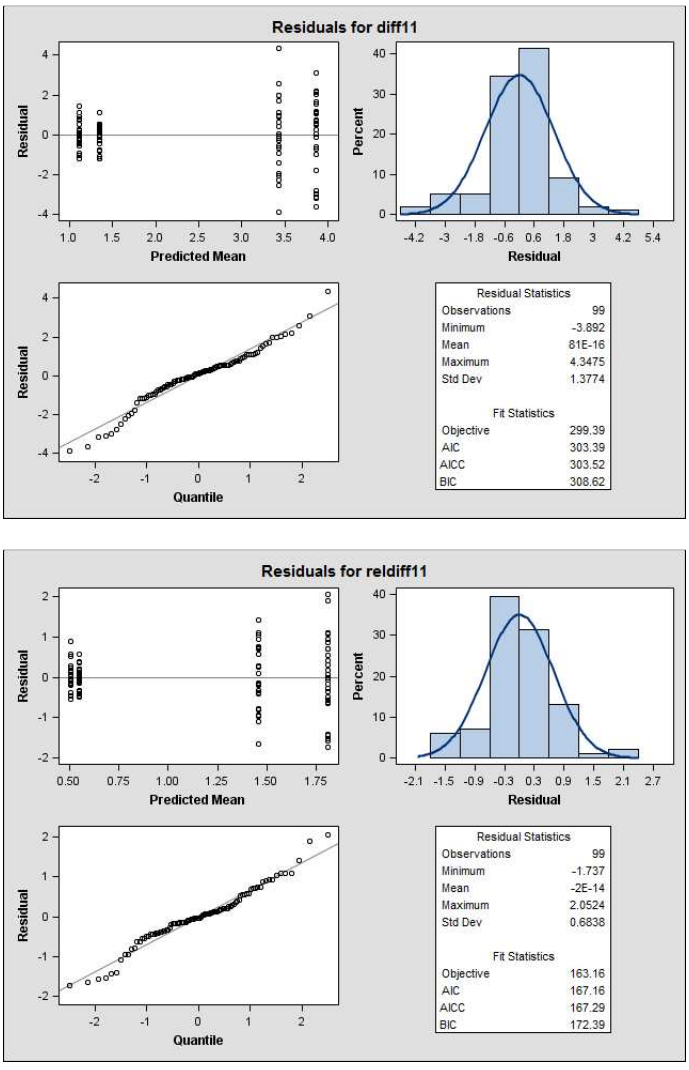


Figure 6. Plots from SAS output showing residuals for the absolute change (top) and relative change (lower) for DHA (22:6n-3), identified as response 11 in the analysis in SAS).

CHAPTER 5: SUMMARY AND DISCUSSION

The purpose of the study was to examine the influence of consuming a DHA supplement with or without food in a meal on the bioavailability of the DHA supplement. All differences between groups could be attributed to the dose of DHA supplemented (i.e., higher dose of DHA resulted in a greater plasma PL DHA response than lower DHA dose) and not to a difference between whether the supplement was consumed with food or in a fasted state. Mean DHA intake at baseline was below the established cutoff point of 100 mg/d for all groups. Prior to DHA supplementation, average plasma PL DHA for the study participants was 2.45% (as relative weight percent of total fatty acids). This is consistent with the normal range for healthy individuals with low DHA consumption and without DHA supplementation (~2.5 to 3.5%), (1). There were significant increases in plasma PL DHA at week 10 compared with baseline for all groups and also greater increases in plasma PL DHA after supplementation with the higher (1,000 mg/d) dose of DHA compared to the lower (200 mg/d) DHA dose. These observations are in agreement with the dose-response of plasma PL DHA following DHA supplementation in which a cross-study analysis of 16 studies using DHA doses ranging from 200 mg to 6,000 mg showed a saturable dose-response (1). The observation that plasma PL DHA increased significantly following supplementation and that the increase depended upon DHA dose together provide an additional indication that participants consumed the DHA supplements as directed. A lack of significant differences between groups related to whether participants consumed the supplement with food or in a fasted state, suggests that consumption of food with an algal DHA supplement does not affect the bioavailability of DHA. Thus, the results of the study do not support our hypothesis that food would influence the bioavailability of DHA when a DHA supplement was consumed with food.

The observation that plasma PL ARA decreased with supplementation of DHA is in agreement with previous research. DHASCO-S resulted in a greater decrease in plasma PL ARA than the decrease in ARA seen with DHA from salmon (32). Another form of algal DHA, DHASCO-T exhibited a linear, dose-dependent decrease on plasma PL ARA, but DHASCO-S demonstrated a more variable decrease in ARA in plasma PL (21).

Dietary factors that might have been expected to influence DHA absorption include meal size and meal composition. Earlier work indicated that the fat composition of the meal a DHA supplement was consumed with affected absorption of the DHA. However, this was primarily only with EE form of DHA. If EE DHA was consumed with a meal containing 44 g of fat, more DHA was absorbed compared with a meal containing 8g of fat. However, the TG form of DHA was equally well absorbed with either the high or lower fat meals (38). Possibly the reason we did not observe a difference in bioavailability between food and fasted groups was that the supplement contained DHA in TG form.

It is interesting to view the results in the context of the few studies that had indirect mention of supplementation and food. Potential support for an effect from food was indicated indirectly in a few studies. In these instances, there appeared to be an effect on DHA bioavailability related to food. Arterburn et al. (21), found an equal response in plasma PL DHA with 465 mg/d DHA in the snack bar versus 600 mg/d DHA via capsule, while Hawthorne et al. (27), was able to detect a difference in DHA response between different DHA dosage groups when providing low doses (50mg/d or 100 g/d) of DHA in orange juice.

A bioequivalence study evaluating plasma PL DHA response to the same dose of DHA (600 mg/d) provided by either a DHA supplement or from approximately 2 ounces of salmon suggests that food does not influence DHA bioavailability (32). The study found equivalent plasma PL DHA responses as a result from the same amount of DHA provided from either fish or DHA supplement (32). Other findings from this same study showed that

DHA supplementation with algal DHA versus from salmon resulted in significantly different ($P < 0.05$) response in other plasma PL FAs (32) The salmon DHA group had significantly higher ($P < 0.05$) plasma PL EPA and DPA than the algal DHA group after DHA consumption. This is likely a result of presence of EPA in fish, but not in algal DHA. In contrast, algal DHA caused what appeared to be a greater decrease in decrease in ARA, although the difference was not significant, only a very slight increase in EPA and a decrease in n-3 DPA in plasma PLs (32). These same patterns from the algal DHA were observed in the present study. There were significant decreases with DHA supplementation in plasma PL ARA and n-3 DPA, and increases in EPA and DHA. The slight increase in plasma PL EPA observed following supplementation with DHA is likely because the DHA supplement did contain a small amount of EPA.

Previous research indicated a decrease in TG as a result of DHA intake of 1000 mg/d over 8 weeks in adults with CAD and elevated TG (26). However, in the current study, we failed to observe any effect of DHA supplementation on TG. Since the participants in our study had normal TG on average prior to starting DHA supplementation, possibly this explains why we did not observe a decrease in TG after 8 weeks of 1000 mg/d DHA in our healthy participants. In the groups consuming 1000 mg/d DHA, there was an increase in total cholesterol, LDL and HDL. Other work supports that DHA increases HDL, but in contrast to our results, DHA had no effect on LDL. A study provided 1.62 g/d of DHA in the form of algal oil capsules (DHASCO from Martek Biosciences) to healthy adult males and females for 6 weeks (39). They observed significant ($P < 0.05$) increases in HDL from 1.20 mmol/L (46.3 mg/dL) to 1.40 mmol/L (54 md/dL), no change in LDL or total cholesterol and a decrease in TG from 0.96 mmol/L (85 mg/dL) to 0.80 mmol/L (70.8 mg/dL). Thus, they did find a decrease in TG following DHA supplementation even in participants with normal TG

(39). One possible reason why we did not observe a similar decrease in TG is because of the difference in DHA dose: 1620 mg/d in their study versus 1000 mg/d DHA in our study.

There were potential limitations for this study. There are multiple considerations involved with studies utilizing human participants. This was a free-living study therefore the participants were allowed to consume their usual diet. Participants were directed to not vary their normal intake with the exception of avoiding fish, seafood and any n-3 supplements. Using current dietary assessment methodology, such as the weighed diet record, participants may alter their usual intake (40). Food frequency questionnaires are known to overestimate and weighed diet records to underestimate actual intake (41). However, the FFQ used in this study had been validated by the Campbell laboratory for use with pregnant women (r=0.68; unpublished data) to quantify habitual daily DHA intake for the previous month. Measures to assess diet as a means of estimating any influence are imperfect.

Lifestyle factors other than diet may also have an influence on ability to test only the effects desired. Physical activity or sedentary behaviours vary between participants. Inherent in any human study is individual variation. The data from this study revealed large ranges in some of the responses. The absolute change in DHA plasma PLs after supplementation for all groups combined, varied from a slight decrease (-0.457%) to a large increase (7.783%). Changes in clinical lipid results also varied greatly from baseline to study completion (total cholesterol range: decrease of 39 mg/dL to increase of 60 mg/dL; LDL ranged from -42 mg/dL to 137 mg/dL, HDL ranged -17 to 32 mg/dL, and TG ranged from -289 mg/dL to 257 mg/dL change). This represents large variations between participants. How much of the variation is a result of biological variability versus a result of external factors is difficult to assess for certain.

A potential future question would be to explore if specific components of diet (macronutrients: fat, carbohydrate, protein) affect bioavailability of DHA in any way. It would

also be interesting to see if future research examines influence of food on other forms of supplemental DHA, such as fish oil (TG), krill oil (PL), concentrated fish oil (rTG, EE), or prescription n-3 (EE) to evaluate if these are bioequivalent. Dyerberg et al. (12), suggests that possibly there is an influence of simultaneous food, specifically fat-rich meals, and supplement consumption on bioavailability based on the observation that some studies show similar plasma PL FA results following supplementation of DHA in different forms (TG vs EE for one example).

Potential relevance of the findings from this project would be the ability to help guide future recommendations for supplementation. The ability to establish more specific recommendations regarding DHA supplementation carries practical application implications in both research and clinical settings. Our results indicated there was no difference when the supplements were consumed with a meal or when fasted. Thus, this simplifies supplementation in research studies because less care would be needed to ensure participants follow instructions regarding diet and supplements to optimize absorption. Additionally, not having to consume a supplement under specific conditions (such as with or without a meal) may improve subject compliance as well as increasing patient compliance in clinical settings. Without influence of concomitant food intake with consumption of a DHA supplement, it can be concluded that observed variation between participants in a study are not a result of whether they consumed the supplement with or without food.

APPENDIX I: STUDY MATERIALS

This appendix contains copies of the materials used in the DHA Supplement Study including consent form and data collection tools.

Study Materials:

-Recruiting fliers and recruiting email draft

-Consent form

-Medical hx questionnaire

-DHA screener

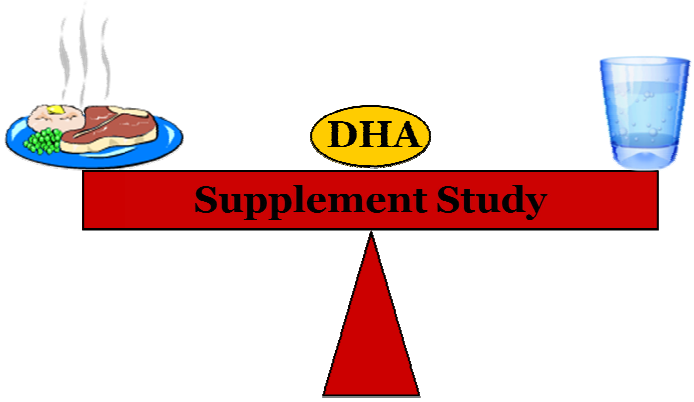
-FFQ

-3dDR instructions, example and food log page

-Supplement instructions

-Supplement instruction sheets

-Data collection sheets (used to record basics at visits: participant weight, number of supplements returned, any participant observations or complaints)



Purpose: To determine if the absorption of DHA, a heart healthy type of fat, is influenced by the presence of food

To be eligible for the study you need to meet the following qualifications:

- Male;
- Between 18 and 45 years of age;
- Non-smoker;
- Eat very little DHA, a fatty acid that is primarily found in fish such as salmon or fortified foods; and
- Not have any gastrointestinal, lipid disorders or other chronic disease.

There are 7 study sessions required.
Eligible participants will be compensated. Participation is voluntary.

For further information: **Call the recruitment team at 515-294-8673 or email:**

dhasupplement@iastate.edu

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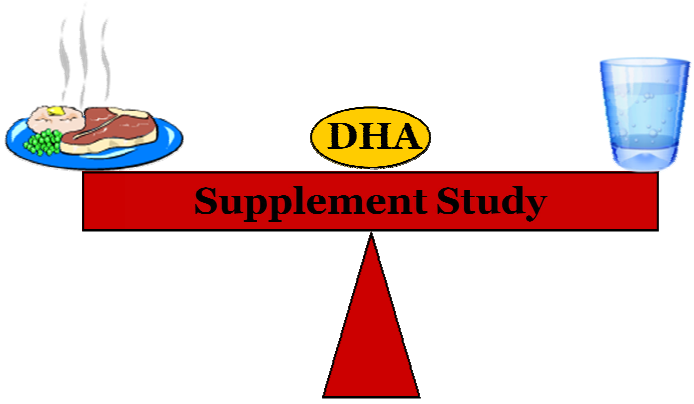

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DHA Supplementation Recruiting Email Template

Thank you for your interest in the DHA Supplement Study! My name is Stephanie Kratzer and I am a member of the recruitment team for the DHA Supplement Study here at Iowa State University.

Here is more information about the DHA Supplement Study:

We are conducting a research study to determine if the amount of DHA absorbed is influenced by the presence of food. DHA is an essential omega 3 fatty acid required by the body. You will be supplied with a DHA supplement to take daily for 8 weeks along with instructions of how to take it. You will also be asked to avoid eating fish or taking any other fish oil or DHA supplement during this time, but you will not be asked to make any other changes to your daily habits. If you qualify for our study, it would require six visits over a 10 week period with meetings at weeks 0, 2, 6, and 10. We anticipate beginning this study in March.

We will collect the following info at weeks 0 and 6:

- A fasting blood sample.
- Diet info that includes a 1-hour food interview and logging your food intake for three days.

We will collect the following info at weeks 2 and 10:

- A fasting blood sample.

To qualify for our study you must be:

- Male between 18 and 45 years of age
- Not a smoker
- Eat very little DHA; a fatty acid that is primarily found in fish such as salmon, or fortified foods
- Not have any history of gastrointestinal or lipid disorders, or other chronic disease.

For your participation, you will receive $70 upon completion of the study.

I am attaching the consent form which provides more detailed information. I'll be happy to answer any more questions that you have.

Please email or call 515-294-8673 if you have further questions. Also, let me know whether you are or are not interested. If you are interested, could you please let me know the phone number where you prefer to be reached? I look forward to hearing from you soon.

Thanks!

Stephanie Kratzer
Recruitment Team
DHA Supplement Study
Iowa State University
515-294-8673
DHAsupplement@iastate.edu

CONSENT FORM FOR:
DHA supplement study

This form describes a research project. It has information to help you decide whether or not you wish to participate. Research studies include only people who choose to take part—your participation is completely voluntary. Please discuss any questions you have about the study or about this form with the project staff before deciding to participate.

Who is conducting this study?

Christina Gayer Campbell, PhD, RD
Associate Professor, Nutrition
Department of Food Science and Nutrition
Mailing Address: 220 MacKay Hall
Physical Address: 1105 Human Nutrition Science Building
Iowa State University
Ames, IA 50011-1123
515-294-4260**;** ccampbel@iastate.edu.

Why am I invited to participate in this study?
You are being asked to take part in this study because you are a healthy man living in the communities in and around Ames, IA who has shown interest in our study by responding to our recruiting efforts. You have been selected to participate based on several criteria including:

- Between the ages of 18-45 years old;
- Not a smoker;
- Eat very little DHA; a fatty acid that is primarily found in fish such as salmon or fish oil supplements; and
- No gastrointestinal or lipid disorders, or other chronic disease.

What is the purpose of this study?
The purpose of this study is to assess the absorption of a DHA supplement in healthy men when consumed with water or your main meal of the day. DHA is a specific type of fat that is primarily found in fatty fish such as salmon. It has been shown to be important throughout the lifespan particularly for heart health.

What will I be asked to do?
If you agree to participate, you will be randomly assigned to one of four groups. All groups will receive a control (a combination of corn and soybean oil) supplement for 2 weeks followed by a DHA supplement taken daily for 8 weeks along with directions to consume the supplement with water and refrain from eating for 90 minutes OR to take the supplement with your main meal of the day. All supplements are provided by Martek, the leading manufacturer and distributor of vegetarian sources of DHA.
Summary
Your participation in this study will last 10 weeks. If you agree to participate you will be asked to schedule a preliminary meeting to review the consent form, complete a health history form and answer ~10-12 questions regarding your current DHA intake. This will determine your eligibility for the study. If you are eligible to participate, prior to taking any supplements you will be asked to schedule a 60-90 minute meeting to obtain one fasted blood sample, participate in a food interview about the foods that you have consumed during

the past month, and receive instructions about the dietary intake data collection period. You will be asked to meet with a member of the project staff at HNSB 2008 or 2023 on the Iowa State University campus. You will be asked to return for additional blood samples at weeks 2, 6, and 10. Additionally you will complete another food interview and 3 day diet record around week 6. At weeks 0 and 6 you will wear an activity monitor on your upper arm for 7 days to document your current level of physical activity.

Specifics
During the initial visit you will be given a dietary scale, and 3-day food log. You will have a weight measurement taken and a fasted **blood** sample obtained via venipuncture by a trained and experienced phlebotomist. There will be a total of 4 blood draws. Consenting to this study allows the investigators to utilize frozen blood samples for future analysis.

You will be asked to consume the control supplement and given instructions to consume the supplement on an empty stomach and to refrain from eating or drinking anything but water for 90 minutes OR to consume the supplement with your main meal of the day. You will need to return any unused supplements at the 2nd blood draw. At the 2nd blood draw you will be given the DHA supplement, either 200 mg or 1000 mg. Both of these doses have been selected based on current recommendations (200 mg) or the amount used in many research studies (1000 mg). At the 3rd blood draw you will again return any unused supplements and be given another supply for the final month. At the 4th blood draw you will again return any unused supplements.

The **3-day food log** requires you to weigh and record all food and beverages consumed for 2 weekdays and one weekend day. You will be given detailed instructions on how to properly complete the forms and tips on accurately weighing food. You will be provided with a dietary scale, at no cost to you, for use during the study to facilitate the process. You may perceive this to be a tedious process; however it is the most accurate means of collecting dietary intake information. You will not be given a diet to follow; observations are made on what you typically choose to eat.

You will be provided with an **activity monitor** that is worn on the upper arm. The activity monitor will be worn with for seven days, 24 hours a day to ensure the best possible data collection. The monitor is water resistant needs to be removed when showering and swimming. This activity monitor has been used in many studies at ISU with minimal complaints.

You will need to arrange a time with a member of the project staff to pass in your 3-day food record and dietary scale at the end of the data collection period.

At any time you are invited to discuss concerns that you have about the study protocol however the project staff will not make any physical activity or diet/food recommendations. Please *maintain* your current usual intake, and physical activity habits. Other than taking the control and DHA supplement, we ask that you **refrain from eating fish or any food fortified with DHA** (e.g. Silk DHA soy milk, Smart Balance spread, Egglands best eggs) or taking a fish oil or DHA supplement for the duration of the study. It is very important that you adhere to this dietary guideline. We reserve the right to dismiss you from the study for failure to comply with the study guidelines we have presented here.

What are the possible risks and benefits of my participation?
Risks – You will be consuming an vegetarian based DHA supplement rather than fish oil. There are minimal risks associated with these DHA supplements however there are random published comments that reveal rare gastrointestinal side effects and/or nausea. These comments are much less frequent than those associated with fish oil supplements.

Approximately 3 Tablespoons of blood will be removed by putting a needle in a vein of your arm on 4 occasions. This is a standard method used to obtain blood for tests. There is momentary pain at the time the needle is inserted into the vein but other discomfort should be minimal. In about 10% of the cases, a small amount of bleeding under the skin will produce a bruise. The risk of infection is less than 1 in 1,000.

Benefits – You may not receive any direct benefit from taking part in this study. Research has shown omega-3 fatty acids to have a protective effect on the heart, as well as being important for brain and visual development in infants. We hope that this research will benefit society by generating data that may contribute to dietary guidelines. Upon completion of the study, you will receive a summary of your baseline (week 0) data.

How will the information I provide be used?
The findings of this study will be shared throughout the scientific community via oral and poster presentations at scientific meetings, and published research articles.

What measures will be taken to ensure the confidentiality of the data or to protect my privacy?
Records identifying participants will be kept confidential to the extent allowed by applicable laws and regulations. Records will not be made publicly available. However, federal government regulatory agencies**,** auditing departments of Iowa State University, and the ISU Institutional Review Board (a committee that reviews and approves research studies with human subjects) may inspect and/or copy your records for quality assurance and analysis. These records may contain private information.

To ensure confidentiality to the extent allowed by law, the following measures will be taken: subjects will be assigned a unique code and letter that will be used on forms instead of their name. If the results are published, your identity will remain confidential. The data obtained from the study will be regarded as privileged and confidential. Your privacy will be maintained in any future analysis and/or presentation of the data with the use of coded identifications for each participant's data. All data will be stored in a locked file cabinet with access only by the principal investigator and project director. Additionally, any data entered into the computer will be available with restricted password only. This data will be kept in a locked file in the PI's lab (HNSB 2023) until the results of the study have been published. Identifiers will not be stored with the data; they will be in a separate locked filing cabinet.

Will I incur any costs from participating or will I be compensated?
You will be responsible for the cost of transportation to and from the research facility while participating in this study (e.g. gas money, bus fare). You will be compensated for participating in this study. Upon **completion** of the study (denoted as the final blood draw) **and** the **return** of equipment (Timbuk2 bag and dietary scale) you will be **compensated $70** for a **complete dataset** (4 blood draws, 2 food interviews, and 2 3-day food logs)(2 food

records*$15 each; 2 food interviews*$10 each; 4 blood draws*$5 each). If you withdraw from the study early you will be compensated for the items you have completed.

What are my rights as a human research participant?
Participating in this study is completely voluntary. You may choose not to take part in the study or to stop participating at any time, for any reason, without penalty or negative consequences. Your choice of whether or not to participate will have no impact on you as a student/employee in any way. You may skip any question during a questionnaire (e.g. medical history, food-focused interview). You may withdraw consent in person or by phone with the principal investigator, Christina Campbell at any time. Please feel free to ask any questions or express your concerns regarding this study. The investigator will attempt to answer all questions. Contact Dr. Christina Campbell at 515-294-4260.

What if I am injured as a result of participating in this study?
Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thielen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance.

Whom can I call if I have questions or problems?
You are encouraged to ask questions at any time during this study.

- For further information about the study contact the principal investigator Christina Campbell at 515-294-4260 or the study coordinator Stephanie Kratzer at 515-294-8673. To schedule blood draw appointments, contact Stephanie Kratzer at 515-294-8673 or email dhasupplement@iastate.edu

- If you have any questions about the rights of research subjects or research-related injury, please contact the IRB Administrator, (515) 294-4566, IRB@iastate.edu, or Director, (515) 294-3115, Office of Research Assurances, 1138 Pearson Hall, Iowa State University, Ames, Iowa 50011.

Consent and Authorization Provisions

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the written informed consent prior to your participation in the study.

Participant's Name (printed) _____

_____ _____
(Participant's Signature) (Date)

Investigator Statement

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Consent) (Date)

Medical History Questionnaire

Please answer the following questions to the best of your knowledge. All information provided here is completely confidential. Please ask for clarification if needed.

Subject Name:_____
(Last) (First) (MI)

Phone Number we can reach you at: _____

Email address_____

Address:_____

Date of Birth:_____

Age: _____yrs_____mo Handedness: Right Left

Weight:_____lbs Height:_____ft_____in

Typically, how many times per week do you exercise?_____

What is the typical length of your exercise sessions?_____

Type of activities you typically do when you exercise:

_____

_____

_____

Race (circle):

1. American Indian or Alaska Native
2. African American
3. Caucasian
4. Asian
5. Hispanic
6. Other (specify):_____

Marital Status (circle):

1. single
2. married
3. divorced/separated
4. widowed

Education Level

What is the last grade in school that you completed? (circle) Please specify if a two year school.

1. Elementary 01 02 03 04 05 06 07 08
2. High School 09 10 11 12
3. College 13 14 15 16
4. Graduate/Professional School 17+

Employment:

Are you currently a student? Yes No

If so, what type of degree are you pursuing?

_____

What is your occupation?_____

If employed how many hours a week do you work?_____

Income:
What is your household annual income (circle)

1. No income
2. $1 to $10,000
3. $10,001 to $20,000
4. $20,001 to $30,000
5. $30,001 to $40,000
6. $40,001 to $50,000
7. $50,001 to $75,000
8. $75,001 or more

Medical History (circle any, and give age at diagnosis):

	Age
1. Diabetes	____
2. Thyroid Disease	____
3. Cirrhosis	____
4. Hepatitis	____
5. Gall Stones	____
6. Kidney Stones	____
7. Nephritis	____
8. Cancer (specify)	____
9. High Blood Pressure	____
10. Angina	____
11. Allergies (specify)	____
12. Goiter	____
13. Cardiovascular Disease	____
14. Depression requiring medication	____
15. Insomnia requiring medication	____

Do you donate plasma, platelets, or any other blood product? Yes No

Diet History:
a. Are you a vegetarian? Yes No
b. If yes, circle one of the following:

i. Lacto-ovo (consume milk, milk products and eggs)
ii. Ovo (consume eggs, but no milk or milk products)
iii. Lacto (consume milk and milk products, but no eggs)
iv. Vegan (consume no animal products)

On average, how many alcoholic beverages do you consume per week?
_____
Approximately how many ounces are consumed per beverage? _____
What type(s) of alcohol do you most commonly consume?
_____

Medication History:
Please list any medications and/or supplements you are currently taking or have taken regularly in the past year. Please indicate how long you took the medication. If none, please write NONE here:
_____

	Currently taking	Have taken in past 3 months	Have taken in last 6 months	Have taken in last 12 months
Prescription Medication				
Vitamin Supplements				
Herbal Supplements				
Dietary Supplements (Creatine, Protein, etc)				
Other (Ibuprofen, Advil, Aspirin, Aleve, Tylenol, etc.)				

Campbell Nutrition Research Lab, Iowa State University, Ames
Dietary intake screening questionnaire

Subject code _____ **Week** _____
Height _____ __ **Interviewer** _____
Weight _____ **Date** _____

How would you describe your diet?

I eat both meat and fish	_____
I avoid fish, but eat meat	_____
I'm a vegetarian and include dairy and eggs in my diet	_____
I'm a vegetarian and include dairy but not eggs in my diet	_____
I'm a vegetarian and include dairy, eggs, and fish in my diet	_____
I'm a vegan, avoid all animal products	_____

In the past month, have you consumed (food group)? What type (reduced fat, fortified, whole grain, etc), how much (ounces, cups, tablespoons, etc) and how often (how many times per day, week or month) was it consumed?

Food	**Description**	**Amount**	**Frequency**		
			Times/day	**Times/week**	**Times/month**
Tuna					
Albacore or white canned in water			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Albacore or white canned in oil					
Light canned in water					
Light canned in oil					
Fatty Fish (salmon, herring, sardines, mackerel, trout)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Lean Fish (tilapia, cod, halibut, catfish)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Food	Description	Amount	Frequency Times/day	Frequency Times/week	Frequency Times/month
Locally caught fish (perch, bass, pike)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Shellfish (shrimp, scallops, oysters, clams, mussels)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Other seafood (specify)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Oils (include cooking and baking) (soybean oil, canola oil, olive oil, flaxseed oil, walnut oil)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Omega-3 fortified foods (eggs, soy milk, orange juice, Smart Balance (spread, milk, sour cream, etc), other)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Supplements (fish oil, flaxseed oil, etc)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Other (specify)			1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Nutrition Research Lab, Iowa State University, Ames

Subject:_____

Height: _____ **Interviewer:_____**

Weight: _____ **Date: _____**

Eating Preferences

Did you restrict any of the following foods?

Food	Yes or No	If yes, why?
Meat (pork, beef, poultry)		
Fish		
Eggs		
Dairy		

Eating Frequency

How often did you eat the following meals per week for the past month?

	Number of Days per Week							
	7	6	5	4	3	2	1	0
Breakfast								
Snack, morning								
Lunch								
Snack, Afternoon								
Supper								
Snack, after Supper								
Snack, before bed								
Other:								

Food Frequency

In the past month, have you consumed (food group)? What type (reduced fat, fortified, whole grain, etc), how much, and how often was it consumed?

Milk and Cream	Fat content (whole, 2%, 1%, buttermilk, skim)	Fortification/description	Amount (cups)	Times per day	Times per week	Times per month
Milk, cow's				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Milk, cow's, flavored				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Powdered				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Evaporated				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Sweetened condensed				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Rice Milk				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Almond Milk				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Soy Milk				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Milk, goat's, fresh or canned				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cream				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Whipped cream				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Half and Half				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Non-dairy creamer				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Non-dairy whipped topping				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Sour Cream				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Ice Cream, Vanilla				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Ice Cream, Flavored				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Sherbet				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Soy ice cream				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Rice ice cream				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other:				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Cheese and Yogurt	**Fat content (Part-skim, 1%, 2%, whole, light)**	**Description**	**Amount (ounces)**	**Times per day**	**Times per week**	**Times per month**
Cheddar				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Colby				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
American, processed				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Mozzarella				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Monterey Jack				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Swiss				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cottage Cheese				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Ricotta				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Feta				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Blue Cheese				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Parmesan				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cream cheese				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Yogurt Cheese				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Goat cheese				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Soy cheese				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Yogurt, plain				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Yogurt, flavored				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Yogurt drink				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Frozen yogurt				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other:				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Eggs	**Fortified**	**Description**	**Amount (items)**	**Times per day**	**Times per week**	**Times per month**
Whole				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Egg white only				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Egg Substitute				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Desserts/Baked Goods	Fat content (Regular, Reduced Fat, Light, Fat Free)	Description (Whole wheat, etc)	Amount (items)	Times per day	Times per week	Times per month
Pudding				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Custard				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cheesecake				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cake				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Pie				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cookies				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Brownies/Bars				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Fruit Breads				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Doughnuts				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Waffles				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Muffins				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Pancakes				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Poptarts				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other:				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Dips/Dressing	Fat content (Regular, Reduced Fat, Low-cal, Fat Free)	Description	Amount (ounces)	Times per day	Times per week	Times per month
Guacamole				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Salsa				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Hummus				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Ranch				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Thousand Island				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Italian				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
French				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Flavored Vinaigrette				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Oil and Vinegar				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other:				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Fats and Oils (cooking, spreading)	Fat content (Regular, Reduced Fat, Light, Fat Free)	Description	Amount (ounces)	Times per day	Times per week	Times per month
Butter				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Margarine/Oleo				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Yogurt spread (ie Brummel &				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Brown)						
Olive Oil				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Canola Oil				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Vegetable oil				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Flax seed oil				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Sesame seed oil				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Coconut oil				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Vegetable shortening				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Lard				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Mayonnaise				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Miracle Whip				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Peanut Butter, regular or natural				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other nut butters				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Avocado				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Olives				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other fat/oil				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Meat and Poultry	**Description (cut, skinless, bone-in, ground, etc)**	**Amount (ounces)**	**Times per day**	**Times per week**	**Times per month**
Chicken (white or dark meat)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Turkey (white or dark meat)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Game bird			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Beef (hamburger, tenderloin, pot roast, round steak, strip steak, filet, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Pork (tenderloin, chops, ham, shoulder roast, loin roast, bacon, sausage, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Fried/Breaded pork tenderloin sandwich or fritter			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Hot dogs, sausage, brats			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Lamb			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Game meat			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Lunch Meat (bologna, salami, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Fish and Shellfish	Description (canned in water/oil, fresh, breaded/fried)	Amount (Ounces)	Times per day	Times per week	Times per month
Tuna, canned or fresh			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Lean fish (Tilapia, cod, halibut)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Fatty fish (Salmon, mackerel, trout, herring)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Fried, breaded fish or shrimp			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Locally caught (Perch, bass, pike)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Shellfish (shrimp, mussels, clams, oysters, scallops)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Sushi			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other seafood			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Fruit	Description (type, fresh, canned in syrup or juice, dried)	Amount (Cups)	Times per day	Times per week	Times per month
Apples			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Grapes, raisins			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Melon (watermelon,			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

cantaloupe, etc)					
Berries (straw-, black-, blue-, cranberries, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Citrus (orange, tangerine, grapefruit, lemon, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Tropical (banana, pineapple, kiwi, mango, papaya, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Stonefruit (peaches, apricots, plums, cherries, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Vegetables	**Description (Type, fresh, frozen, canned, fried/breaded)**	**Amount (Cups)**	**Times per day**	**Times per week**	**Times per month**
Green Leafy (Kale, spinach, lettuce)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Yellow/Orange (corn, pumpkin, squash, sw. potatoes, carrots)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Red/Purple (tomatoes, eggplant, peppers, beets)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Tomato Sauce			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Green (Peas, soybeans, cabbage, broccoli, beans, celery, peppers, cucumber)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
White (Potatoes,cauliflower, onions, turnips, mushrooms, radishes)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Beans (Kidney, black, pinto, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Vegetarian meat substitute (tofu, seitan, TVP)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Cereals and Grains	**Description**	**Amount (cups)**	**Times per day**	**Times per week**	**Times per month**
Rice			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Pasta, couscous			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Quinoa (“keen-wa”)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cornmeal (masa, polenta)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other grains (Barley, bulgur, cracked			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

wheat, millet)					
Flax			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Oatmeal			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cream of Wheat/Farina			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Cream of Rice			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Grits (hominy)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Hot whole grain			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Added flax			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Highly fortified breakfast cereals: (Total, Smart Start, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
High fiber breakfast cereals: (raisin bran, All bran, Kashi)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other breakfast Cereals: (Corn flakes, wheat flakes, puffed rice, shredded wheat)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Granola			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other grains/cereals			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Breads	Description (Type, white, wheat, whole grain)	Amount (items)	Times per day	Times per week	Times per month
White, wheat			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Whole wheat, whole grain			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Pita			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Bagels			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
English muffins, crumpets			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Biscuit			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Buns or rolls			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Tortillas, taco shells			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Snacks and Candy	Description (Type, whole grain, reduced fat)	Amount (Ounces)	Times per day	Times per week	Times per month
Crackers (graham, saltines, cheese, wheat thins, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Potato chips			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Corn chips			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Popcorn, microwave			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Popcorn, air popped			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Pretzels			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Rice cakes, crisps			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Soy chips			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Granola bars			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Energy bars, meal replacement bars			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Nuts (almonds, walnuts, peanuts, pecans, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Seeds (sunflower, pumpkin)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other Snacks			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Candy and Sweeteners	**Description**	**Amount (items)**	**Times per day**	**Times per week**	**Times per month**
Candy (chocolate, Twizzlers, jelly beans, mints, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Candy continued…			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Honey, Agave nectar, Stevia			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Artificial Sweeteners (splenda, equal, etc)			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Pre-packaged, prepared foods	Description	Amount	Times per day	Times per week	Times per month
Lasagna			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Macaroni and cheese			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Hamburger or tuna helper			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Frozen dinners			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Tacos			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Burritos			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Pizza			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Stir-fry			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Soup			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Casseroles			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Rice-a-roni or pasta mixes			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other			1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Beverages	Fortified	Description	Amount (ounces)	Times per day	Times per week	Times per month
Orange Juice				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Other 100% fruit juices				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Sweetened juices				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Vegetable juice				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Non-carbonated beverages (Kool-aid, tang)				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Carbonated beverages				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Sports Drinks				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Flavored water				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Tea				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Coffee				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Meal replacement drinks (Carnation instant breakfast, Slim fast, ensure)				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
Energy drinks				1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Fast Food Consumption

Description	Amount	Times per day	Times per week	Times per month
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Other foods consumed in the past month, not previously mentioned.

Description	Amount	Times per day	Times per week	Times per month
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Supplements and Vitamins

Description	Amount/Dosage	Times per day	Times per week	Times per month
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3
		1 2 3 4 5 6 +	1 2 3 4 5 6 7	1 2 3

Directions for
3-Day Weighed Diet Records

- Please use the scale provided to weigh all food that you eat during your 3 day recording period.

- Keep your food record current. List all foods and supplements immediately after they are weighed. Do not wait until the end of the day to record entries.

- Please print all entries.

- Be as specific as possible when describing the food or beverage:
  - Include the method of preparation used (boiled, baked, broiled, fried, grilled, steamed, raw, etc); *example: pork chop, center cut, no bone, grilled*
  - Include a well detailed description of the food item (fresh, canned, packed in heavy or light syrup, packed in water or oil, skinless, boneless, cut of meat, brand name); *examples: peaches in heavy syrup, tuna in oil, broiled T-bone steak, microwave heated canned corn*
  - Include label with the nutritional information for any unusual items or if unsure how to record

- Include the name of restaurant if eating out

- Report only the portion of food that was actually eaten*; example: T-bone steak, grilled -100g (do not include the weight of the bone)*

Example: 100g t-bone- 30 g bone=70g actual food consumed
1- 500 mg multivitamin

- Weigh food left on plate that you did not eat and subtract from original total

- Record amount in either grams or ounces (wt) –please be consistent

- Remember to record condiments (ketchup, soy sauce, mustard, ranch dressing, salt, etc) as well as any fats used in cooking (oils, butter, margarine, etc), it is acceptable to measure these (Tbsp, tsp etc)

- Please try not to alter your normal diet during the period that you keep this record …… **Thank you!!!!!!**

- If there are any questions please contact:
  Stephanie Kratzer @ (515) 294-8673; kratzer1@iastate.edu

Date: ___Wednesday, March 21, 2007___

For Official Use Only
Subject ID:

Time	Food	Constituents	Description	Weight
9 am	Daily Supplements:	Multivitamin	One a Day multivitamin	1-500 mg capsule
9am	Grape Nuts		Post Brand	120g
9am	Sugar		White	3g
9am	Milk		1%	106g
9am	Blueberries		Frozen, unsweetened	50g
9am	Orange Juice		Tropicana, no pulp, calcium added	120g
11:30 am	Almonds		Raw, unsalted, Kirkland brand	60g
1:00pm	Sandwich	Bread	Whole Wheat, Wheat Montana	45g
1pm		Sprouts	Alfalfa	5g
1pm		Cheese	Tillamook Sharp Cheddar	33g
1pm		Ham	Hillshire Farms Honey Ham	15g
1pm	Cottage Cheese		Low fat 2% small curd	55g
1pm	Apple Juice		From concentrate, Apple Tree brand, 100% juice	

82

Date: _____

For Official Use Only
Subject ID:

Time	Food	Constituents	Description	Weight
	Daily Supplements:			

83

Supplement Instructions

Control (weeks 0-2)

Food

Take one supplement capsule daily with your main meal of the day.

Return all unused supplement capsules when you come for your next appointment.

Fasted

Take one supplement capsule daily with water first thing in the morning before eating. Then do not eat anything for 90 minutes (1.5 hours) afterward.

Return all unused supplement capsules when you come for your next appointment.

Experimental (weeks 2-6 & 6-10)

Food 200 DHA

Take one supplement capsule daily with your main meal of the day.

Return all unused supplement capsules when you come for your next appointment.

Food 1000 DHA

Take five supplement capsules daily with your main meal of the day.

Return all unused supplement capsules when you come for your next appointment.

Fasted 200 DHA

Take one supplement capsule daily with water first thing in the morning before eating. Then do not eat anything for 90 minutes (1.5 hours) afterward.

Return all unused supplement capsules when you come for your next appointment.

Fasted 1000 DHA

Take five supplement capsules daily with water first thing in the morning before eating.

Then do not eat anything for 90 minutes (1.5 hours) afterward.

Return all unused supplement capsules when you come for your next appointment.

If there are any questions, please contact:

Stephanie Kratzer: 515-294-8673; kratzer1@iastate.edu

Supplement Instructions

Take one supplement capsule daily with your main meal of the day. Return all unused supplement capsules when you come for your next appointment.
If you have any questions, please contact:
Stephanie Kratzer: kratzer1@iastate.edu, (515) 294-8673

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21

Supplement Instructions

Take one supplement capsule daily with water first thing in the morning before eating. Then do not eat anything for 90 minutes (1 ½ hours) afterward. Return all unused supplement capsules when you come for your next appointment.
If you have any questions, please contact:
Stephanie Kratzer: kratzer1@iastate.edu, (515) 294-8673

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21

Supplement Instructions

Take one supplement capsule daily with your main meal of the day.
Return all unused supplement capsules when you come for your next appointment.
If you have any questions, please contact:
Stephanie Kratzer: kratzer1@iastate.edu, (515) 294-8673

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35

Supplement Instructions

Take five supplement capsules daily with your main meal of the day.
Return all unused supplement capsules when you come for your next appointment.
If you have any questions, please contact:
Stephanie Kratzer: kratzer1@iastate.edu, (515) 294-8673

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35

Supplement Instructions

Take one supplement capsule daily with water first thing in the morning before eating. Then do not eat anything for 90 minutes (1 ½ hours) afterward. Return all unused supplement capsules when you come for your next appointment.
If you have any questions, please contact:
Stephanie Kratzer: kratzer1@iastate.edu, (515) 294-8673

Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Day 8	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
Day 15	**Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 20**	**Day 21**
Day 22	**Day 23**	**Day 24**	**Day 25**	**Day 26**	**Day 27**	**Day 28**
Day 29	**Day 30**	**Day 31**	**Day 32**	**Day 33**	**Day 34**	**Day 35**

Supplement Instructions

Take five supplement capsules daily with water first thing in the morning before eating. Then do not eat anything for 90 minutes (1 ½ hours) afterward. Return all unused supplement capsules when you come for your next appointment.
If you have any questions, please contact:
Stephanie Kratzer: kratzer1@iastate.edu, (515) 294-8673

Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Day 8	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
Day 15	**Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 20**	**Day 21**
Day 22	**Day 23**	**Day 24**	**Day 25**	**Day 26**	**Day 27**	**Day 28**
Day 29	**Day 30**	**Day 31**	**Day 32**	**Day 33**	**Day 34**	**Day 35**

Subject: _____ Date: _____
Group: _____ Week: _____
Weight: _____ Number of supplements returned: _____
Concerns/side effects?

Subject: _____ Date: _____
Group: _____ Week: _____
Weight: _____ Number of supplements returned: _____
Concerns/side effects?

Subject: _____ Date: _____
Group: _____ Week: _____
Weight: _____ Number of supplements returned: _____
Concerns/side effects?

APPENDIX II: SUPPLEMENT INFORMATION

This appendix contains copies of the certificates of analysis for the placebo and experimental supplements supplied by Martek Biosciences.

Certificates of analysis:

-DHA

-Placebo (control)

Martek®

Martek Biosciences Corporation

CERTIFICATE OF ANALYSIS

DHASCO-S® Capsules

Lot Number DHA L2F0029A

Physical Description

Appearance: 500 mg softgel clinical capsules containing DHASCO-S®

Chemical Analyses	Units	Results
Capsule Fill Weight	mg	524.8
Peroxide Value	meq/kg	1.94
Free Fatty Acid, as Oleic	%	0.05
DHA Content	mg/capsule	200.30

Fatty Acid Profile	Units	Results
8:0	%	0.12
9:0	%	< 0.1
10:0	%	0.12
12:0	%	0.28
11:0	%	< 0.1
14:0	%	7.93
14:1	%	< 0.1
15:1	%	0.13
16:0	%	22.47
16:1	%	0.30
17:0	%	< 0.1
18:0	%	0.62
18:1n-9	%	0.26
18:1n-7	%	0.27
18:2n-6t	%	< 0.1
18:2n-6c	%	0.28
18:3n-3	%	< 0.1
18:3n-6	%	0.29
20:0	%	0.18
21:0	%	< 0.1
20:1n-9	%	< 0.1
20:2n-6	%	< 0.1
20:3n-6	%	0.46
20:4n-6	%	0.9
20:5n-3	%	2.53
22:0	%	0.4
22:5n-3	%	0.4
22:5n-6	%	16.62
22:6n-3	%	40.5
24:0	%	0.24
Others	%	4.7

Analysis Completed By: Central Analytical Laboratories

Released By: [signature]

Date: 5/19/2004

rev.5/19/2004

555 Rolling Hills Lane
Winchester, Kentucky 40391

(859) 744-0920
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Martek®
Martek Biosciences Corporation

CERTIFICATE OF ANALYSIS

Corn / Soy Placebo, Orange, 530 mg Capsule

Lot Number: **6650413725**

Physical Description					Conforms
Appearance:	530 mg, Size 10 Oval capsule containing a mixture of Corn and Soy Oil				yes
Color:	Orange				yes
Chemical Analyses	**Units**	**Minimum**	**Maximum**	**MDL**	**Results**
Capsule Fill Weight	mg	504	557	0.1	513.80
Free Fatty Acids	%		<0.4	0.005	0.10
Peroxide Value	meq/kg		<5	0.1	1.79
Microbial Analyses					
Total Plate Count	CFU/g		< 1000	10	<10
E. Coli	CFU/g		ABSENT in 10g	10	<10
Yeast	CFU/g		< 100	10	<10
Mold	CFU/g		< 100	10	<10
Salmonella			ABSENT in 25g		Negative
S. aureus	CFU/g		ABSENT in 10g	10	<10
Fatty Acid Profile					
10:0	%			0.1	0.82
12:0	%			0.1	<0.1
14:0	%			0.1	<0.1
16:0	%			0.1	10.67
16:1	%			0.1	0.10
18:0	%			0.1	3.06
18:1 n-9	%			0.1	26.53
18:1 n-7	%			0.1	1.08
18:2 n-6	%			0.1	50.52
18:3 n-3	%			0.1	2.95
20:0	%			0.1	0.43
20:3 n-6	%			0.1	<0.1
20:4 n-6	%			0.1	<0.1
20:5 n-3	%			0.1	<0.1
22:5 n-6	%			0.1	<0.1
22:5 n-3	%			0.1	<0.1
22:6 n-3	%			0.1	<0.1

Analysis Completed By: Central Analytical Laboratory

Analytical Review By: [signature] **Date:** 7-21-06

Product Release By: [signature] **Date:** 7-21-06

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