EFFECTS OF DOCOSAHEXAENOIC ACID SUPPLEMENTATION ON LIPIDS, LIPOPROTEINS AND INFLAMMATORY MARKERS FOLLOWING HEAVY PHYSICAL TRAINING IN DIVISION I FOOTBALL ATHLETES

A Dissertation

by

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ABSTRACT

Dietary docosahexaenoic acid (DHA) (22:6 n-3) has been linked to many health benefits in sedentary populations, positively altering lipid profiles and reducing inflammation. The prospective impacts of DHA supplementation in an athletic population during intensive physical training are less clear. The first investigation describes inflammatory responses and the second describes lipid and lipoprotein responses during intensive physical training with DHA supplementation in football athletes.

Sixty NCAA Division I football players (20 ± 1.5 years, 187.4 ± 6.1 cm, 105.7 ± 18.9 kg) were randomly assigned to 2 g•day^{-1} DHA (n=28) or corn-oil placebo (n=32). Blood samples were collected at voluntary summer training (Summer), 30 days after Summer (Pre-camp), and 24 days after Pre-camp (Post-camp). Selected cytokines (multiplex assay), WBC #, percent leukocytes, total cholesterol (TC), triglycerides (TG), LDL, HDL, IDL, and VLDL cholesterol (-C) and lipoprotein particles were analyzed. One sample t-tests (α=0.05) were used to assess differences in percent change of cytokine concentration, leukocyte concentration, lipoprotein concentration, particle numbers, and density at each time point; independent t-tests (α=0.05) were used for differences between groups at Summer.

Eotaxin and monocyte chemoattractant protein-1 (MCP-1) elevations were significantly attenuated in the DHA group during preseason camp compared to Placebo ($P < 0.05$). Regulated on activation, normal T cell expressed and secreted (RANTES)

was significantly elevated in both groups ($P < 0.05$); however, the percent change increase in the Placebo group was 2-fold that of the DHA group. White blood cell counts decreased at Post-camp ($P < 0.05$) in both groups. Pre-camp percent change TG was significantly ($P < 0.05$) increased only in the Placebo group. Post-camp percent change TG and HDL particle number in the DHA group was significantly ($P < 0.05$) reduced. LDL_4 number significantly increased in the DHA group (Post-camp, $P < 0.05$), and the Placebo group decreased in LDL-C (Pre-camp, $P < 0.05$). Both groups had increased HDL_{2b}-C and HDL_{2a}-C at Pre-camp ($P<0.05$). Pre-camp LDL_3-C and Post-camp LDL_4-C increased in the DHA group ($P<0.05$). RLP-C increased in the Placebo group (Pre-camp, $P<0.05$). Pre-camp HDL density and Post-camp LDL density decreased in the Placebo group. The DHA group decreased HDL density during preseason, but LDL density remained constant. Summer IDL-C was significantly ($P < 0.05$) higher in the DHA group. Percent change VLDL number was significantly ($P < 0.05$) increased during preseason camp. There was no difference in lipoprotein-a and C-reactive protein between groups. TC, HDL-C, and RLP number did not change over time nor differ between groups. Pre-camp homocysteine increased, while Post-camp insulin significantly ($P < 0.05$) decreased in the DHA group.

These investigations further our knowledge of a particular omega-3 fatty acid (DHA) as a potential lipid mediator to mitigate cardiovascular risk, as well as an inflammatory modulator for possible overtraining. Adequate dosage for the anti-inflammatory and lipid profile improving effects of DHA in a sedentary population is still unclear for this particular athletic population.

DEDICATION

I would like to dedicate my dissertation to my wife, Catherine. Through thick and thin, you have always been there and we will continue to grow long after this. To Ethan, my brilliant son who amazes me every day with the vast knowledge you possess. To my son, Reid, who will be one of the most confident men I will ever know. To my daughter, Alice, my little princess, that binds all of us together in fun and laughter. Finally, to the little one, Helena, you have come into an astonishing world with a loving family.

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"Somewhere, something incredible is waiting to be known."
-Carl Sagan

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Chapter I will provide a study proposal with review literature. Chapters II and III are intended to serve as separate manuscripts to be submitted for publication in a peer-reviewed journal. Chapter IV provides suggestions and limitations for future studies.

This document will highlight the interaction of docosahexaenoic acid (DHA) and its potential effects on Division I football athletes during strenuous training. To the author's knowledge, this study was the first to highlight this particular omega-3 supplementation in an elite group of football athletes during a rigorous training period. Two specific and complementary aims were identified with the goal of clarifying the potential benefits of algal DHA in this athletic population. The literature review links the descriptive interactions among exercise, inflammation, blood lipids, and polyunsaturated fatty acids. The resultant information will prove useful to researchers, practitioners, athletes, and the public.

Polyunsaturated fatty acids (PUFA) have been of great interest for several years in relation to risk reduction and possible prevention of multiple diseases, including diabetes, cardiovascular disease, metabolic syndrome, asthma, Alzheimer's and other nervous system disorders [30, 130, 162, 178]. In particular, total dietary intake of omega-3 fatty acids (FA) is a focus of current dietary research. Omega-3 FAs have a basis in animal models for acting as neural protective/recovery agents following head trauma, like concussion [10, 217], partly stemming from anti-inflammatory compounds produced

from DHA, such as D-Series resolvins and neuroprotectins [25, 32, 97]. When DHA and other omega-3 FAs are discussed in context of sports medicine, it is often concerning supplementation in concussion prevention and treatment [15, 158]. American football is known, in research and also in the public media, as being a sport with the potential for numerous concussion incidents, as well as other injuries [4, 48, 56].

American football is a high intensity sport that requires speed, strength, and power. The ability to make a variety of plays is necessary. A high level of athleticism is needed to play the sport, as not only does the athlete need to be able to pass, run, and avert a tackle, but must also perform in situations that lead to a collision with other players. These impacts result in many injuries each season [3, 48, 56, 75, 98, 138]. Herbenick et al. [75] found that of out of 1,199 documented injuries (a study spanning 2002-2005), 38.7% of those injuries occurred during preseason or the first week of season.

In preparation for the college season, football players participate in preseason camp, previously known as two-a-days, where they undergo weeks of training with numerous conditioning, resistance exercises, and sport practice sessions regulated by legislation by the National Collegiate Athletic Association (NCAA) [134]. Even with training guidelines, there still remains a possibility that a football player, preparing for the upcoming season, could be over-trained during this preparatory period of training. Overtraining syndrome (OTS) is defined by an increase in training volume (weeks or months) with a concomitant decrease in performance [180] which can also include the accumulation of non-training stressors that show not only physiological but also psychological symptoms [69]. Indicators of overtraining can vary from study to study, as

well as from variations in coaching style. These indicators can include: decreased muscular strength, decreased work capacity maximums, loss of coordination, persistent high fatigue ratings, decrease in the quality of sleep, and/or decreased maximal heart rate [114, 121, 180].

Moderate exercise can lead to attenuation in illness rates, that is, a drop in frequency of both viral and bacterial infections [139, 140]. Conversely, when exercise becomes chronic and/or too strenuous, an inverse effect appears and the chance for infections increases [21, 139, 140, 150, 152, 153]. All the physical work football teams perform to prepare for the season and to maintain performance throughout the season can have a cumulative effect which could potentiate a state of over-inflammation. That is, with all the seasonal practice, the excess amount of exercise could lead to an over-trained state which could also be associated with over/prolonged inflammation.

A strategy to combat this state of over-inflammation is nutritional intervention. One nutrient of particular interest is the omega-3 fatty acid, DHA, to aid in regulation of the inflammation process. Regulation and modification of inflammation could have the potential to allow the athlete to recover sooner and adapt more efficiently to additive stimulus.

Inflammation

Inflammation is a natural response to stress (physical, chemical, or psychological) by the body. It is the first step in the body's healing process, in which repair cells are directed from the blood into the injured tissue. Inflammation in response to exercise is essential to the sustainability and adaption of the working skeletal muscle

[109, 145, 150, 153]. In this document, inflammation and immune function will be linked together to demonstrate the communication and action following a stress, such as multiple exercise bouts. Acute (short-lived) inflammation is necessary, but problems arise when inflammation persists (chronic inflammation). Besides rheumatoid arthritis or tendonitis, chronic inflammation accompanies many other disease states, such as atherosclerosis, coronary heart disease, diabetes, obesity, and certain forms of cancer [97, 132, 156, 162].

The two main phases of inflammation are the acute response phase and resolution phase. During the acute response phase, there are several characteristics associated with repair signaling, including increases in blood flow and vasodilation, as well as chemical messengers that affect transient time and vascular permeability. There are many cell types of numerous functions and interactions that take place during this first phase. A cellular overview consists of neutrophils moving into the damaged area with maturing monocytes (into macrophages) ensuing, of which proliferation will occur and redirection/reprogramming of indigenous macrophages to handle cellular deconstruction and the rebuilding process [172].

The second phase of inflammation is the resolution phase. The reversal or removal of the inflowing granulocytes is considered by some to be a marker of inflammation resolution, as the macrophage and lymphocyte population of the tissue returns to baseline concentrations that were present prior to the increased cell population from the initiation phase of inflammation [62]. Excessive stimulation of neutrophil production and function can change a skeletal muscle repair model into a damaging

process until some intervention can rectify the initiation process and proceed to the resolution phase [197]. If the resolution phase cannot be initiated and the acute response phase is prolonged, then the body is placed into a state of chronic inflammation, whether it is low-level or high-level.

Prostaglandins, or lipid mediators, play an integral role in the resolution phase, as they regulate how leukocytes are removed from the tissue area [118, 172, 212]. The various roles of lipid mediators in the inflammation process make lipids and their constituents prime candidates for supplemental intervention to optimize the inflammation process.

The immunological connection between overtraining and how adaptation relates to cytokine formation has been defined as the cytokine hypothesis of overtraining [180]. Microtrauma found in bones, connective tissue, and skeletal muscle has been linked to participation in training and competition [121, 181]. This microtrauma could potentiate the cytokine response necessary to initiate the acute inflammation phase. Continual microtrauma without time to recover could extend acute inflammation to chronic inflammation, ultimately leading to a systemic immune/inflammatory response [180]. Testing for overtraining and distinguishing between overtraining and overreaching is complex and many times symptoms or indicators are anecdotal in nature [69, 126]. The term overreaching can be applied to strenuous activity that requires a recovery period that lasts days or weeks [121]. This continuous training, with insufficient recovery, can extend past overreaching for adaptation and may lead to overtraining syndrome [126, 174]. Furthermore, heavy excessive exercise in the absence of regenerative time, can lead to repeated transient immune dysfunction [57] .

The use of non-steroidal anti-inflammatory drugs (NSAIDs) has been common practice for treating many sports related injuries. Studies have examined the inflammatory responses following heavy eccentric exercise with and without commonly used NSAIDs, ibuprofen and acetaminophen [157, 198]. Prostaglandins are eicosanoid-derived regulators of inflammation [25, 32]. Prostaglandin E_2 (PGE_2), a pro-inflammatory prostaglandin, is associated with the acute phase of inflammation and mediates the influx of neutrophil exudates to the exercised muscle [198]. Opposite in function to PGE_2, is Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which affects the neutrophil chemotaxis during the resolution phase of inflammation [41]. Interestingly, heavy eccentric exercise and the administration of either maximal consumer dosages of ibuprofen or acetaminophen resulted in attenuated $PGF_{2\alpha}$ concentrations and PGE_2 concentrations that were attenuated with acetaminophen, but not affected by ibuprofen, when compared to placebo [198]. Further analysis of macrophage and neutrophil concentrations showed no difference in treatment groups, as all exhibited elevated macrophage count following heavy eccentric exercise; no group had a significant change in neutrophil concentrations pre- or post-exercise, as well as with treatment groups [157]. The attenuated $PGF_{2\alpha}$ without change in leukocyte concentration effects suggests that NSAIDs do not alter the acute phase of inflammation but have the possibility to blunt the resolution phase [157, 198]. Additionally, this alteration in cellular repair timing has been shown to attenuate protein synthesis of the stimulated muscle [41, 157, 198].

Researchers have investigated other possible interventions to regulate the prolonged initiation phase of inflammation, including nutrient compounds with possible

anti-inflammatory effects. Compounds include fatty acids, fish oils, quercetin, carbohydrate isoforms, antioxidants, nitrites, and bicarbonates [26, 92, 136, 139]. The use of polyunsaturated fatty acids and their role in resolvin and protectin formation are major topics of research addressing inflammation and immunomodulation [29, 30, 59, 97, 172, 208]. Later in this chapter, the importance of resolvins and protectins derived from different omega-3 fatty acids will be explored.

Inflammation and Exercise

Inflammation is an essential element of the training and preparation that all athletes undergo to become bigger, faster, and stronger [109]. The acute response phase is necessary for repair and has the potential to effect muscle adaptation [109, 120, 145]. Intracellular neutrophil populations (from cytokine signaling) begin to increase immediately post exercise and remain elevated for several hours after, during the acute phase of inflammation [152]. Acute bouts of exercise have resulted in reductions in inflammation [13, 140, 175]. Following a resistance training session with 3 x 10 repetitions at 60-70% repetition-max, untrained individuals had increased leukocyte formation (66%) which returned to baseline concentrations within 30 minutes, compared to almost double that when exposed to aerobic exercise of similar caloric expenditure [175].

Routine, moderate exercise has been linked to reductions of proinflammatory cytokines and chemokines, fibrinogen, C-reactive protein, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) [57, 99, 117, 147, 184]. Issues arise when overtraining occurs or time for recovery is limited [137, 140]. Brenner et al. [19] states that strenuous physical activity can elicit mild clinical injury which could potentiate excessive

immunosuppression and inflammation comparable to clinical sepsis. In contrast, a 2005 review on the role of exercise in cytokine regulation explained that the exercise cytokine response was different from responses present in severe infections [156]. The main difference was with contracting skeletal muscle. In this instance, there is less of an initial proinflammatory effect of TNF-α, which, in a sepsis situation, would lead to a lower production of IL-6, but this was not the case [156].

Additionally, IL-6 from muscle has a different cascade of responses (including acting both pro and anti-inflammatory), acting as a myokine, which can be elevated post-exercise from resistance or endurance training [156]. IL-6 is considered the most prominent cytokine messenger originating either in the muscle cell or of systemic origin during physical activity [99]. IL-6 has both pro-inflammatory and anti-inflammatory properties [55, 57, 100, 156]. Extracellular IL-6 acts a proinflammatory messenger, directing neutrophils to the site of repair while intracellular IL-6 (from muscle tissue mononuclear leukocytes) signals the up-regulation of anti-inflammatory cytokines IL-1ra, IL-8, IL-10 while inhibiting TNF-α (promoting the resolution phase of inflammation) [55, 99, 100, 145, 156]. The inhibition of TNF-α by IL-6 has been described as a glucose and lipid modulator during and after exercise with termination corresponding with glycogen resynthesis [99].

It has been suggested that heavy exertion (i.e., prolonged endurance exercise) leads to a compromised immune system [140]. Upper respiratory tract infections have been linked to prolonged heavy exertion training and show elevations in both IL-6 (proinflammatory response) and IL-1ra (anti-inflammatory response) concentrations [140].

Two and a half hours of intense running by 30 marathoners led to a 5.5-fold increase in IL-6 immediately post-run, as well as IL-1ra increasing 127% 1.5 hours post-run [136]. For an athlete, training and competition is sometimes combined with malnutrition, weight loss, mental stress, and lack of sleep, which can all be detrimental to the immune system [140].

Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFA) are fatty acids that contain at least two carbon-carbon double bonds. The naming convention for unsaturated fatty acids follows numbering from the methyl end of the chain, instead of the carboxylic acid end. The methyl end is also termed the "omega" end and denoted as ω-# or n-# (Fig. 1). The number associated with n or ω is the location of the first carbon-carbon double bond starting from the methyl end.

PUFA are often deemed "good" fats for improving lipid profiles and reducing cardiovascular disease risk. The two families of omega-6 and omega-3 fatty acids are located in most phospholipid bilayers of cellular membranes [125]. Of the known PUFAs, omega-6 and omega-3 fatty acids have both negative and positive impacts on inflammation and blood lipid profiles [125]. Both of these fatty acid groups are essential – that is, must be consumed in the diet, because they cannot be synthesized de novo in animals [125, 176, 178, 207]. Arachidonic acid (AA) (20:4n-6) and its essential precursor linoleic acid (LA)(18:2n-6) are considered to be proinflammatory, and are found in lymphocyte and monocyte membranes in high concentrations [25, 30, 31, 125]. The products from AA include eicosanoids, reactive oxygen species, and adhesion molecules [25, 30, 31,

[125]. The three main omega-3 PUFA; eicosapentaenoic acid (EPA)(20:5n-3), DHA (22:6n-3), and their essential precursor α-linolenic acid (ALA)(18:3n-3), are considered to be less inflammatory and are involved in the resolution of inflammation [30, 32]. Linoleic and α-linolenic acids are competitive inhibitors of the metabolism of each other, and their derivatives compete for inclusion into phospholipids of cell membranes. The integration of omega-3 fatty acids into phospholipid cell membranes can affect membrane compositions of multiple tissues [60, 76], as well as impact stress kinetics and inflammation signaling through alterations in lipid rafts and inhibition of receptor sites [28, 30, 32].

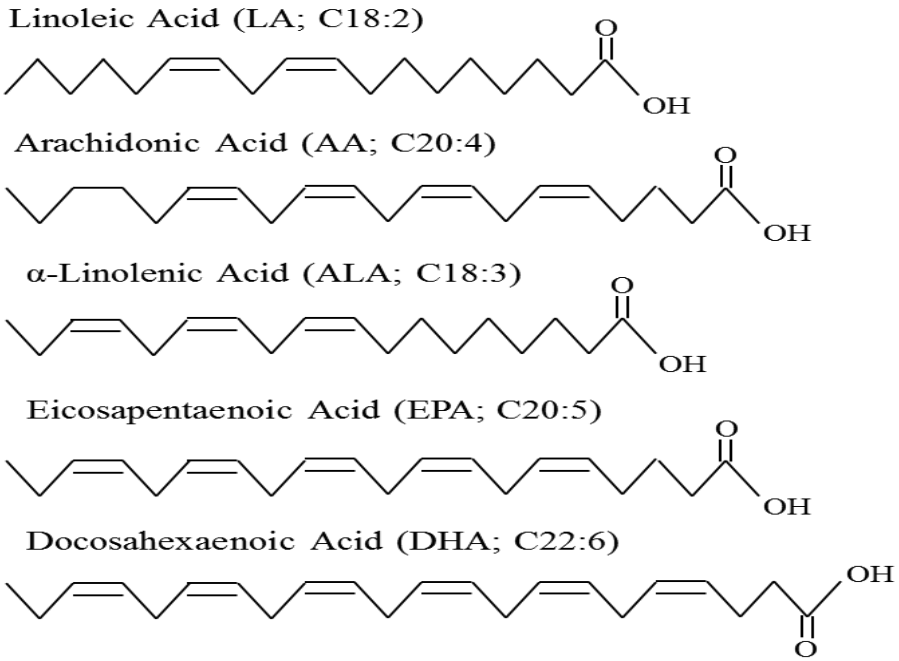


Figure 1. Bond-line structures for polyunsaturated fatty acids: LA, AA, ALA, EPA, and DHA. Number following C represents number of carbons. Number after ":" represents number of double-bonds. LA and AA have the first double-bond at 6th carbon from methyl end (ω-6). ALA, EPA, and DHA have the first double-bond at 3rd carbon from methyl group (ω-3).

Linoleic acid is the major PUFA found in Western diets. Second to that is ALA. Both LA and ALA sources are found in plants, while marine organisms are the major sources of ALA derivatives. ALA serves as a precursor for EPA and DHA, while LA serves as a precursor for arachidonic acid (AA). Mammals lack the capacity to synthesize EPA and DHA because of an inability to desaturate 16- or 18-carbon FAs that are more than nine carbons from the carboxyl end [183]. The conversion of ALA to EPA or DHA is limited in humans because of the inadequate amount of delta-6 desaturase, and delta-5 desaturase [14, 88, 94, 176]. There is a competitive inhibition of the delta-5 and delta-6 desaturase enzymes by LA on ALA to EPA; the result is increased production of AA [207]. Delta-6 desaturase is a rate limiting step of this pathway [14]. Additionally, the consumption of trans-fatty acids impedes the production of AA, EPA, and DHA by negatively affecting the elongases and desaturases associated with the metabolism of LA and ALA [177]. Though limited, brain, liver, intestinal mucosal, and retina endoplasmic reticulum tissue membrane are the primary sites for both delta-5 and deta-6 desaturases [119]. The main sites of omega-3 and omega-6 fatty acid storage are in the phospholipid layers of cytoplasmic lipid bodies, glycerides, organelle membranes and cell membranes [168].

In a rested state, phospholipids contain more PUFAs than monounsaturated fatty acids (MUFAs), while the opposite is true for triglycerides in humans. Following exercise training, muscle triglyceride content can be elevated with increased total PUFA in the triglyceride by decreasing total MUFA carrying very low density lipoprotein (VLDL) [5, 201, 213]. Nikolaidis and Mougios [141] concluded that the rate of uptake for

unsaturated fatty acids in skeletal muscle is greater than that of saturated fatty acids entering the same tissues during exercise. The effects of acute and chronic exercise on the plasma fatty acid profile have been demonstrated in several studies [83, 86, 128, 201, 213]. Rodent models [8, 9] using chronic exercise have demonstrated effects on adipose tissue such as decreasing delta-9 desaturase activity (lowering MUFA formation) while favoring elongase activity (increasing PUFA formation) in skeletal muscle [141].

The incorporation of PUFA, such as DHA, into erythrocyte cell membranes can be used as a systemic marker of overall fatty acid content in tissues [187], since de novo synthesis of erythrocyte phospholipid membrane free fatty acids does not occur. Thus, phospholipid constituents are collected from plasma lipids to construct the erythrocyte cell membrane [38]. Studies have reported that, within one month of omega-3 supplementation in humans, omega-3 PUFA concentrations reach a steady state when measuring total plasma lipids, thus incorporation into erythrocytes (a FFA systemic composition tissue marker) will have lower kinetics and represent a longer period of membrane structure and composition [111].

Diet has the potential to alter plasma and erythrocyte cell membrane FA composition [149, 187, 207]. Sun and colleagues [187] compared food frequency questionnaires to plasma and erythrocyte FA concentrations of 306 US women aged 43-69 and determined DHA concentrations in erythrocyte and plasma provided the strongest correlations with those who had ingested a diet high in marine foods. Similarly, Patel and colleagues [149] found plasma phospholipid fatty acids were strongly correlated with the reported intake of omega-3 and omega-6 fatty acids in food frequency questionnaires

from diabetic patients. Analyzing 4,902 plasma phospholipid fatty acid samples, in a study comparing consumption of plant-derived ALA intake with intake of fish, Welch and colleagues [207] found an increase in DHA and EPA in the fish-eaters compared to the non-fish-eaters.

Erythrocyte cell membrane FA composition, especially n-3 fatty acids, has been proven to be a reliable detection method for long-term consumption of fatty acids [149, 186, 187]. Though not as highly correlated as adipose tissue samples [63], researchers have shown a higher correlation of erythrocyte PUFA content with intakes based on food frequency questionnaires than with plasma PUFA content [149, 187]. In a population of US women, it was determined that plasma FA composition corresponds with PUFA intake of past weeks, while erythrocyte PUFA content reflects intake of past months [187]. The difference in the ability to detect long-term versus short-term PUFA intake may be due to the fact that erythrocyte half-life is one hundred-twenty days, which is greater than the half-life of circulating plasma lipoprotein [6]. Cao and colleagues [34] reported elevated DHA concentration in erythrocyte cell membranes for as long as 16 weeks post-supplementation of a 2g •day^{-1} fish oil (1296 mg EPA + 864 mg DHA) following 8 weeks of supplementation in a sedentary population; plasma phospholipid DHA concentration decreased sooner than erythrocyte cell membrane DHA concentration once supplementation ceased (within two weeks post-supplementation).

Both exercise and diet may combine to affect plasma lipids and erythrocyte membrane composition. Results of exercise training are inconsistent among populations of sedentary individuals, sprinters, and long-distance runners. When compared, the

sedentary participants and sprinters had higher ratios of saturated to unsaturated fatty acids in erythrocyte cell membranes, while the long-distance runners' unsaturated fatty acids were higher in total PUFA concentrations [96].

Polyunsaturated Fatty Acid Sources and Common Diets

Anthropologists have determined that ancient wild game and livestock had higher omega-3 contents than present day diets. Livestock diets for cattle have changed, causing a shift from omega-3 FA to omega-6 FA in meat, but the food industry is compensating. Supermarkets now carry omega-3-enriched eggs and cereal, as well as DHA-enriched milk. Other sources include walnuts, kale, soy beans, Brussels sprouts, collard greens, winter squash, and tofu. Wild animal carcasses have more total FA, increasing total omega-3 FA than farm raised animals. Differences can be attributed to vegetation available. The major sources of omega-3 fatty acids today in humans are canola oil, flax seed, Salmon, Halibut, and other cold water fish, or direct fish oil supplementation. To avoid concern about mercury and other metal concentrations found in fish, algal (water algae)-based oil supplements come in purified forms. Researchers have suggested that healthy diets should have a 1:1 or 1:2 ratio of omega-3 to omega-6, but ratios common to the typical US diet vary within a 1:6 to a 1:20 range [32, 33]. In 2000, U.S. omega- 3 intake was only 1.6 g•day^{-1} (0.04 g•day^{-1} EPA, 0.07 g•day^{-1} DHA) compared to that of 15.9 g/d omega-6 [53].

The American Heart Association issued recommendations for consumption of omega-3 PUFA [115]. Those individuals without documented coronary heart disease are advised to consume at least two oily fish meals per week [115]. This level of dietary

consumption would provide approximately 500 mg•day^{-1} of combined EPA + DHA [72]. Those with documented coronary heart disease are advised an intake of at least 1 g•day^{-1} of combined EPA+DHA, whether by oily fish or through supplementation [115]. Individuals needing to lower triglycerides are recommended to ingest 2 – 4 g•day^{-1} of combined EPA + DHA [115]. The EPIC-Norfolk cohort stated that vegans and non-fish vegetarians and meat eaters might have slightly different rates of enzymatic processes for DHA formation, and the total EPA and DHA fatty acid membrane incorporation is lower than that of fish or fish oil eaters 5% EPA and 0.5% from DHA [207].

Recommendations for macronutrients in an athlete's diet have varied from expert to expert. In 2009, Wagner [202] noted experts in sports nutrition generally recommend ranges of 55-65% carbohydrate, 15% protein, and the rest of the calories from fat for athletes. Specific to American football, Holway and Spriet [82] reviewed nutritional strategy studies (involving 3-day recall) and found macronutrients for college players to range 39-53% carbohydrate, 16-22% protein, 23-41% fat, with possible variations between positions. There are few data describing specific PUFA amounts in their diet of an athletic population. Associations to a Western diet are usually extended to the American sport athlete, which would be low in PUFA content. Posner [160] reported fat intake for North Americans was approximately 38% of total energy consumed. For the United States, 66% of the fat consumed comes from an animal source, while the rest is from plants [92]. This ratio leads to a diet with higher saturated fat consumption than unsaturated fat consumption. A study by Clark et.al. [40], comparing preseason to postseason eating habits of Division I female soccer players, showed larger amounts of

total fat (grams) consumed at preseason, though percent energy contribution was 2% lower. Additionally, it was noted that even with changes in total caloric intake, carbohydrate intake was still not sufficient for optimizing glycogen stores. Instead, high fat and high protein diets were reported, which were less nutrient dense, in addition to the lower percent carbohydrate intake. [40]

Effects of Polyunsaturated Fatty Acids on Inflammation

Research on polyunsaturated fatty acids role in chronic inflammation and immune response, especially omega-3 fatty acids anti-inflammatory effects, continues to grow [25-32, 36, 59, 97, 132, 162, 209]. Of particular interest is the effect of omega-3 fatty acids on cytokine production and signaling. Cytokines are proteins that mediate both phases of the inflammation process. There are cascades of signaling performed by the cytokines and many times found to be "redundant signaling", as some cytokines share the same the effect [97]. Serving as precursors to cytokines, the formulation and placement of the lipid mediators influences the release and initiation of different granulocyte cytokine signaling [30, 32, 97, 132]. AA competes with EPA and DHA for phospholipid membrane space which can influence inflammatory signaling [28, 29, 119, 177].

Arachidonic acid freed from the cell membrane leads to the development of various types of proinflammatory eicosanoids. AA can be converted by cyclooxygenases (COX-1 & COX-2) into 2-series prostaglandins (i.e. PGE_2) and thromboxanes. AA can also be metabolized by lipoxygenases (15-LOX, 12-LOX, and 5-LOX) to create 4-series leukotrienes and hydroxyeicosatetraenoic acid. [21, 32] During the acute phase, series-2 prostaglandins promote not only neutrophil infiltration, but also

neutrophil phagocytosis [166]. Superoxide anions are byproducts from neutrophils and macrophages breaking down cellular debris [172, 197]. If the intracellular concentration of superoxide anions remains elevated, non-damaged cellular structures will break down which can result in further injury [197].

In contrast, EPA and DHA are metabolized by the cyclooxygenases and lipoxygenases resulting in eicosanoids with either lesser potency or opposite functions than their AA derivative counterparts (2-series prostaglandins) [29]. EPA is converted by COX-2 into 3-series prostaglandins, thromboxanes, and E-series resolvins. Interaction with 5-LOX leads to 5-series leukotrienes. Similarly, DHA can give rise to D-series resolvins and neurprotectins. [25, 32, 97] Refer to Figure 2 for a flow diagram of the two phases of inflammation and the roles of AA, EPA, and DHA.

The following summarizes three possible mechanisms of how DHA influences inflammation: (1) membrane lipid raft theory with cascading effects on selective eicosanoid production opposite that of AA; (2) the use of the PUFA as fuel for neutrophils and leukocytes; and (3) intracellular inhibition of cytokine gene expression [205]. First, the physical cell membrane alteration shifts lipid rafts in such a way that it reduces binding sites for cytokine receptors, thus blocking or inhibiting the receptors [36]. It is also suggested that the unique structure of DHA lends itself to integrating into the cell membrane and changing properties including fluidity, resident protein function, phase behavior, and ion permeability [36]. Additionally, DHA intake can take the place of AA in the phospholipid bilayer of the cell, which reduces the number of pro-inflammatory cascade signaling mediators. Four and a half months of algal DHA has

been shown to increase mean plasma phospholipid DHA by 193% and EPA by 50%, while decreasing AA concentrations by 25% [135]. Some suggest that the need for fatty acids is up-regulated during periods of injury where fatty acids act as an energy source, as well repair mediators [195].

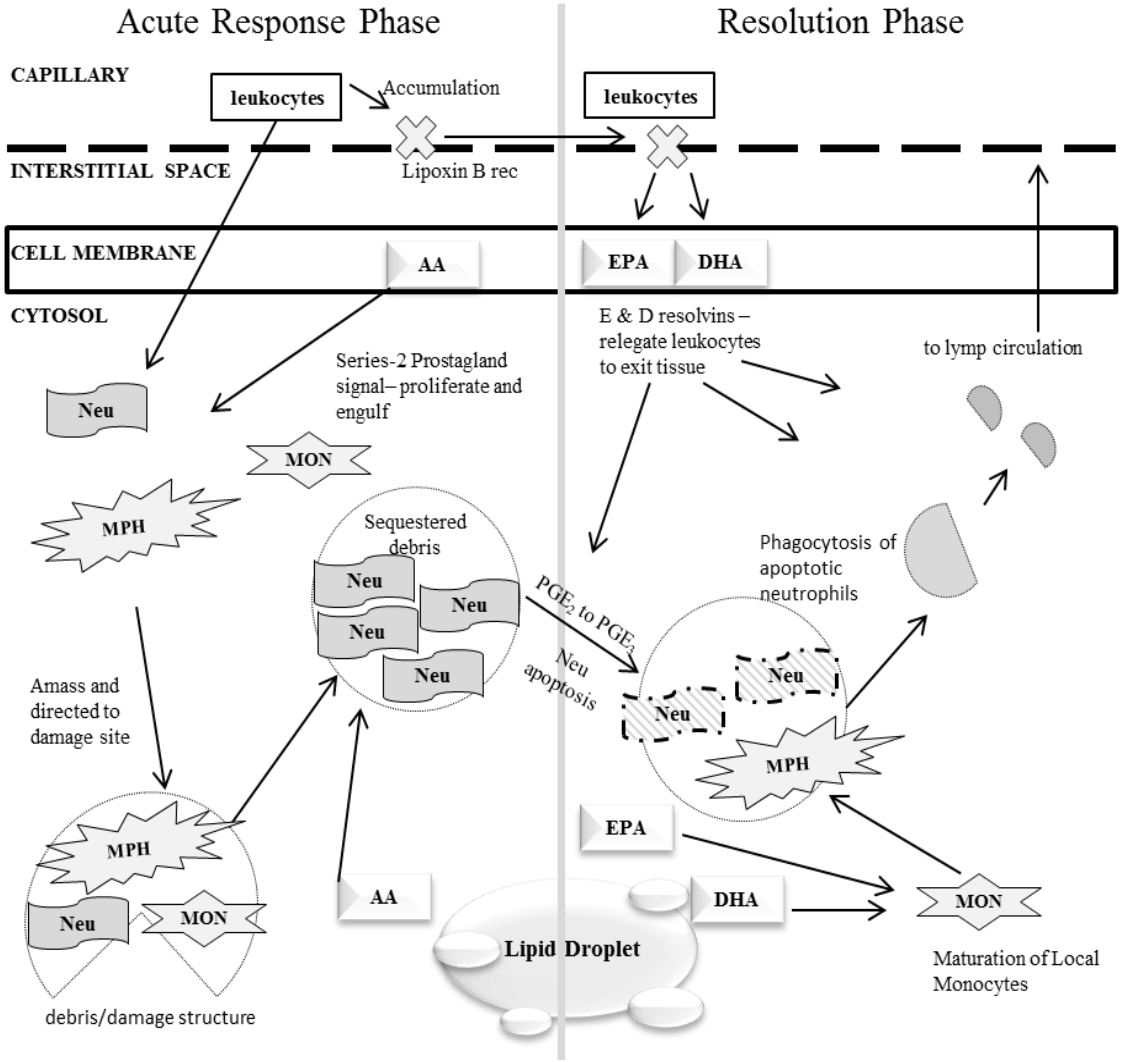


Figure 2. Acute and resolution phases of inflammation. Upon mechanical stress, extracellular leukocytes enter cell near area of damage (acute phase of inflammation). The influx of leukocytes activates the lipopoxin B receptor which works in tangent with omega-3 fatty acids in phospholipid membrane to attenuate the increase in intracellular leukocyte population. Arachidonic acid (AA) derives prostaglandin PGE which signals phagocytosis and endocytosis of debris/damage structures. Once sequestered, neutrophil (Neu) proliferation is disabled and Neu apoptosis is initiated (additionally signaling from intracellular AA). Resolution of inflammation is promoted by prostaglandins and eicosanoid communicators from docosahexaenoic acid (DHA) and eicosapentaenoic acid EPA signal the monocyte (MON) maturation into cell specific macrophages (MPH) to remove apoptotic Neu from damaged site until intracellular leukocyte concentrations return to homeostasis.

As many of these cytokines directly or indirectly affect neutrophil and monocyte/macrophage function, the effect of DHA must be considered when examining the production of free radicals by inflammatory cells. Free radical production in the skeletal muscle serves multiple functions during the inflammatory process [109]. Besides reducing responses to proinflammatory cytokines, DHA and other omega-3 fatty acids have been demonstrated to show reductions in monocyte and neutrophil chemotaxis properties [132]. These free radicals assist in the degradation of damaged cellular material [109]. Moreover, the free radicals act as trigger mechanisms for redox sensitive signaling pathways that promote skeletal muscle hypertrophy [109]. Monocyte/macrophage function during the resolution phase of inflammation functions to induce proliferation and differentiation of skeletal muscle cells; this activation is induced by IFN-γ [109]. Additionally, it is thought that DHA could incorporate into neutrophil membranes at similar rate as in erythrocytes and, in turn, reduce superoxide production [78]. These elevations in macrophage population have usually been reported days after a resistance exercise session [109]. DHA is converted into resolvin D series, as well as protectin D series lipid mediators that promote the resolution phase, working to bring about tissue homeostasis with regards to granulocyte population [172].

Figure 3 diagrams potential key cytokine signaling in skeletal muscle. During the acute phase, monocyte chemoattractant protein-1 (MCP-1) and interferon gamma (IFN- γ), promote the maturation of monocytes into macrophages [173, 185]. Tumor necrosis factor alpha (TNF- α) promotes apoptosis of the sequestered neutrophil and structural debris [33, 145, 184]. Interleukin-6 (IL-6) signaling, during the acute phase,

directs mature macrophages to engulf damaged tissue and recruit more macrophages (173). Interleukin-1 (IL-1) works in conjunction with IL-6 to signal neutrophil migration to macrophage sites [33]. Membrane bound, interleukin-1 beta (IL-1b) stimulates TNF- α to direct post-used neutrophils for apoptosis [33, 173, 185]. To indirectly regulate both the maturation of macrophages and sequestration of leukocytes out of the cell, interleukin-15 (IL-15) influences the balance of IFN- γ and TNF- α [185].

During the resolution phase, interleukin-1 receptor alpha (IL-1ra) inhibits IL-1b and reduces concentrations of TNF- α [33, 145, 184]. IL-6 takes on an anti-inflammatory characteristic, as it promotes the maturation of monocytes to drive the neutrophil remnants out of the cell and into the lymphatic circulation [33, 100, 145, 184]. Interleukin-10 (IL-10) and interleukin-13 (IL-13) suppress macrophage functions which can lead efflux of intracellular leukocyte debris [33]. Eotaxin also influence the efflux of leukocytes out of the cell into circulation [173].

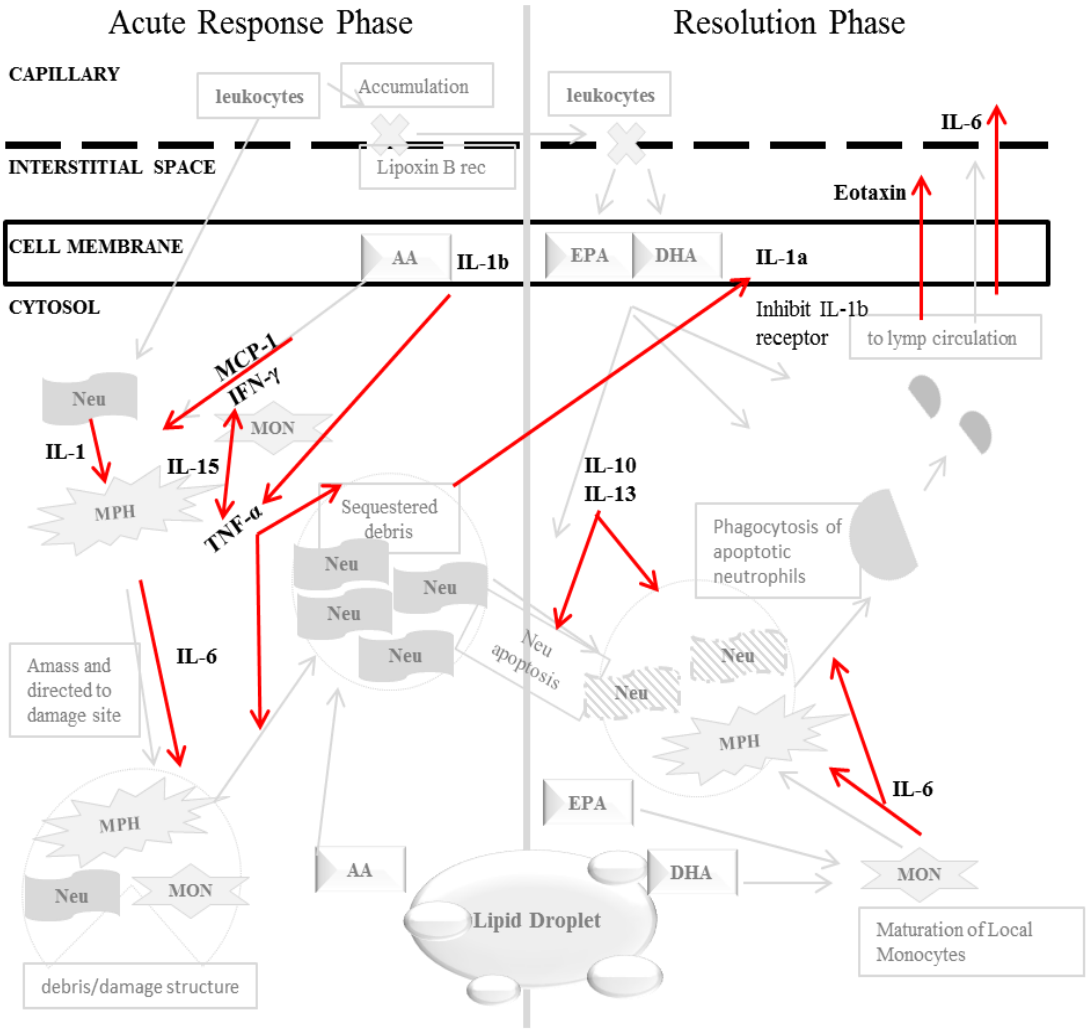


Figure 3. Cytokine signaling of potential key messengers. During the acute phase, monocyte chemoattractant protein-1 (MCP-1) and interferon gamma (IFN- ɣ) promote macrophage formation. Tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) signal the sequestration of cellular debris. Interleukin-1 (IL-1) promotes macrophage formation as well as enhanced IL-6 signaling. TNF- α is up regulated by interleukin-1 beta (IL-1b) and interleukin-15 (IL-15); IL-15 also augments IFN- ɣ. During the resolution phase, Eotaxin and IL-6 promotes efflux of cellular debris. Interleukin-1 receptor alpha (IL-1ra) inhibits IL-1b (initial leukocyte influx). IL-6, interleukin-10 (IL-10), and interleukin-13 (IL-13) signal packaging and phagocytosis of cellular debris.

Lipoproteins, Exercise, and Polyunsaturated Fatty Acids

Lipoproteins. Couriers of cholesterols and triglycerides are known as lipoproteins and are classified by density, which also contributes to their distinct roles in lipid metabolism. Figure 4 depicts a flow schematic for lipoprotein metabolism. Major lipoprotein fractions, from most dense to least dense, include: high density lipoprotein

(HDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), VLDL, and chylomicrons. Part of the identification of each lipoprotein is the type of apolipoprotein associated with it. Apoliporoteins act as regulators to conformation/composition and movement of cholesterol esters and triglycerides for each lipoprotein. Though many forms exist, isoforms of Apolipoprotein A and B are the primary signalers for lipid metabolism [37]. Starting in the gut, apolipoprotein A1 is associated with HDL formation and migration of cholesterol to the liver while Apolipoprotein B48 is the primary signal reference protein of chylomicrons as lipoprotein lipase removes triglycerides for use in peripheral tissues before the remnant chylomicrons reach the liver. Conversely, another form of Apolipoprotein B, B100 is active on lipoproteins leaving the liver, with VLDL leading to IDL to LDL as triglycerides are extracted. [37, 65]

HDL and its subfractions function to return cholesterol (reverse cholesterol transport) from peripheral tissues to the liver and adipose tissue. HDL also works as a reference lipoprotein that can donate cholesterol to chylomicrons, VLDL, IDL, and LDL through the action of cholesterol ester transfer protein (CETP), as well as contribute to coenzymes for membrane receptors for influx of lipids into cells. Key signaling involves communication of the lipoprotein surface proteins, apolipoproteins, and the enzyme lecithin cholesterol acyl transferase (LCAT) to convert cholesterol into cholesterol esters. Nascent HDL-C is initially comprised of apolipoprotein A1, which acts as a receptor to collect cholesterol esters. As nascent HDL-C accumulates more cholesterol esters, the esters are packed tightly by LCAT creating a cholesterol-dense

HDL_3-C in conjunction with ATP binding cassette A1. HDL_3-C via LCAT further incorporates more esters from surrounding tissues creating a less dense (not packed as tightly) but larger HDL_2-C, which is the mature form of HDL-C and more buoyant, which will be transported back to the liver [37, 65, 161]. At this point, HDL-C reverts back to nascent HDL-C and can begin scavenging for cholesterol esters; the cholesterol delivered back to the liver can either be discarded in bile salts through the digestive system or packaged with triglycerides and sent to peripheral tissue in the form of VLDL-C [37].

Lipoprotein lipase (LPL) is used to hydrolyze/breakdown lipoproteins. Bonding to the endothelium, LPL can sequester circulating VLDLs and extract triglycerides through hydrolysis and capillary uptake for storage in adipose tissue as well as muscle, and the remnant chylomicron can be redirected back to the liver and converted to LDL. LPL works similarly in extraction from VLDL to skeletal muscle tissue (triglycerides for storage/energy, such as fatty acids); and extracting phospholipids for membrane repair. Interestingly, absorption of fats by the intestine, and the transportation and delivery of fats to skeletal muscle are both executed primarily by VLDL, and especially chylomicrons [91, 92, 143]. Cholesterol from bile salts (reabsorption), along with dietary forms, can be absorbed through the intestinal lumen and packaged by the endoplasmic reticulum and Golgi apparatus, along with apolipoprotein B48, to form chylomicrons in circulation; the other alternative is packaging of absorbed cholesterols from digested sterols that are then transferred through the ATP binding cassette A1 channel to nascent HDL-C [37]. In combination with CETP, cholesterol collected by HDL-C can not only be

directed to the liver, but also to VLDL-C, which can go directly to peripheral tissue, or be further transferred to IDL-C and LDL-C for peripheral tissue use.

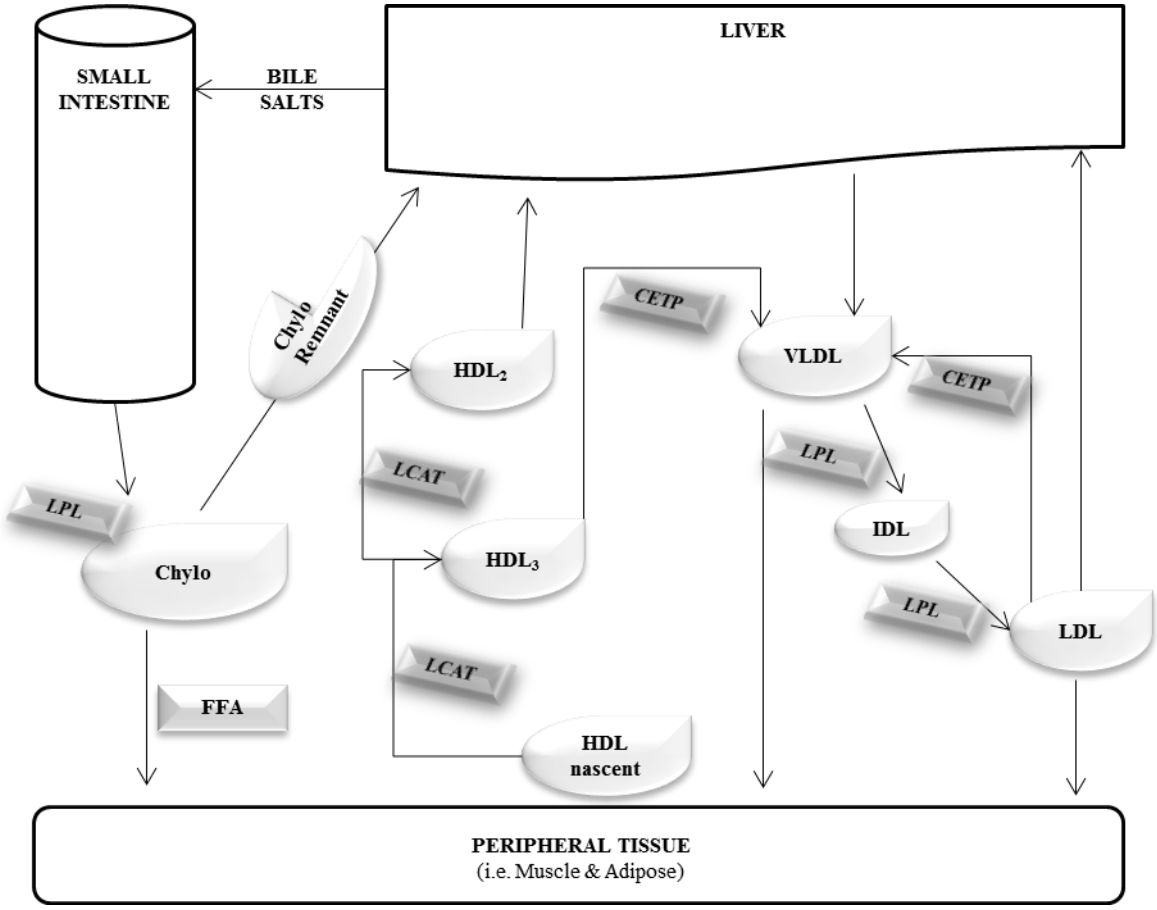


Figure 4. Lipoprotein transport. Chylomicron (Chylo), very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL); high density lipoprotein (HDL). HDL donates apo proteins as well as acts in reverse cholesterol transport. Nascent HDL to HDL_3 to HDL_2 to liver.

Exercise and Lipids. Cardiovascular risk is decreased when individuals engage in habitual exercise [(105)], which is associated with decreased LDL-C and triglycerides as well as increased HDL-C. In general, exercise training has been shown to decrease plasma triglycerides [(105)]. Research using exercise interventions has noted that the

intensity of exercise is not the main determinant in lipid and lipoprotein alterations; exercise volume could cause greater changes [51]. Exercise volume in terms of caloric expenditure with a threshold of 1200-1500 kcal/week seems to be essential for alterations to blood lipid and lipoprotein profiles [51, 58]. Studies using energy expenditures ranging from 1200-2200 kcals per week led to reductions of 5 to 38 mg/dL of triglycerides and increased HDL-C concentrations of 2 to 8 mg/dL [51].

Acute exercise has been demonstrated to reduce triglycerides by 14-50%, while following a regular exercise regime has been reported to have reductions of 4-37% [51]. Acute aerobic exercise increases HDL_3-C content while decreasing triglyceride and phospholipid content [61]. Notably, Crouse et. al. [42] reported an exercise session of 350 kcal expenditure at 50 to 80% VO_{2max} to cause elevations in HDL-C 24 hours post-exercise in an hypercholesterolemic population; suggesting a different starting point(exercise volume) for altering lipoprotein profiles for specific populations. In trained subjects, a single exercise session can elicit 10-25% increases in HDL and reductions in triglycerides [50]. Following a 350 kcal training session, untrained individuals with hypercholesterolemia demonstrated an increase in HDL-C concentration the next day after the exercise session [42]. HDL-C can increase by 4-22% following training with increases ranging from 2-8 mg/dL [51]. Durstine and colleagues [51] found that the potential for HDL alterations is more likely with training greater than 12 weeks. Again, it is theorized that increases in HDL-C are in a dose dependent relation to volume. Ferguson et. al. [58] found that 800 kcal exercise session did not

affect HDL-C and that exercise sessions at 1100 kcal or above resulted in increases of HDL-C 24 hours post exercise in untrained subjects.

The effects of resistance training on blood lipid and lipoprotein concentrations have been mixed [105, 106, 110, 179, 193]. LDL-C reductions, without alterations in triglyceride or HDL-C, have been noted with resistance training [18]. In contrast, other resistance exercise training regimes have shown no change in LDL-C, while HDL-C increased and triglycerides decreased [189].

It is possible that subclasses of LDL-C could be changed with training, while leaving total LDL-C unchanged [188]. Caloric restriction increases LDL large particles while decreasing small particle LDL, demonstrating a change to more buoyant lipoprotein forms [211]. Another exception to this caloric expenditure threshold seems to be VLDL, which is more affected by dietary interventions (such as low fat or calorie deficient), than by an exercise intervention [51].

Several studies have shown there to be no change in cholesterol except when associated with a decrease in percent bodyfat [5, 43, 188]. LPL activity has been reported to be elevated after a variety of acute exercise training regimes [188]. With exercise, LDL particles become richer in cholesterol and increase in size [85]. Exercise reduces LDL mass and increases HDL_2 mass [210]. It has been suggested that LPL activity following training is responsible for the transfer of LDL mass to smaller HDL particles, leading to the larger mass of HDL [105, 113]. Superko [188] suggests the main LDL component being transferred is cholesterol, as LPL activity and HDL cholesterol concentrations have been strongly correlated.

Endurance training has been shown to lead to elevated LCAT activity, but some studies have demonstrated mixed results [123, 192]. The same mixed results apply to CETP, as trained individuals show increased concentrations of CETP, but those who had associative weight loss following a training program had reductions in CETP [67, 171]. Durstine and colleagues [51] concluded that endurance exercise training (whether in cross-sectional or longitudinal studies) results in a decrease in triglyceride concentrations relative to baseline concentrations. Additionally, Durstine and colleagues [51] concluded that endurance exercise training has yet to be shown to alter plasma cholesterol (in absence of dietary intervention), unless prolonged. Figure 5 depicts a flow schematic long chain fatty acid transport and cell membrane integration.

Fat Store Utilization. The fatty acid composition of adipose tissue could affect several systemic functions, as adipose tissue has endocrine and paracrine abilities [141]. Lower body sub-cutaneous adipose tissue has an attenuated lipolytic rate during exercise [7, 84]. It has been suggested that 10-15% of fat oxidation during exercise is derived from intramuscular triglycerides (IMTG) [84]. Extramyocellular triglycerides are considered to be a lesser source of free fatty acids than IMTG, as they are spaced between muscle fibers in relatively small lipid vacuoles [84]. Fat utilization is enhanced following endurance exercise training [81]. This use of fat appears to be attributed to an increase in rate of oxidation, but not mobilization [84]. Diet can affect fatty acid oxidation and liberation from adipose tissue during exercise [84].

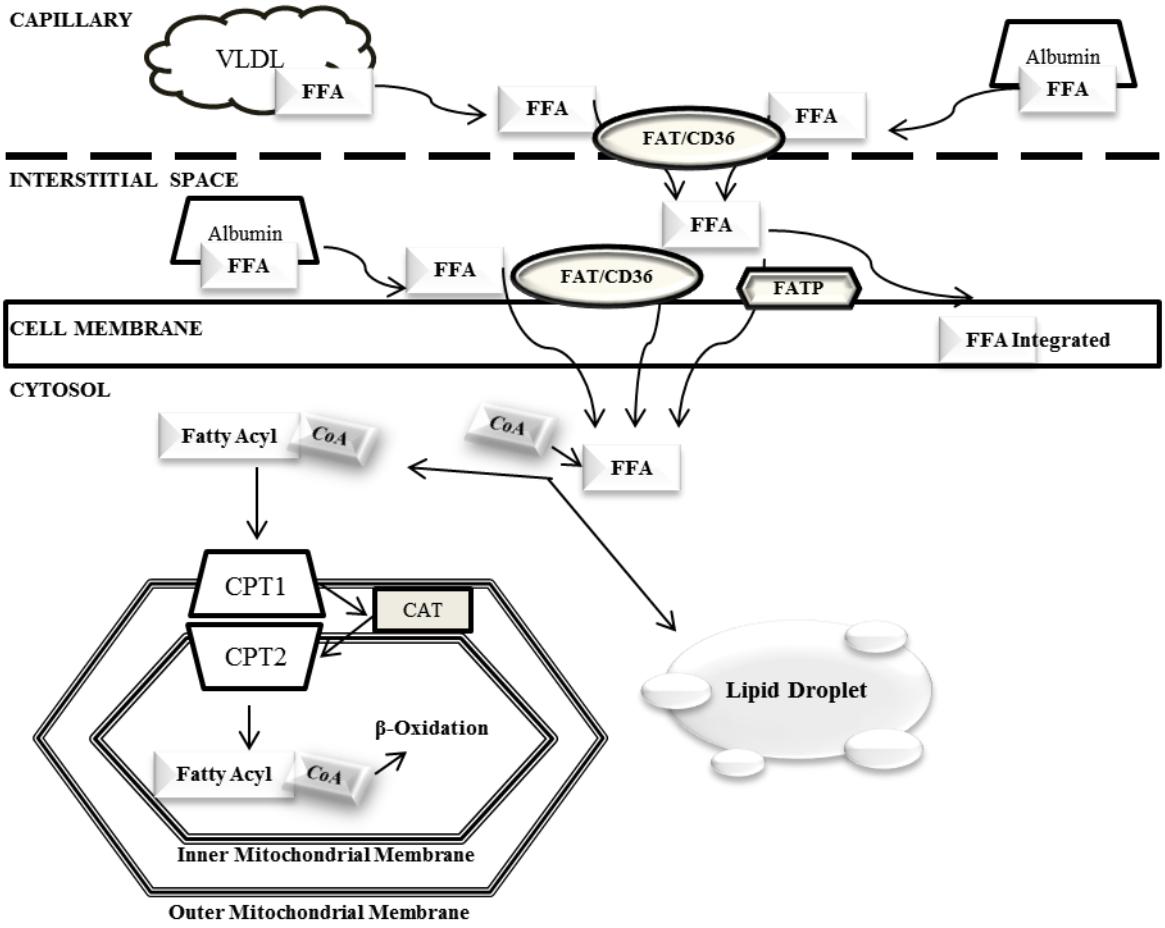


Figure 5. Long chain fatty acid transport and integration into the cell. Hepatic VLDL and albumin bound FFA are transported and received by FAT/CD36 of capillary beds. Within the cellular interstitial space FAT/CD36 as well as FATP transport FFA through cell membrane, during this point FFAs can integrate into cell membrane phospholipid bilayer and act as mediators in the inflammation process. Fatty acids in the cytosol are transferred in the form of fatty acyl-CoA to lipid droplets in the muscle or through a series of shuttling facilitated by CPT1, CAT, and CPT2 to reach the inner mitochondrial membrane for β - oxidation.

Diet and Lipids. Schwingshackl and Hoffmann [170] produced a systemic review and meta-analysis of low-fat, low protein, and high protein diets on metabolic markers including lipoproteins. Notably, the strongest correlation to any alteration in lipoprotein profiles was with high protein diets and increased HDL-C concentrations. Not as clearly elucidated, was the connection between high protein diets and reduced triglyceride

concentrations. Effects or lack of effect on LDL-C and VLDL-C varied greatly from study to study and the authors noted the possible influence of increased dietary fat intake in subjects who eat high protein diets, compared to low protein diets. [170] Miller et. al. [127] compared the Atkins (high fat, low carbohydrate), Mediterranean South Beach (high polyunsaturated fat), and Ornish (low fat, high carbohydrate) diets over a 20-week period (4 weeks of each diet, with a 4 week washout period in-between). Additionally, diets were adjusted weekly to insure no weight gain or weight loss throughout the study. Interestingly, the South Beach and Ornish diets were associated with reductions in total cholesterol and LDL-C, while the Atkins, with its higher saturated fat content, showed increases in both total cholesterol and LDL-C. Moreover, the Atkins diet also resulted in subjects having impaired brachial artery flow-mediated dilation. [127] Grundy and colleagues [66] conducted a shorter, two-week diet intervention with three different diets consisting of high saturated fatty acid and high cholesterol (High Sat+Chol), high monounsaturated fatty acid and low cholesterol (High Mono), and low fat with high carbohydrate (Low Fat). Both Low Fat and High Mono reduced total cholesterol and LDL-C. Low Fat also reduced HDL-C while High Mono maintained HDL-C concentrations, making this diet advantageous to those needing to reduce total cholesterol and LDL-C, while preserving the cardioprotective factor of HDL-C. [66] Besides macronutrient composition, other contributing effects of a dietary intervention are important to consider, for example, whether or not the diet resulted in weight loss of the subjects. The majority of dietary interventional studies conducted that were

associated with beneficial changes in lipoproteins and blood lipids, had either a decrease in body fat and/or body weight [46, 49, 127, 193, 210, 211].

Polyunsaturated Fatty Acids and Exercise. Supplementation of polyunsaturated fatty acids has been linked to positive cardiovascular health outcomes and enhanced fat metabolism, which has the potential to augment the benefits of physical activity, including decreased body fat and oxygen consumption efficiency at submaximal exercise [103, 130, 133, 154, 155, 176, 203]. Additionally, supplementation of omega-3 FAs, specifically DHA and/or EPA, can suppress effects of endogenous omega-6 FAs and compete for positioning on cell membranes, leading to decreased platelet aggregation, increased dilation of blood vessels, and as well as increased circulatory responses, such as blood flow and vascular conductance [23, 131, 196, 203].

Peoples et. al. [155] noted lower heart rate, rate pressure product, and whole body oxygen consumption at submaximal workloads in healthy individuals supplementing with 8 g•day^{-1} fish oil when compared to supplementation with 8 g•day^{-1} olive oil and exercising at similar incremental workloads (up to 55% VO_{2max}). In a follow-up study, Peoples et. al. [154] demonstrated higher oxygen efficiency (less oxygen consumption to maintain similar levels of force and duration until fatigue) in contracted Wistar rat hindlimbs when fed omega-3 FA rich diets, compared to omega-6 FA rich diets. Additionally, Walser and colleagues [204] examined the effects of supplementing 3 g•day^{-1} EPA + 2 g•day^{-1} DHA or safflower oil control over a six week period on vasodilation and blood flow during rhythmic exercise (30% of maximal handgrip tension). Main determinants measured were mean arterial pressure, heart rate, and

brachial artery vascular conductance (diameter and flow). Notably, mean arterial pressure and heart rate were not significantly different between groups, while the EPA+DHA group had significant increases in brachial artery diameter, blood flow, and conductance during exercise. [204] Conclusions from this study suggest increased erythrocyte deformality from the integration of the omega-3 FAs into the erythrocyte cell membrane [144, 196, 204]. This integration of omega-3 FAs reduces the stiffness of the cell, which, in turn, has been linked to a decrease in the peroxidation of membrane lipids and heightened oxygen and nutrient transport during submaximal exercise [196].

The dynamics of the phospholipid layers are determined by fatty acid composition [141]. Such dynamics include the properties of saturated fatty acids, which create a rigid state (tightly-packed hydrocarbon chains) and, with increased unsaturation, MUFAs and PUFAs create a less-dense membrane (fewer hydrophobic attractions) [141]. The longer hydrocarbon chains, such as omega-3 FA, display increased fluidity [141]. Hulbert et al. [87] have suggested a membrane pacemaker theory to describe the effects of metabolic rate, as determined by MUFA and PUFA membrane composition. The pacemaker theory states that a phospholipid membrane that has more unsaturated fatty acids will not only have increased substrate transport, but also increased ionic transfer, resulting in more efficient ionic leak pumps (including Na+ and K+ transfers). The authors suggest that the carbon double bonds of the unsaturated fatty acids found on mitochondrial membranes have a greater energy transfer potential (greater intermolecular collisions) with membrane bound proteins. This translates into a faster energy metabolism compared to membranes that have more saturated fatty acids. [87]

Exercise has the potential to alter systemic free fatty acid composition. During exercise, abdominal sub-cutaneous fat provides the majority of plasma free fatty acids to the skeletal muscle [84]. Cell membrane bound omega-3 FAs have been termed fuel regulators because of the enhanced signaling function to increase lipolysis and b-oxidation, compared to cell membranes predominately comprised of omega-6 FAs [196].

Brilla and Landerholm [20] conducted a 10 week study comparing exercise with supplementation of 4 g•day^{-1} omega-3 FA (from fish oil) and without supplementation on serum lipids and aerobic fitness sedentary males. Following one-hour aerobic exercise bouts, three times per week, there was no difference in lipid profiles or body composition, in comparison to the control group. [20] If omega-3 FAs were to augment the exercise performed, perhaps the exercise volume threshold was not met to elicit changes in lipids and body composition. Interestingly, Warner et. al. [206] reported that after 12 weeks, there was a decrease in percent body fat and an increase in VO_{2max} in hyperlipidemic subjects who ingested 50 mL of fish oil while exercising three times per week for one hour at an intensity of 50-80% VO_{2max}. The group that had the combination of exercise and fish oil had increases in HDL-C and reductions in LDL-C, LDL protein, and apo-B. The fish oil only group did not show the same lipid reductions that the fish oil plus exercise group exhibited, but did have similar increases in HDL-C. [206]

Effects of Omega-3 Fatty Acids on Lipids and Cardiovascular Disease Risk Factors

Evidence for supplementing omega-3 polyunsaturated fatty acids suggests that there are many cardiovascular protective effects. These include anti-atherosclerotic

effects, beneficial circulating lipid and lipoprotein composition changes, and alterations in response to exercise [103, 130, 131, 133, 176]. Some have used red blood cell omega-3 fatty acid content as a variable risk factor for CVD [103]. Moreover, remnant-like particle-cholesterol indexed against ingestion of DHA has been correlated with less atherogenic effects on vessels [103]. Independent lipid risk factors for the development of CVD include elevated total cholesterol and LDL cholesterol, triacylglycerols and low HDL cholesterol, and total and small dense LDL particles [115, 129, 167]. Additionally, unique markers for the identification of risk factors for cardiovascular disease have been adopted. These include a decreased ratio of plasma EPA to AA, increased plasma concentrations of remnant-like particle cholesterol (RLP-C), and a decreased n-3 index (erythrocyte EPA + DHA content) in the red blood cell membranes [103]. The n-3 index is comprised of the sum total of EPA and DHA as a percentage of the total fatty acid content [72]. An n-3 index of < 4% was associated with a 10 fold greater risk of death from coronary heart disease, compared to an n-3 index of > 8% [41]. A reduction in the inflammatory response is correlated with an increase in the ratio EPA to AA [164]. DHA (not EPA) has been linked to decreasing resting blood pressure and heart rate by 5.8/3.3 (systolic/diastolic) mm Hg and 3.7 bpm (daytime), respectively [131]. This same group of researchers determined that there must be endothelium-independent mechanisms that explain the selective blood pressure lowering effects of DHA; as coinfusion of acetylcholine with L-NMMA as well as sodium nitroprusside (both endothelium-independent dilators) had enhanced vasodilatory responses with DHA supplementation [130].

Additionally, supplementing with 4 g•day^{-1} DHA for 6 weeks has led to a 20% reduction in triglycerides, a 29% increase in HDL_2 cholesterol and an 8% increase in LDL cholesterol in mild hyperlipidemic men [133]. At a smaller dosage of 1.52 g•day^{-1} DHA, Maki et. al. [122] were able to demonstrate a reduction in LDL cholesterol carried by small dense particles. Past research has demonstrated small dosages of 0.7 g•day^{-1} DHA to alter resting lipoprotein concentrations [191], and an increased dose of 2 – 4 g•day^{-1} of EPA + DHA has been shown to lower serum triglycerides in a sedentary population [115]. The relatively small dosage of 0.7 g•day^{-1} DHA elevated LDL cholesterol by 7%, but particle size change was not mentioned [191].

Egert et al. [52] conducted a study in which normolipidemic participants were supplemented with either ALA (4.4 g•day^{-1}), EPA (2.2 g•day^{-1}), or DHA (2.3 g•day^{-1}). Fasting serum triacylglycerol concentrations decreased significantly in each of the three interventions, EPA (-0.14 mmol/L), DHA (-0.30 mmol/L), and ALA (-0.17 mmol/L). DHA intake increased serum HDL cholesterol, in contrast to ALA and EPA intake which had no effect on HDL. It was reported that possible interconversion of DHA to EPA led to differential enrichment in LDL due to isolated dietary EPA, ALA, and DHA intakes. [52]

In a study of hypertriglyceridemic men, Kelley et al. [104] found that supplementation with DHA (3 g•day^{-1}) lowered fasting and postprandial triacylglycerol concentrations by 25-30%. Additionally, the n-3 index was increased, while lowering RLP-C, following the DHA supplementation for 90 days [103]. RLP-C concentrations, especially those triglyceride-rich, have been associated with increased atherogenic risk

(atherosclerosis) [103, 104]. Mechanisms proposed for these changes by omega-3 FA in RLP-C include: improving vascular reactivity, altering inflammation status, and decreasing platelet aggregation [115]. Harris [73] suggested the additional intake of omega-3 fatty acids could affect hepatic triglyceride metabolism through the attenuation of hormone-sensitive lipase, acetyl-CoA carboxylase FA synthase, while enhancing mitochondrial and peroxisomal β-oxidation. Park [148] demonstrated that intake of 4 $g \cdot day^{-1}$ of either DHA or EPA resulted in increased chylomicron triglyceride clearance via increased LPL activity causing smaller chylomicron particle sizes. For the athletic population, this translates into a possible greater availability of energy which could lead to better endurance or recovery.

Implications

The published literature mentioned above supports the assertion that the supplementation of DHA may attenuate inflammation and improve lipid profiles during strenuous training. The specific aims will develop a model to explore the effects of incorporation of 2 $g \cdot day^{-1}$ algal DHA during voluntary summer training camp through the more rigorous (higher work volume) preseason training camp in Division I football players. The underlying goal is to identify whether algal DHA does decrease or modulate inflammatory markers that would be elevated during preseason camp training. The modulation of inflammatory markers could reduce the risk of over-inflammation or chronic inflammation. Additionally, lipid profiles may improve with algal DHA supplementation in the football players, leading to reduced cardiovascular risk.

Purpose & Specific Aims

The central purpose of this study is to determine if DHA supplementation in American football athletes impacts lipid profiles, leukocyte counts, and inflammation markers.

Specific Aim #1 (Chapter II) – Inflammation and Immune Function. Hypothesis: Pro-inflammatory cytokine concentrations will be attenuated and leukocyte counts (neutrophil, monocyte, macrophage) will be suppressed with 2 $g \bullet day^{-1}$ DHA supplementation in collegiate Division I American football athletes during voluntary summer training through preseason training camp.

Specific Aim #2 (Chapter III) – Lipids and Cardiovascular Disease. Hypothesis: With the potential benefits of DHA improving blood lipid profiles, it is hypothesized that 2 $g \bullet day^{-1}$ DHA will reduce cardiovascular disease risk by modifying lipid profiles including increased HDL-C (specifically HDL_2-C) and decreased triglycerides as well as LDL-C in collegiate Division I American football athletes during preseason camp.

CHAPTER II

EFFECTS OF DOCOSAHEXAENOIC ACID ON INFLAMMATORY MARKERS IN DIVISION I FOOTBALL PLAYERS DURING HEAVY PHYSICAL TRAINING

Introduction

Regulation and modification of the inflammatory response to exercise has the potential to accelerate an athlete's recovery and enhance adaptation to the physical stress they encounter. Inflammatory responses can occur in the absence of overt injury in skeletal muscle. Regular exercise training can lead to a balance of inflammatory and anti-inflammatory responses [57]. The initial response of inflammation is necessary for repair and has the potential to effect muscle adaptation [109, 120, 145]. Acute exercise, more specifically mechanical loading of skeletal muscle, can activate the inflammation cascade of cytokine signaling and granulocyte accretion as a normal part of the response to exercise [109, 172]. Moderate exercise training has been linked to a reduction in illness and increased resistance to minor infections [152]. In the well-rested athlete, resting immune function concentrations are similar to those of a non-athlete, with the exception that athletes demonstrate higher natural killer cell activity, and slightly lower neutrophil activity [152]. However, intensive exercise with absence of sufficient regenerative time can lead to transient immunosuppression, which increases risk for infection [57]. An increase in training volume with a concomitant decrease in performance, known as overtraining,[180] includes the accumulation of non-training stressors shown in

physiological symptoms such as: decreased muscular strength, decreased work capacity maximums, loss of coordination, persistent high fatigue ratings, decrease in the quality of sleep, and/or decreased maximal heart rate [69, 114, 121, 180]. Delayed recovery from intensive physical activity may lead to an over-trained state, which may be associated with prolonged or unresolved inflammation. From an athletic perspective, inflammation is an essential part of the training and preparation that all athletes undertake to improve performance [109]. The acute response phase is necessary for repair and has the potential to effect muscle adaptation [109, 120, 145].

Immune system regulation may be of particular concern to American collegiate football athletes during their preseason of repetitive, intensive practice sessions with minimal recovery. American college football has been described as a sport characterized by short rest periods with intervals of high-intensity exertion which, when coupled with environmental stress, cause heavy physiological strain [47, 89]. The intensity of preseason football camp is evidenced by the Hoffman et. al. [80] report of elevated creatine kinase concentrations, an important marker of skeletal muscle damage, ten days following preseason camp training.

Various dietary interventions have been explored in an attempt to find ways to regulate inflammation [26]. Interventional compounds include polyunsaturated fatty acids (PUFAs), fish oils, quercetin, carbohydrate isoforms, antioxidants, nitrites, and bicarbonates [26, 92, 136, 139]. In this regard, the role of PUFAs in acute and chronic inflammatory responses, especially the role of omega-3 fatty acids (FA) as an anti-inflammatory agent, continues to gain attention among researchers [25-32, 36, 59, 97, 132, 162,

[209]. Of particular interest is the effect of omega-3 FAs on cytokine production and signaling. Cytokines mediate both the initiation and resolution phases of the inflammatory process. Both phases are essential for cellular repair, growth, and adaptation [109]; but a limiting of repair/adaptation occurs if the system remains in the initiation phase and the progression into the resolution phase is attenuated [118, 172]. Furthermore, omega-3 FAs have a basis in animal models for acting as neural protective/recovery agents following head trauma, like concussion [10, 217], partly stemming from anti-inflammatory compounds produced from docosahexaenoic acid (DHA)(22:6n-3), such as D-series resolvins and neuroprotectins [25, 32, 97]. The most abundant omega-3 FAs comprising marine oil are eicosapentaenoic acid (EPA)(20:5n-3) and DHA. DHA acts as a precursor to specific eicosanoids that function as lipid mediators for the production of cytokines.

Relatively fewer studies have examined the impact of DHA and EPA supplementation on inflammation associated with exercise, when compared to studies examining the more generalized health benefits of omega-3 FA. Jouris et al. [93] reported that arm circumference swelling and soreness were decreased after eccentric exercise following only a week of supplementing with 3 $g \cdot day^{-1}$ fish oil (2g EPA and 1g DHA). Additionally, six weeks of supplementation with 4.5 $g \cdot day^{-1}$ (half EPA, half DHA) lowered resting tumor necrosis factor alpha (TNF-α) and C-reactive protein concentrations in non-athlete, exercised-trained men [16].

Furthermore, there is research on the impact of DHA alone, without EPA, on inflammation [44, 45, 102, 219]. Zhao et. al. [219] demonstrated significant decreases of

proinflammatory cytokines interleukin – 6 (IL-6), interleukin – 1 beta (IL-1β), and TNF-α in human monocytic THP-1 cells when incubated with DHA. De Caterina et. al. [44, 45] found decreased leukocyte adhesion molecules/mediator signaling and cytokine signaling on adult human saphenous vein endothelial cells, notably IL-6, interleukin-8 (IL-8), and TNF-α. Exercise was not a part of these inflammation studies.

The effects of supplementing with DHA as an exercise anti-inflammatory compound, in absence of EPA, remains to be fully elucidated. Kelley et. al. [102] found that in young, healthy men, supplementation with 6 g•day^{-1} DHA, over 90 days, resulted in decreased natural killer cell activity, as well as reduced proinflammatory prostaglandin E2 and leukotriene B4 production. This reduction could be beneficial to those with chronic inflammatory diseases. Additionally, Hill et. al. [79] reported overweight and obese subjects, with at least one additional cardiovascular risk factor, who consumed 6 g•day^{-1} fish oil (with 2 g n-3 PUFA, 1.6 g DHA) had effective suppression of neutrophil chemotaxis and adherence over the course of 12 weeks; which emulated the results of 12 weeks of moderate aerobic exercise. One study did note no difference with DHA supplementation on neutrophil function, with eight weeks of training, in volunteer soccer players; though they were also consuming a Mediterranean-type diet during the study [124].

To the authors' knowledge, there is no research published to date examining the putative anti-inflammatory effects of algal DHA supplementation during intense exercise training in Division I football athletes. Our objective was to document the effects of DHA on cytokine concentrations and immune markers in American collegiate football

players during the period of voluntary summer training through preseason training camp. Common consumer dosages have ranged from 1-4 g of fish oil (with varying EPA to DHA ratios) as a possible health prophylactic. We hypothesized supplementation of 2 $g \bullet day^{-1}$ algal DHA would act as a chronic anti-inflammatory agent and reduce overall inflammation, as marked by either no-change or decreased pro-inflammatory cytokine and white blood cell concentrations.

Methods

Study Design and Dietary Intervention. Approval for this double-blind, placebo controlled study was given by the university Institutional Review Board for Research with Human Subjects. Sixty male football athletes were recruited for participation from the student-athlete members of a NCAA Division I football team. Subjects abstained from long-term anti-inflammatory or anti-hypertensive drug therapy. Volunteers were randomly assigned (double blinded) to two groups, ingesting either 2 $g \bullet day^{-1}$ of algal DHA (n = 28) or a corn oil placebo (n = 32) (DSM, Columbia, MD). During the supplementation, three measurement time points were established: the beginning of voluntary summer training (Summer), approximately 30 days after the beginning of Summer coinciding with the start of mandatory preseason training camp (Pre-camp) in August, and at the end of preseason training camp (Post-camp), 24 days after Pre-camp.

Participant Exclusion. To reduce potential outside sources of omega-3 ingestion, candidates for the study were excluded either by: 1) consumption of more than two servings of fish per week, or supplementation of omega-3 fatty acids and/or fish oil or 2) injury or the diagnosis of a concussion during the study, leading to reduced physical

activity. Figure 6 (consort diagram) provides participant distribution and exclusion from final analysis for the study. Data from forty-three subjects were used for analysis (age = 20 ± 1.5 years, height = 187.4 ± 6.1 cm, weight = 105.7 ± 18.9 kg), demographics are shown in Table 1.

TABLE 1. Subject demographics

	Placebo	DHA
N	21	22
Age (yrs)	20.1 ± 1.4	20.5 ± 1.6
Height (cm)	186.6 ± 6.9	188.1±5.2
Weight (kg)	104.0 ± 18.1	107.3 ± 20.0
BMI (kg/m^2)	29.7 ± 3.9	30.2 ± 4.8

Baseline at beginning of summer; No significant differences between groups. Values are mean ± SD.

Supplementation Protocol. Supplement compliance during the study was monitored by research staff member observation of the consumption of capsules, following weekday strength and conditioning training sessions. For weekend supplementation, packets containing the prescribed capsules were sent home with the athlete and the packets were collected the following Monday and counted for capsule ingestion. During this Summer to Pre-camp training, table snacks (i.e. fruits, nuts, protein/cereal bars) were available only before and after conditioning and strength training sessions, with the athlete being responsible for other food throughout the day. Preseason camp dinners and training table snacks (post workout) were provided in accordance with NCAA guidelines. Supplements were distributed and consumed at preseason camp dinners under visual supervision of research staff. Athletes were instructed not to consume more

than one serving of fish per week for the entire duration of the study. A registered dietician conducted a 24-hour dietary recall at each time point to ensure accuracy, and dietary data was analyzed with Nutribase 8.0 software [142].

Physical Training. One to three voluntary strength training and one to two anaerobic conditioning sessions were performed by the football players each week, as directed by their strength and conditioning coach, during the Summer to Pre-camp period. Conditioning sessions were approximately 0.75 hours, usually following a one hour strength training session. Preseason camp (Pre-camp to Post-camp) introduced the athletes to two-a-day, or multiple practice and training sessions per day, conforming to NCAA limitations [134]. The frequency and volume of strength training and conditioning sessions were increased, while incorporating agility, sport-specific and position-specific practices. Comparable to a study of similar training in college football players [80], preseason camp training in our study was deemed, by coaching and research staff, to be high intensity and high volume, resulting in a high workload.

Blood Sampling and Preparation. Blood was collected at each time point following at least eight hours of overnight fasting and prior to any exercise. While seated, one 8.5 mL serum vacutainer with a clot activator, and one 4 mL whole blood (2K EDTA) vacutainer were drawn from an antecubital vein. Samples were placed into ice, allowed to clot for 30 minutes, followed by refrigerated centrifugation for 30 minutes (2,000 x G) for serum separation. One mL aliquots of serum were then frozen at -80 ° C until cytokine assays could be performed. The 4 mL (2K EDTA) vacutainer of whole blood was sent to St. Joseph's Hospital (Bryan, TX) for CBC analysis by complementing

optical and hemoglobin flow cell with impedance transducer measurements (Abbot CELL-DYN Sapphire, Abbot Diagnostics, Abbott Park, IL).

Measurement of Cytokines. Assessment of cytokines from plasma was performed with two custom Milliplex multiplex cytokine assay kits (MPXHCYTO, EMD Millipore, Darmstadt, Germany). One set of kits was used to detect concentrations of monokine induced by gamma interferon (MIG) while another set of kits was used to detect 23 other cytokines: IL-1β, IL-1ra, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-13, IL-15, IL-17, TNF-α, IFN-α, IFN-γ, GM-CSF, MIP-1 α, MIP-1β, IP-10, Eotaxin, RANTES, MCP-1. All samples were run in triplicate for each respective time point and followed manufacturer's guidelines and averages were assessed. Inter-assay variation was controlled by having all triplicates and time points for the subject on the same assay plate (account for intra-assay variation), analyzed with a Luminex 200 (Luminex Corporation, Austin, TX). Quality controls were added to each plate along with serum matrix for each well following the cytokine multiplex manufacturer's instructions. Cytokine standards were made of increasing concentrations: 3.2, 16, 80, 400, 2000 pg/ml.

Statistical Analysis. For analyses, difference scores were first calculated for each athlete and for each variable as; 1) Pre-camp minus Summer, and 2) Post-camp minus Summer. These calculated difference scores were subsequently converted to percent changes from Summer, then analyzed via one sample t-tests ($\alpha = 0.05$) to determine if the percent changes within group were significantly different from zero. Percent change differences between the DHA and Placebo groups were determined by independent t-tests at Pre-

camp and Post-camp. Independent t-tests were used to determine if there was a difference between groups' baseline values at Summer.

Results

The percent changes from Summer and the average values for the cytokines detected are listed in Table 2. Only data for Eotaxin, IL-6, IL-8, IL-17, RANTES, MCP-1, IFN-γ, and TNF-alpha could be used, due to low detection of the other cytokines analyzed. Inter-assay and intra-assay percent coefficient of variation for these cytokines are as follows (inter-assay, intra-assay): Eotaxin (28%, 20%), IL-6 (39%, 27%), IL-8 (23%, 24%), IL-17 (30%, 22%), RANTES (33%, 14%), MCP-1 (30%, 21%), IFN-γ (31%, 22%), and TNF-alpha (25%, 19%). Comparison of similar assay kits using Luminex-based bead analysis have shown that with multiple bead analytes per well (13 or more), replicate variation coefficients ranged from 18-44% for many analytes, as well as at least three analytes were undetectable in serum samples [214]. There were no differences detected in the initial cytokine concentrations between the DHA and Placebo groups at Summer (Table 2).

Percent change from Summer increased at Pre-camp and Post-camp for all cytokines measured in the Placebo group, but reached statistical significance only for Eotaxin, MCP-1, and RANTES (Figures 7, 9, 10). The increase in the DHA group for IFN-γ reached significance at Post-camp, and for RANTES at both Pre-camp and Post-camp (Figures 8 and 10).

By comparison, the Placebo group exhibited a significantly higher percent change in Eotaxin than the DHA group at Pre-camp ($P<0.05$) (Fig. 7). In addition, the

percent change in MCP-1 concentrations were higher at Pre-camp ($P<0.05$) and Post-camp ($P<0.05$) in the Placebo group compared with the DHA group (Fig. 9). Overall, there was a clear trend of a blunting effect in the DHA group as RANTES percent change increased by 54.0% and 48.6% compared to placebo's 117.1% and 97.0%, respectively (Table 2 and Fig. 10). Though statistical significance was not met for each analyte, the percent change of cytokine concentrations in the Placebo were greater by 1.7 to 14.0 fold at Pre-camp as well as 1.9 to 8.9 fold at Post-camp, compared to the DHA group.

There were no treatment effects detected for percent neutrophils, percent lymphocytes, or percent monocytes. The percent change of white blood cells was significantly elevated at Pre-camp by 12.3% in the DHA group, compared to 6.8% in the Placebo group. Post-camp percent white blood cells were noted to have significant decreases in both DHA and Placebo group from Summer (11.1% and 11.9%, respectively).

TABLE 2. Immune and inflammatory markers

	Time Point			Percent Change from Summer	
	Summer	Pre-Camp	Post-Camp	Pre-Camp	Post-Camp
Eotaxin					
DHA	55.4 ± 5.0	58.7 ± 5.7	55.8 ± 4.5	7 ± 6	8 ± 8
Placebo	41.0 ± 6.3	53.7 ± 7.4	50.3 ± 6.6	57 ± 24 *†	44 ± 16 *
IFN-γ					
DHA	49.3 ± 16.0	39.1 ± 12.2	51.0 ± 13.1	29 ± 25	71 ± 27 *
Placebo	36.7 ± 12.2	68.6 ± 29.0	69.5 ± 24.0	372 ± 270	307 ± 217
IL-6					
DHA	39.5 ± 15.5	26.2 ± 9.9	23.6 ± 7.6	130 ± 83	112 ± 64
Placebo	27.0 ± 8.7	28.9 ± 11.7	32.0 ± 10.3	219 ± 171	217 ± 134
IL-8					
DHA	27.7 ± 7.1	19.9 ± 4.1	21.7 ± 4.2	20 ± 21	70 ± 52
Placebo	29.5 ± 11.1	40.3 ± 15.0	38.2 ± 12.9	136 ± 72	135 ± 66
IL-17					
DHA	21.8 ± 7.0	13.2 ± 3.5	14.6 ± 4.2	18 ± 21	121 ± 108
Placebo	33.9 ± 15.6	52.9 ± 26.5	42.2 ± 16.2	252 ± 148	227 ± 132
MCP-1					
DHA	262.3 ± 30.8	291.2 ± 32.0	239.0 ± 13.7	23 ± 11.43	5 ± 8
Placebo	190.7 ± 17.6	300.9 ± 24.8	239.1 ± 16.4	74 ± 18 *†	40 ± 13 *†
RANTES					
DHA	3247.0 ± 444.4	3614.6 ± 392.0	3599.9 ± 436.4	54 ± 26 *	49 ± 23 *
Placebo	3069.7 ± 556.3	4503.8 ± 500.0	4102.3 ± 499.4	117 ± 34 *	97 ± 36 *
TNF-α					
DHA	7.7 ± 1.2	7.4 ± 0.8	7.8 ± 0.8	4 ± 6	15 ± 10
Placebo	7.1 ± 1.9	8.0 ± 1.7	9.2 ± 3.0	38 ± 23	41 ± 21

Cytokine Placebo N=21, DHA N=22; except for RANTES Placebo N=20. Percent Change from Summer calculated from individual scores. Time Point Cytokine - (pg/ml ± SEM). * denotes P<0.05 % percent change from Summer; † denotes P<0.05 percent change difference between DHA and Placebo.

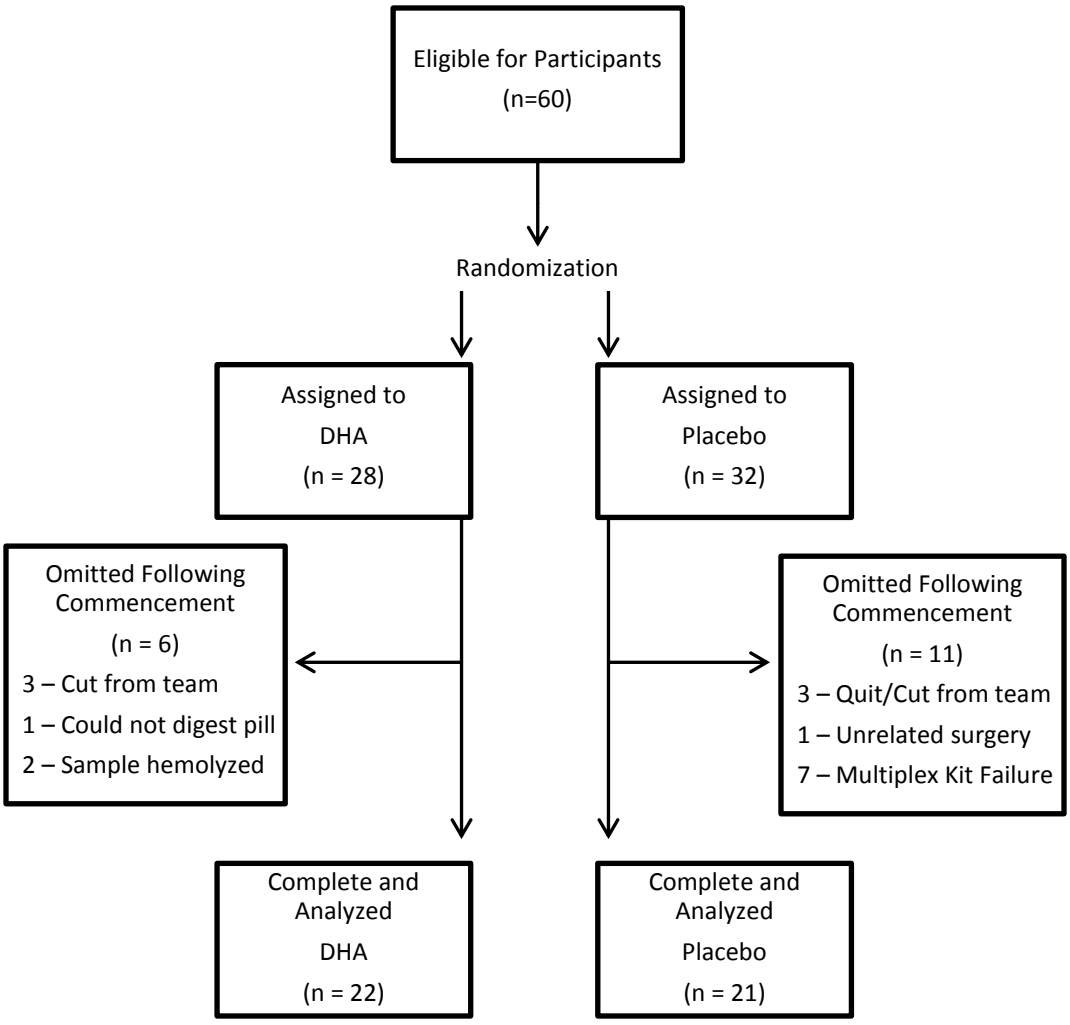


Figure 6. Study consort diagram.

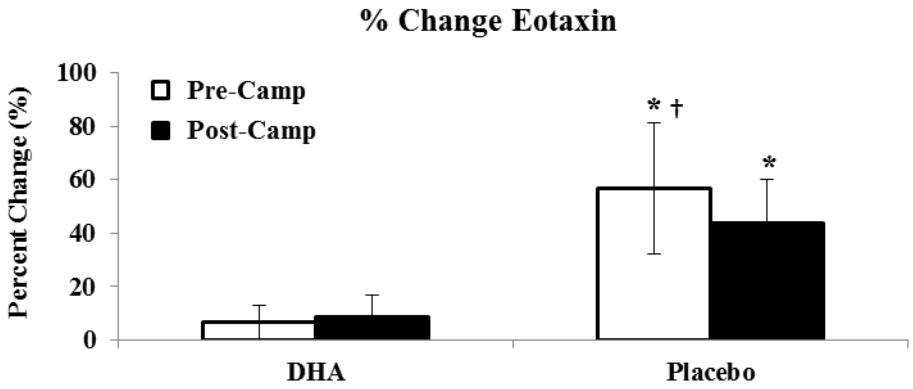


Figure 7. Change serum Eotaxin concentration. * Placebo Pre-camp P=0.030; Post-camp P=0.015. † DHA vs Placebo Pre-camp P=0.049 Placebo (N=21), DHA (N=22).

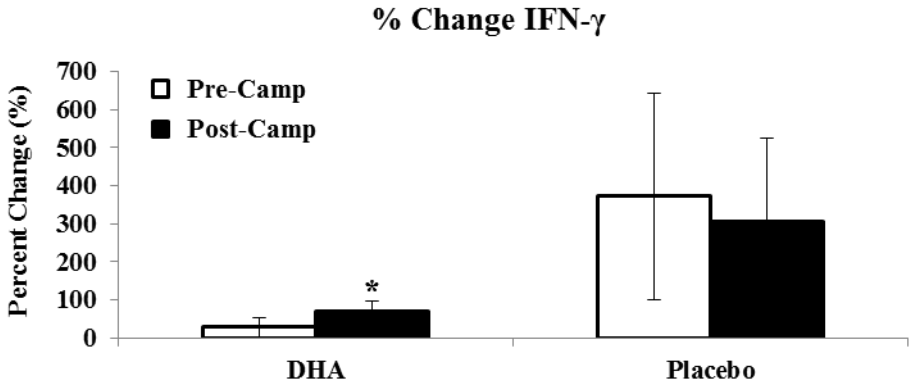


Figure 8. Change serum Interferon-gamma concentration. * DHA Post-camp P=0.015. Placebo (N=21), DHA (N=22).

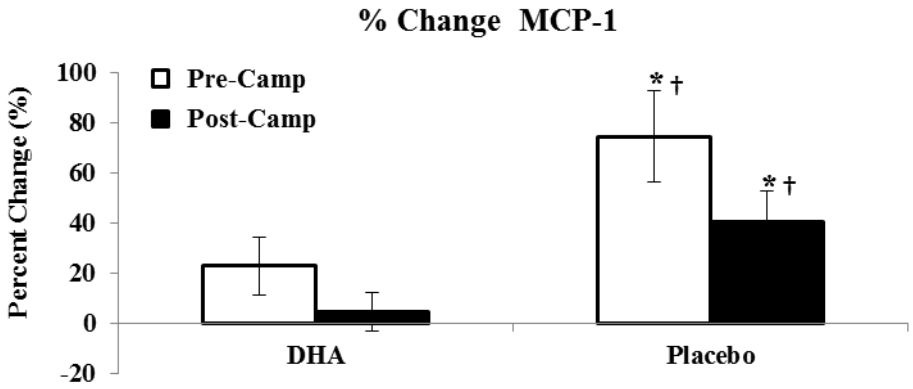


Figure 9. Change serum MCP-1 concentration. * Placebo Pre-camp P<0.001; Post-camp P=0.005. † DHA vs Placebo Pre-camp P=0.019; Post-camp P=0.016. Placebo (N=21), DHA (N=22).

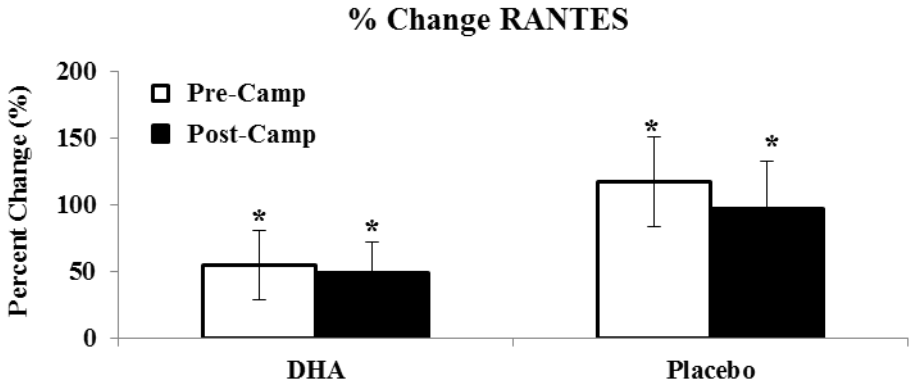


Figure 10. Change serum RANTES concentration. * DHA and Placebo Pre-camp and Post-camp P<0.05. Placebo (N=20), DHA (N=22).

Discussion

This is the first study to examine the possible anti-inflammatory effects of 2 g•day^{-1} algal DHA in Division I football athletes during a period of intense training. Results indicate that athletes are more susceptible to higher elevations in inflammatory

cytokine markers during preseason camp, which is likely related to this increased workload. In comparison to cytokine concentrations measured in healthy adults ≥ 18 years-old [108], our study had lower baseline values for IFN- γ, RANTES, and Eotaxin while MCP-1 concentrations in both groups were at least 4.6 times greater. This difference in baseline could be due to differences in training experience and training status. Our subjects' inflammatory baseline values may be explained by previous training or stimulus, compared to healthy adults that may not undergo the same amount or type of training experienced by an athlete. It should be noted that the healthy adult study was comparing age effects on cytokine concentrations; the ≥ 18 years-old group was comprised of 9 males and 26 females and had a median age of 36 years, with minimum and maximum ages at 21 and 86, respectively [108]. A population with a higher median age has the potential to have age-related, chronic low-grade inflammation which could create higher baseline cytokine concentrations than those in our study population [22].

Eotaxin and MCP-1 significantly increased in the placebo group while the anti-inflammatory effects of DHA were demonstrated by maintaining concentrations similar to Summer baseline (Fig. 7 and 9). The percent change in both cytokines was lower in the DHA group than in the Placebo group, but only MCP-1 was significantly lower at Post-camp compared to the Placebo group. Elevated MCP-1 has been associated with the formation of atherosclerotic lesions; the reduction in the DHA group may be cardioprotective [218]. Eotaxin reductions have been associated with exercise training, while elevations in eotaxin have been strongly correlated with obesity and/or allergen-

induced asthma [39]. For other cytokine markers, a trend existed in which DHA appeared to attenuate cytokine concentrations.

Chronic high-intensity training, as occurs in football, could lead to a prolonged initial inflammation phase [180, 182]. When overtraining occurs, athletes have the potential to remain in the initial inflammation phase, and are slow to progress into the resolution phase [121, 180, 181]. If numerous cytokine concentrations remain elevated, it may indicate that the body is in a state of repair and has not yet recovered, or that the body is under constant stress (not yet reaching homeostasis or baseline concentrations) [109, 180]. In our study, the increased workload from Summer baseline through Post-camp has the potential elevate pro-inflammatory cytokine concentrations.

IL-17 can induce expressions of IL-6 and RANTES, directly or indirectly, through vascular endothelial cells and migrating fibroblasts [185]. Though statistical significance was not met, the percent change of IL-17 from Summer to Pre-camp for the DHA group was minimal compared to Placebo group (Table 2). In comparison, a two-hour exercise bout at 60% VO_{2max} on a cycle ergometer has been shown to elicit significant increases in both IL-6 and TNF-alpha [19]. Similarly, in our study, the increased workload from summer to preseason camp demonstrated a trend of higher concentrations of TNF-α (more pro-inflammatory) for the Placebo group compared to the DHA group (though not significant). Additionally, it should be noted that blood samples were collected at least 12 hours following training sessions -- this extended elevation post-exercise represents prolonged inflammation. Studies on acute moderate aerobic bouts, as well as acute moderate resistance exercise bouts, usually see reductions

in the elevated cytokine concentrations such as IL-6 and TNF-alpha within three to six hours [99].

Other researchers note that chronic elevations of IL-6, and C-reactive protein could be a feedback result of increased systemic TNF-α production [147, 180, 184]. During the initiation phase, IL-6 is pro-inflammatory, as it stimulates production of TNF-α; while in the resolution phase, it inhibits the endotoxin-induced TNF-α production [57]. The placebo group had a steady increase in IL-6 over the course of study, almost doubling the percent change of the DHA group (Table 2). IL-6 is known as a myokine and has been reported to affect the sensation of fatigue (increased IL-6 correlating with increased fatigue) during and following exercise. The more contractions or time under tension of the exercised muscle correlates with increased expressions of IL-6 [100]. The metabolic status of the exercised muscle can determine the effectiveness of IL-6 being released [57, 156]. Elevations in IL-6 are thought to be the result of the influx of calcium ions from the sarcoplasmic reticulum [55, 100]. Glycogen content of the exercised muscle determines the potency at which IL-6 influences lipolysis and lipid oxidation [55, 156]. Additional factors which can influence IL-6 expression include exercise intensity, duration, and mass of muscle recruited. This response following exercise has been shown even without muscle damage. [156]

Researchers have demonstrated that exercised-trained (regularly aerobic and resistance trained) men who ingested 2.2 g•day^{-1} EPA and 2.2 g•day^{-1} DHA had lower resting TNF-α and C-reactive protein concentrations following six weeks of supplementation. Conversely, the supplementation was not effective in moderating

similar cytokine concentrations during low intensity exercise training. [16] Those who undertake excessive exercise or long durational exercise (e.g. marathon or triathlon) might benefit from the anti-inflammatory effects of DHA [16, 17]. Since training and competition in American football has higher intensities and varying volume compared to a recreational population, and potentially different workloads from an endurance-trained population, additional research into appropriate dosages of DHA for football athletes is needed.

There are confounding factors to consider in this study and future measurements of inflammation in football athletes. In a 2000 review [151] of cytokines and exercise, it was noted that the magnitude and variation of cytokine concentrations can be affected, not only by duration of exercise, but also the specificity and sensitivity of assay kits used. The accumulation of work performed by the athletes during these training periods, as well as the large multiplex cytokine kit could increase variation. All samples were run in triplicate and all wells per subject and time point were on the same plate. Also, the larger stature of football athletes could impact results. Many non-exercise-related studies have used subjects of lesser weight, with dosages of EPA and DHA similar to the 2 g•day^{-1} used in the present study [54, 115, 163]. One possibility is that, due to their large body mass, football players could metabolize DHA to a greater extent than someone of a smaller body mass. Also, diet can affect fatty acid oxidation and liberation during exercise [84]. Validation of DHA incorporation, through analysis of erythrocyte phospholipid membrane fatty acid composition, marked significant increases of DHA in the DHA group [142]. However, it is possible that the erythrocyte uptake of DHA might

be limited [119]. Thus, excess ingested DHA (accounted for in an adipose tissue biopsy) might not be accounted for in the erythrocyte composition of a larger sized individual. Adipose tissue lipid storage has been shown to have sizable capacity for omega-3 PUFAs [111]. In lieu of an adipose biopsy to compliment an erythrocyte sample, additional data [142] found significant elevations in plasma DHA free fatty acid composition from Summer baseline and continuing throughout Post-camp (Pre-camp 2.32% and Post-camp 3.30% increases).

The impact of intensive preseason football training is widespread, with over 640 university teams comprised of 70 to 125 players per team competing annually [1]. The preliminary findings of this study may be of benefit to sport dieticians, athletic trainers, and team physicians who administer DHA for prolonged inflammation or post-concussion therapy. Eotaxin and MCP-1 were blunted with DHA supplementation during the strenuous preseason football camp. Future studies are needed to explore the possibility of maximal dosing and adequate fueling during heavy exertional training, to ensure DHA is being used, not as a fuel, but as a membrane component and/or intracellular eicosanoid communicator. These follow-up studies might shed light on other pro and anti-inflammatory cytokines that could be impacted by dietary DHA and proper exercise.

CHAPTER III

EFFECTS OF DOCOSAHEXAENOIC ACID ON LIPID PROFILES IN DIVISION I FOOTBALL PLAYERS DURING HEAVY PHYSICAL ACTIVITY

Introduction

In the United States, 38% of all deaths are attributed to cardiovascular disease [(101)]. Concern over the cardiovascular health of American football players has prompted much research regarding cardiovascular risk factors among this athletic population [(12, 24, 64, 70, 95, 112, 200)]. The large body size of football athletes has raised questions about an associated increase in the incidence of cardiovascular disease (CVD) compared to an aged-matched population [(200)]. A body mass index (BMI) > 28.4 kg/m^2 is correlated with a higher incidence of CVD, regardless of the presence of extra lean mass [(12)]. The combination of increased in-game playtime and a BMI of ≥ 30 kg/m^2, predicted a two-fold increase in CVD mortality [(12)]. The statistics are particularly profound among linemen. When compared to the general population, retired NFL linemen (both offensive and defensive) were 52% more likely to die from heart disease, in contrast to 46% lesser CVD mortality for retired non-lineman players [(11)]. In order to accommodate rule changes over the past three decades, which have restricted blocking, player size and body mass has increased [(95, 112)]. Consequently, case 2 obesity was indicated for over a quarter of the players in the NFL in 2003 [(70)]. Limitations have been

noted when using BMI as an identifier of obesity among football players. Lambert et. al. [116] reported clear delineations of college football player body composition comparing BMI and measurement techniques such as skin fold and DXA; BMI measures indicated obese measures for linemen, while skin fold and DXA did not indicate obese status. The latter measures accounted for the muscle mass of the athletes when calculating percent body fat and total body composition. Of all the team positions, NFL linemen have been reported to have the highest total cholesterol and triglyceride concentrations and low density lipoprotein (LDL) cholesterol concentrations, while having the lowest high-density lipoprotein (HDL) cholesterol concentrations [64].

In college, consumption of larger amounts of food to increase body mass, especially as traditional American football players or redshirt freshman,[107] could affect body composition (increased percent body fat), which in turn could be correlated with changes in lipid profiles. One of the times when food consumption is increased is during preseason camp. The intensity of preseason training requires increased fueling. Questions still exist on how high-level athlete lipid profiles respond to dietary interventions when combined with heavy physical training.

Independent risk factors for the development of CVD include: elevated total cholesterol and LDL cholesterol, triacylglycerols and low HDL cholesterol, and total and small dense LDL particles [115, 129, 167]. The preponderance of evidence for omega-3 polyunsaturated fatty acids suggests that there are many cardiovascular protective effects of supplementing with DHA. These include anti-atherosclerotic effects as well as improved circulating lipid and lipoprotein composition in response to exercise, such as

lowered LDL-C and increased HDL-C [103, 130, 131, 133, 176]. Research in sedentary subjects has shown triglyceride and total cholesterol reductions with 1 g•day^{-1} algal DHA while increasing HDL concentrations by 5.5% [169]. Additionally, supplementing with 4 g•day^{-1} DHA for 6 weeks resulted in a 20% decrease in triglycerides, a 29% increase in HDL_2 cholesterol and an 8% increase in LDL cholesterol in mild hyperlipidemic men [133]. At a dosage of 1.52 g•day^{-1} DHA, Maki et. al. [122] demonstrated a reduction in LDL cholesterol carried by small dense particles. Notably, 0.7 g•day^{-1} DHA elevated LDL cholesterol by 7%, although particle size change was not measured [191]. In a study of hypertriglyceridemic men, Kelley et al. [54] found that supplementation with DHA lowered fasting and postprandial triacylglycerol concentrations by 25-30%. Moreover, research has shown that the n-3 index was increased while lowering the remnant-like particle cholesterol (RLP-C) following the DHA supplementation for 90 days [103]. RLP-C concentrations, especially those triglyceride-rich, have been associated with increased atherogenic risk [103, 104].

The n-3 index is comprised of the sum total of EPA and DHA as a percentage of the total fatty acid content in red blood cell membranes [72]. Factors associated with cardiovascular risk include a decreased ratio of plasma EPA to AA, increased plasma concentrations of remnant-like particle cholesterol (RLP-C), and a decreased n-3 index (erythrocyte EPA + DHA content) in the red blood cell membranes [103]. An n-3 index of < 4% was associated with a 10 times greater risk of death from coronary heart disease, compared to an n-3 index of > 8% [41].

Cardiovascular risk is decreased when individuals engage in habitual exercise [105], which is associated with decreased LDL-C and triglycerides, as well as increased HDL-C. Studies using energy expenditures ranging from 1200-2200 kcals per week led to reductions in triglycerides of 5 to 38 mg/dL and increased HDL-C concentrations of 2 to 8 mg/dL [51]. Acute exercise has been demonstrated to reduce triglycerides by 14-50%, while following a regular exercise regime has been reported to have reductions of 4-37% [51]. Acute aerobic exercise increases HDL_3-C content while decreasing triglyceride and phospholipid content [61]. The effects of resistance training on altering lipids and lipoproteins have been mixed [105, 106, 110, 179, 193]. LDL-C reductions, without alterations in triglyceride or HDL-C, have been noted with resistance training [18]. In contrast, other resistance exercise training regimes have shown no change in LDL-C, while HDL-C increased and triglycerides decreased [189]. Several studies have shown there to be no change in cholesterol except when associated with a decrease in percent bodyfat [5, 43, 188]. LPL activity has been reported to be elevated after a variety of acute exercise training regimes [188]. With exercise, LDL particles become richer in cholesterol and increase in size [85]. Exercise reduces LDL mass and increases HDL_2 mass [210]. It has been suggested that LPL activity following training is responsible for the transfer of LDL mass to smaller HDL particles, leading to the larger mass of HDL [105, 113].

To the author's knowledge, there has been no research published to date examining possible alterations of lipid profiles with supplementation of algal DHA during heavy physical activity in Division I football athletes. The present study aimed

to determine the effects of DHA on lipid and lipoprotein concentration profiles during a heavy physical training, starting during voluntary summer training camp through mandatory preseason preparatory training camp. With evidence that 0.7 g•day^{-1} can affect lipid profiles and the fact that the subject population for this study was larger in body mass, the dosage of 2 g•day^{-1} algal DHA was explored to investigate if American collegiate football players' lipid profiles would be altered resulting in increased HDL and lower LDL.

Methods

Participants. The study was approved by the university Institutional Review Board for Research with Human Subjects. Sixty student-athlete NCAA Division I football players volunteered for participation. Volunteers were evaluated and excluded on the basis of the use of medications known to alter blood lipids and lipoproteins. Moreover, prior consumption of more than two servings of fish per week, or supplementation of omega-3 fatty acids and/or fish oil were also deemed reason for exclusion. Additionally, if a player became injured or received a concussion during the study, they were excluded. A consortium diagram outlines exclusion throughout the study to final subject numbers used for analysis (Fig. 11). Subject distribution and demographics are reported in Table 3.

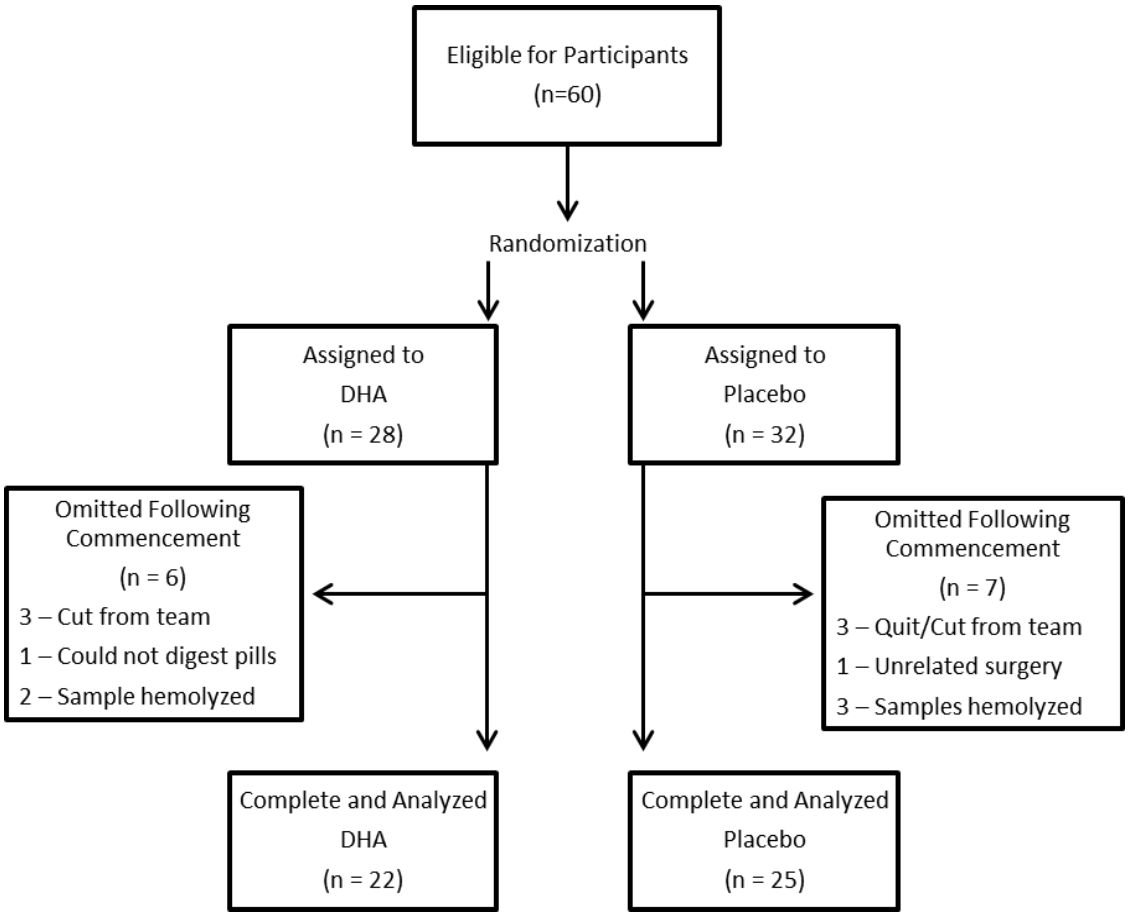


Figure 11. Study consort diagram for lipids.

Study Design. The study was double-blinded with subjects randomly assigned into either 2 g•day^{-1} of algal DHA oil (n=28) or 2 g•day^{-1} corn oil placebo (n=32) (both provided by DSM, Columbia, MD). Measurement and sample collection time points were established at voluntary summer training camp (Summer), beginning preseason training camp (Pre-camp) (30 days after Summer), and post preseason training camp (Post-camp) (24 days after Pre-camp). At each time point, body weight and a 24-hour dietary recall were collected by a registered dietician to ensure accuracy (Table 3). Athletic department staff members observed consumption of capsules following training during

Pre-camp on week days, as well as distributing capsule envelopes to the subject for the weekends, with instructions to return empty envelopes on the subsequent weekday. During voluntary summer training camp, limited training table snacks were available, leaving the athlete responsible for deciding and supplying the majority of his diet. Preseason training camp dinners and training table snacks (post workout) were provided in accordance with NCAA guidelines. During mandatory preseason training camp, research staff distributed supplements at team dinners and athletes consumed supplements in their presence. For the duration of the study, subjects were asked to refrain from consuming more than one meal per week consisting of fish.

Strength and Conditioning Training. During voluntary summer training, one to two anaerobic conditioning sessions and one to three voluntary strength training sessions per week were performed by the football athletes. On conditioning days, conditioning sessions would last 45 minutes followed by an hour of strength training. Training during mandatory preseason training camp increased in both frequency and volume of strength training and conditioning (agility drills) sessions, as well as the inclusion of sport-specific practices, two-a-day style practice/training, as allowed by the NCAA.

Blood Analysis. Resting blood samples were collected in two, 8.5 mL vacutainers (BD SST^{TM} w/ gel 367988) from the antecubital vein, in a seated position, after an overnight fast (≥ eight hour, water allowed *ad libitum*), and before any exercise training session. Following sample collection, samples were immediately plunged into ice and allowed to clot for thirty minutes, followed by refrigerated centrifugation for thirty minutes (2,000 x g) for separation of serum. One mL aliquot of sample was sent to St. Joseph's Hospital

(Bryan, TX) for analysis cholesterol and triglyceride concentrations through a combination of enzymatic assay and spectrophotometry. Another aliquot of serum was immediately transported (over ice) to SpectraCell Laboratories, Inc. (Houston, TX) for lipoprotein density and particle size analysis. The lipoprotein subgroup particle number analysis method was employed to complete a Lipoprotein Particle Profile™ [199]. Separation of lipoprotein particles were conducted through a continuous gradient generated by analytical ultracentrifugation (patent pending method). A separation gradient over a range of d = 1.000 - 1.300 $g \cdot cm^{-3}$ was established through application of a fluorescent dye onto lipoprotein particles. HPLC-type flowmetry technique was utilized for quantifying lipoprotein particles and normalized to a standard cholesterol scale via a proprietary algorithm. List of particles classified include: HDL (HDL number) and buoyant high-density $lipoprotein_{2b}$ (HDL_{2b} number), remnant lipoprotein (RLP number), VLDL (VLDL number), IDL (IDL number), LDL (LDL number), dense low-density $lipoprotein_3$ (LDL_3 number), dense low-density $lipoprotein_4$ (LDL_4 number). A multiple Gaussian fit/integration routine was conducted for specific densities of lipoprotein subgroups (e.g. LDL mean lipoprotein density). Standards have been reported at 2-3% coefficient of variation for this analytical technique.

Statistical Analysis. Percent change from Summer to Pre-Camp and Summer to Post-Camp was calculated and developed a basis for statistical significance testing. Independent t-tests were utilized for baseline comparisons of the Placebo and DHA group lipoproteins. The use of one sample t-tests ($\alpha = 0.05$) on the percent change for the treatment groups at Pre-camp and Post-camp were employed to identify effects of

treatment and training from Summer baseline. Independent t-tests were used to determine if there difference between groups' baseline values at Summer. For the 24-hour recall, independent t-tests were used to determine differences between groups at each respective time point as well as dependent t-tests where used to determine within group difference over time. The use of an $\alpha = 0.0125$ was used for the 24-hour recall data (original $\alpha = .05$ divided by four) to account for the multiple t-tests performed per time point.

Results

Table 3 lists the dietary composition of the subjects throughout the study. Notably, total calories and carbohydrates significantly increased ($P<0.0125$) in both the DHA and the Placebo groups during preseason camp training, while total fat was reduced at both Pre-camp and Post-camp. Protein stayed relatively the same throughout the study in the DHA group and was only reduced at Post-camp in the Placebo group. There was no difference detected in caloric or macronutrient intake between groups.

Table 4 reflects lipoprotein concentrations. Notably, both groups had increased percent change in HDL_{2b}-C and HDL_{2a}-C at Pre-camp. Percent change HDL_3-C was only decreased in the DHA group at Post-camp. The DHA group also had elevations in LDL_3-C (Pre-camp) and LDL_4-C (Post-camp). Additionally, the DHA group had significantly ($P < 0.05$) higher IDL-C at Summer compared to the Placebo group. RLP-C increased from Summer baseline only in the Placebo group at Pre-camp, while the DHA remained relatively unchanged. RLP number did not change throughout the study

and there was no difference between groups. There was no difference in lipoprotein-a (LP(a)) concentrations for either group throughout the study.

Table 5 provides particle numbers and densities for each time point. LDL density decreased in the Placebo at Post-camp, along with decreased HDL density at Pre-camp. The DHA group had no change in LDL density, while having a decreased HDL density at both Pre-camp and Post-camp. Percent change VLDL number was significantly ($P < 0.05$) increased at Pre-camp and Post-camp in the Placebo group, compared to Summer.

Figure 12 shows the variance of total cholesterol concentration during Pre-camp and Post-camp, though no difference was detected with time or by group. There was a significant decrease in Post-camp triglyceride concentration in the DHA group, while the Placebo group increased at Pre-camp (Fig. 13). LDL concentration tended to be higher (significance not met) than Summer, at both time points in the DHA group (Fig. 14). The Placebo group had a significant decrease in LDL concentration at Pre-camp. Percent change of HDL concentration did not significantly change in either group (Fig. 15).

DHA group had a significant percent change decrease in insulin at Post-camp (Table 6). There was no difference between DHA and Placebo groups C-reactive protein concentrations. Both the groups demonstrated a percent change increase at Post-camp. Homocysteine concentrations were only altered in the DHA group, where Pre-camp percent change was significantly higher than Summer.

TABLE 3. Subject demographics and nutritional intake

		Summer	Pre-camp	Post-camp
Age (years)		20 ± 0.11	-	-
Body Mass (kg)				
	DHA	235.67 ± 8.80	236.50 ± 8.66	236.63 ± 8.44
	Placebo	226.56 ± 7.96	227.96 ± 7.78	228.96 ± 8.04
Calories (kcal/d)				
	DHA	3527.22 ± 213.92^{a}	3050.61 ± 195.95^{a}	4526.43 ± 271.88^{b}
	Placebo	3916.25 ± 344.48^{a}	3200.31 ± 145.39^{a}	4412.86 ± 252.63^{b}
Carbohydrate (g/d)				
	DHA	351.91 ± 24.04^{a}	334.13 ± 27.13^{a}	664.30 ± 37.03^{b}
	Placebo	398.39 ± 36.77^{a}	352.78 ± 19.42^{a}	657.39 ± 36.49^{b}
Fat (g/d)				
	DHA	154.78 ± 9.30^{a}	114.52 ± 8.17^{b}	132.00 ± 11.63^{ab}
	Placebo	174.39 ± 16.75^{a}	120.31 ± 5.93^{b}	126.50 ± 10.27^{c}
MUFA (g/d)				
	DHA	21.52 ± 3.21^{a}	9.61 ± 1.43^{b}	17.83 ± 2.92^{ab}
	Placebo	25.11 ± 2.63^{a}	11.94 ± 1.17^{b}	16.79 ± 2.50^{c}
PUFA (g/d)				
	DHA	7.61 ± 1.31^{a}	2.35 ± 0.44^{b}	8.04 ± 1.29^{a}
	Placebo	7.89 ± 1.56^{a}	3.39 ± 0.46^{a}	5.89 ± 0.9^{b}
Saturated (g/d)				
	DHA	61.09 ± 4.49^{a}	45.43 ± 3.22^{ab}	46.39 ± 4.48^{b}
	Placebo	68.57 ± 6.94^{a}	46.69 ± 2.28^{b}	44.61 ± 4.53^{c}
Protein (g/d)				
	DHA	179.78 ± 12.96^{a}	170.61 ± 9.75^{a}	176.96 ± 12^{a}
	Placebo	191.46 ± 17.59^{a}	177.82 ± 7.75^{a}	168.89 ± 11.64^{b}

24-hour recall analyzed by Nutribase 8.0 software; different letter superscript denotes $P<0.0125$ different from other superscript letter (dependent t-test) within group. DHA (N=23), Placebo (N=28), Represented as mean ± SEM.

TABLE 4. Lipid and lipoprotein concentrations

		Summer	Pre-camp	Post-camp
Total Cholesterol				
	DHA	174.45 ± 6.85	182.05 ± 7.85	172.45 ± 6.97
	Placebo	174.92 ± 5.61	172.48 ± 5.97	175.36 ± 6.15
Triglycerides				
	DHA	115.32 ± 11.11	110.59 ± 9.87	85.18 ± 8.06*
	Placebo	100.16 ± 9.88	128.16 ± 11.11*†	95.20 ± 9.55†
LDL-C				
	DHA	103.41 ± 6.38	109.55 ± 6.22	108.14 ± 6.39
	Placebo	100.41 ± 4.13	93.76 ± 4.63*†	105.36 ± 4.42
LDL_3-C				
	DHA	16.25 ± 1.25	20.35 ± 1.67*	17.86 ± 1.59
	Placebo	15.79 ± 1.11	16.76 ± 1.36	14.94 ± 1.15
LDL_4-C				
	DHA	5.63 ± 0.28	6.07 ± 0.30	6.22 ± 0.35*
	Placebo	5.57 ± 0.31	5.85 ± 0.39	5.53 ± 0.28
IDL-C				
	DHA	19.71 ± 1.50 ‡	18.02 ± 1.18	20.15 ± 1.66
	Placebo	15.37 ± 1.66	17.75 ± 1.66	18.88 ± 1.87
RLP-C				
	DHA	22.42 ± 1.69	20.82 ± 1.29	22.93 ± 1.83
	Placebo	18.10 ± 1.76	20.90 ± 1.92*	21.81 ± 2.18
VLDL-C				
	DHA	12.60 ± 1.38	13.03 ± 1.38	12.76 ± 1.34
	Placebo	12.04 ± 1.55	15.33 ± 2.10	14.36 ± 2.36
HDL-C				
	DHA	47.86 ± 2.01	50.41 ± 2.78	47.18 ± 2.01
	Placebo	54.48 ± 4.18	53.32 ± 4.76	50.92 ± 3.04
HDL_{2b}-C				
	DHA	16.67 ± 1.28	19.13 ± 1.47*	16.13 ± 1.30
	Placebo	17.97 ± 2.19	20.45 ± 2.64*	16.76 ± 1.84
HDL_{2a}-C				
	DHA	6.81 ± 0.59	7.98 ± 0.73*	5.31 ± 0.34*
	Placebo	6.50 ± 0.54	8.61 ± 0.73*	6.03 ± 0.46
HDL_3-C				
	DHA	26.91 ± 1.03	25.63 ± 0.00	23.57 ± 0.96*
	Placebo	26.58 ± 0.82	25.76 ± 0.76	25.54 ± 0.94
TC: HDL				
	DHA	3.10 ± 0.13	3.11 ± 0.13	3.54 ± 0.19*
	Placebo	2.88 ± 0.15	2.98 ± 0.17	3.17 ± 0.16*
Lp(a)				
	DHA	38.67 ± 7.23	37.81 ± 6.15	36.09 ± 6.08
	Placebo	43.58 ± 8.41	42.34 ± 7.41	43.71 ± 7.02

Represented as mean (mg/dL) ± SEM; * denotes P <0.05 based from individual percent change from Summer averaged. † denotes P<0.05 percent change between DHA and Placebo; ‡ denotes p <0.05 DHA different from Placebo absolute values at Summer.

TABLE 5. Lipid and lipoprotein particle numbers and densities

		Summer	Pre-camp	Post-camp
LDL#				
	DHA	628.09 ± 35.40	676.61 ± 37.45	674.96 ± 41.72
	Placebo	552.32 ± 28.41	592.46 ± 36.08	597.89 ± 36.97
LDL_3#				
	DHA	154.26 ± 11.84	193.30 ± 15.89*	169.57 ± 15.12
	Placebo	146.32 ± 11.70	159.07 ± 12.90	141.71 ± 10.93
LDL_4#				
	DHA	69.61 ± 3.51	75.17 ± 3.67	76.83 ± 4.35*
	Placebo	68.86 ± 3.86	72.50 ± 4.82	68.36 ± 3.49
LDL Density				
	DHA	1.030000 ± 0.000199	1.030565 ± 0.000294	1.030174 ± 0.000241
	Placebo	1.030643 ± 0.000190	1.030500 ± 0.000184	1.030036 ± 0.000196*
RLP#				
	DHA	95.22 ± 7.15	88.35 ± 5.47	97.26 ± 7.78
	Placebo	77.93 ± 7.46	88.71 ± 8.18	92.68 ± 9.29
VLDL#				
	DHA	48.39 ± 5.32	49.96 ± 5.28	48.91 ± 5.15
	Placebo	46.18 ± 5.92	58.57 ± 8.05*	55.11 ± 9.03*
HDL#				
	DHA	9738.09 ± 396.61	9824.87 ± 428.27	8556.74 ± 321.84*
	Placebo	9718.61 ± 381.90	10091.18 ± 459.15	9246.32 ± 362.14
HDL_{2b}#				
	DHA	1496.17 ± 114.69	1717.96 ± 131.67*	1448.91 ± 116.58
	Placebo	1613.32 ± 197.01	1836.82 ± 237.59	1503.71 ± 165.05
HDL Density				
	DHA	1.099522 ± 0.001126	1.096522 ± 0.000802*	1.097174 ± 0.001064*
	Placebo	1.098750 ± 0.001301	1.096000 ± 0.000984*	1.098679 ± 0.001264

Represented as mean (nmol/L) ± SEM; * denotes P <0.05 based from individual percent change from Summer averaged.

TABLE 6. C-reactive protein, insulin, homocysteine

		Summer	Pre-camp	Post-camp
C-Reactive Protein (mg/dL)				
	DHA	0.16 ± 0.04	0.11 ± 0.04	0.23 ± 0.04*
	Placebo	0.13 ± 0.04	0.06 ± 0.01	0.18 ± 0.04*
Insulin (uIU/mL)				
	DHA	10.98 ± 1.65	7.80 ± 0.94	6.54 ± 0.53*
	Placebo	11.38 ± 1.28	10.38 ± 1.48	8.01 ± 0.65
Homocysteine (umol/L)				
	DHA	9.05 ± 0.77	10.79 ± 1.88*	9.46 ± 1.46
	Placebo	10.69 ± 2.41	10.76 ± 2.45	10.49 ± 2.38

Represented as mean ± SEM; * denotes P <0.05 based from individual percent change from Summer averaged.

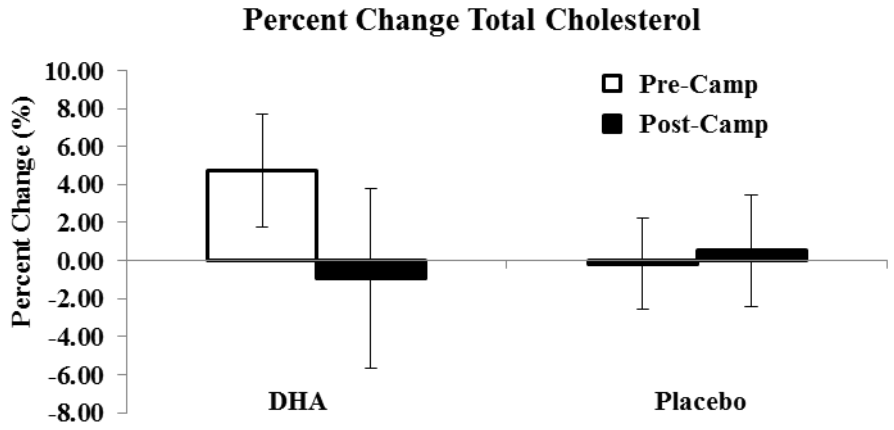


Figure 12. Percent change total cholesterol. Pre-camp represents the change from summer baseline to Pre-camp time point; and Post-camp represents the change from summer baseline to Post-camp time point. DHA (N=23), Placebo (N=28).

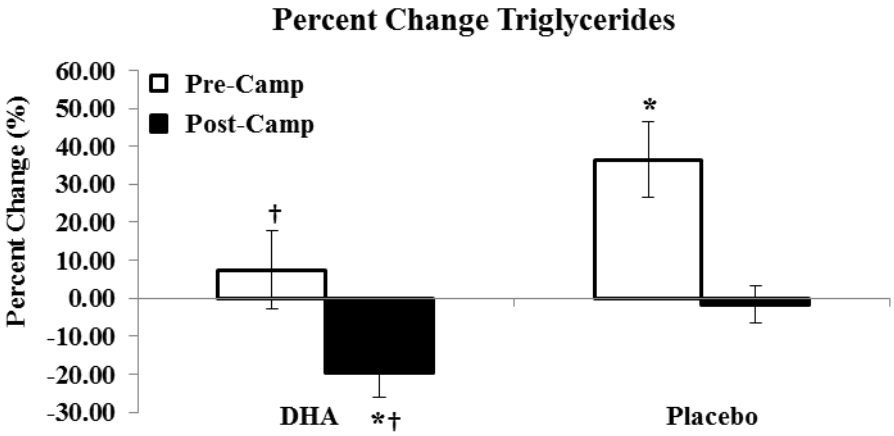


Figure 13. Percent change triglycerides. Pre-camp represents the change from summer baseline to Pre-camp time point; and Post-camp represents the change from summer baseline to Post-camp time point. * denotes P<0.05 significance from Summer. † denotes P<0.05 from Placebo at respective time point. DHA (N=23), Placebo (N=28).

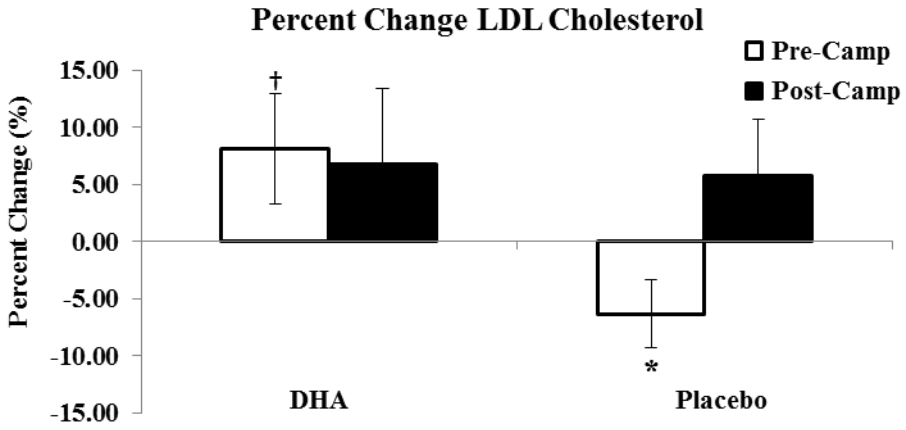


Figure 14. Percent change LDL cholesterol concentration. Pre-camp represents the change from summer baseline to Pre-camp time point; and Post-camp represents the change from summer baseline to Post-camp time point. * denotes P<0.05 significance from Summer. † denotes P<0.05 from Placebo at respective time point. DHA (N=23), Placebo (N=28).

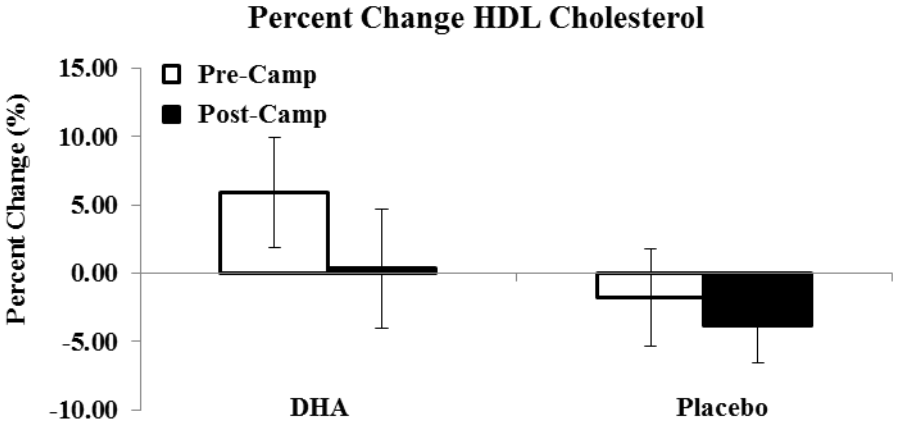


Figure 15. Percent change HDL cholesterol concentration. Pre-camp represents the change from summer baseline to Pre-camp time point; and Post-camp represents the change from summer baseline to Post-camp time point. DHA (N=23), Placebo (N=28).

Discussion

Kirwan et. al [107] examined a similar cohort (American college football without dietary intervention) to the one in our study, over an 8-week training period. The investigators reported an increase in total cholesterol and LDL-C following training; while subjects from our study had significant decreases percent change in LDL-C in the Placebo group (Pre-camp), but no significant change in the DHA group (Fig. 14). Additionally, the DHA group in our study exhibited increases in LDL_3 particle number (Pre-camp) and LDL_4 particle number (Post-camp). Triglycerides were similarly reported as no change following Kirwan et. al.'s [107] study. Our Placebo group did have significant triglyceride elevation at Pre-camp but returned to similar Summer baseline concentrations at Post-camp. The DHA group did not demonstrate a percent change in triglycerides from Summer baseline at Pre-camp, but did have a significant reduction from Summer baseline at Post-camp, which was also significantly different from Placebo at the same time point (Fig. 13).

Triglycerides are transported throughout the body, primarily by chylomicrons and VLDL, to provide energy to specific muscle tissues. During exercise, triglycerides are liberated to deliver free fatty acids to working muscles, as well as to provide energy for post-workout tissue repair. Though not significant, the total amount of fat intake ($g \bullet day^{-1}$) at Pre-camp and Post-camp was lower than Summer (Table 3). Hill et. al. [77] reported, when comparing isocaloric diets, that the diets higher in long-chain or medium-chain triglycerides or fish oil, resulted in altered concentrations of LDL, HDL, and triglyceride concentrations compared to diets lower in fat with no alterations

reported. Following a standard strength and conditioning program and consuming a high-fat, high-calorie diet, redshirt freshman players had elevations in LDL and total cholesterol (+31.8 mg/dl and + 29.1 mg/dl, respectively), as well as increased total body fat (+1.4 kg) [107].

In several studies using fish oil (majority EPA to DHA composition), subjects deemed normotriglycerolemic demonstrated a 25% reduction in serum triglycerides, and hypertriglycerolemic subjects had a 34% reduction [74]. This reduction in triglycerides is similar to our DHA group, which demonstrated a significant reduction at Post-camp (Fig. 13). Notably, Mori et. al. [133] reported significant decreases in serum triglycerides with DHA supplementation, in mildly hyperlipidemic men. The combination of DHA and increased training in our study resulted in decreased serum triglycerides at Post-camp. Besides triglycerides, cholesterol is the other molecule transported by lipoproteins. Our study showed no difference in total cholesterol for either group (Fig. 12). The lack of cholesterol alteration is similar to previous research using fish oil in both normotriglycerolemic and hypertriglycerolemic men [74] and exclusive DHA supplementation in hypertriglycerolemic men [133].

Lipid particle numbers had mixed results over the course of this study for both groups. VLDL particle number significantly increased at Pre-camp and Post-camp in the Placebo group, along with increased triglycerides at Pre-camp. Increased VLDL particle number could be the result of increased energy demands due to training [106], as well as the reduction of total fat intake from Summer. Other studies have demonstrated EPA supplementation reduces VLDL concentrations, while DHA supplementation does not

affect VLDL [130, 133]. During voluntary summer training, LDL_3 particle number tended to be increased at Pre-camp in both groups (significant for the DHA group), while at Post-camp, the DHA group still displayed a higher total amount of LDL_3 particle number following preseason training camp. The increase in LDL peak particle size has been related to increased aerobic exercise [215], which enhances cholesterol transport to phospholipid cell membranes. Changes in LDL concentrations were only significantly decreased at Pre-camp in the Placebo group, though the DHA and the Placebo groups tended to have higher LDL concentrations at Post-camp (Fig. 14). Similar to previous studies [107], HDL concentrations were unaffected (Fig. 15), though particle number was decreased in the DHA group following preseason training camp (Table 5).

Mechanisms proposed for altering lipid profiles by omega-3s include improving vascular reactivity, altering inflammation status, and decreasing platelet aggregation [115]. Harris [73] suggests the additional intake of omega-3 fatty acids could affect hepatic triglyceride metabolism through the attenuation of hormone-sensitive lipase, acetyl-CoA carboxylase FA synthase while enhancing mitochondrial and peroxisomal B-oxidation. Additionally, Park [148] demonstrated an increased chylomicron triglyceride clearance via increased LPL activity and smaller chylomicron particle sizes. For the athletic population, this translates into a possible greater availability of energy which could lead to increased endurance or more efficient recovery.

Our study also highlights lipid particle number and density fluctuations in football athletes from Summer through preseason camp training, in preparation for the season. These fluctuations may be due to increased energy demands of training sessions

and sports practice, necessitating an increased need for cholesterol mobilization for cellular (skeletal muscle) membrane and structural repair. Practitioners and dieticians who supplement athletes with DHA should consider adequate caloric and macronutrient needs in order to limit the possibility of the omega-3 fatty acid of being used as fuel in β-oxidation. Further investigation is needed to examine differing dosages of DHA, as well as diets with higher fat content, to determine if either factor mitigates lipid profile alterations during heavy physical activity.

CHAPTER IV

CONCLUSIONS

The primary purpose of this research was to document the effects of algal DHA, combined with intense physical training, on inflammatory and lipid responses associated with such training, in Division I football players. The first study describes changes in inflammatory cytokines during intensive physical training with DHA supplementation in football athletes. The second study describes lipid and lipoprotein responses during intensive physical training with DHA supplementation. From our current study, the knowledge of the incorporation of DHA into plasma and tissue in this power-dominated athletic population, coupled with the potential anti-inflammatory effects of DHA, could positively alter lipid profiles, immune functions, body composition, and inflammatory issues such as insulin resistance, or recovery from injuries (e.g. concussions); these beneficial effects remain to be demonstrated.

There are large numbers of athletes who train every day, in various sports, and at various levels. In the U.S. alone, over 7 million high school athletes, over 450,000 college athletes, and nearly 15,000 professional athletes compete each year [2, 90, 190]. Though our study of DHA supplementation focused on Division I college football players, other athletes of different sports who participate in intense training regimes could benefit from DHA supplementation as well. Buckley et al.[23] found that supplementation with DHA-rich fish oil (6 g•day^{-1}) improved cardiovascular function in elite Australian Rules football players, by means of lower heart rates at submaximal

exercise. Even though reduced cardiovascular risk factors were seen with this type of supplementation, endurance performance and recovery were deemed uneffected in the athletes. [23] Walser and Stebbins [203] reported the combination of 2 g•day^{-1} of DHA, plus 3 g•day^{-1} of EPA improved stroke volume and cardiac output during low and moderate work-intensity exercise in recreationally-fit, healthy subjects, following 6 weeks of supplementation. Moderate exercise with fish oil (1.6 g•day^{-1} DHA) supplementation has also been linked to improved immune function and reduced cellular inflammation in sedentary individuals. Following twelve weeks of supplementation and moderate aerobic exercise (3 sessions of walking for 45 minutes at 75% maximum heart rate), leukocyte function and cytokine production were altered to improve body composition, reduce cardiovascular risk, and lessen inflammation.[78]

The anti-inflammatory and neuroprotective characteristics of DHA and exercise working in concert are especially important among the collegiate athletic population. Though researchers using animal models have demonstrated the neuroprotective effects of DHA on neural tissue following head-trauma [216], the ability of DHA to affect human neuropathies is not known [146]. Wu and colleagues [216] reported that, not only does the combination of DHA and exercise complement each other to enhance brain development in rats (marked by elevated brain-derived neurotropic factor); but also following brain trauma in rodents, DHA provided a resistive effect to oxidative stress and promoted cognitive capacity and enhanced neuroplasticity [217].

Significantly, in 1998, the Center for Disease Control and Prevention (CDC) reported that there are approximately 300,000 traumatic brain injuries related to sport

each year [194]. From 2001-2005, the CDC reported 207,830 patients with nonfatal sport-related traumatic brain injuries, of which the highest number of incidents were recorded in young athletes [35]. Guskiewicz and colleagues [68] found a 6.3% occurrence of concussions after tracking 19 Division I, three Division II, and three Division III football teams over three years. Moreover, it was concluded that football players who had three or more concussions are three times more likely to have another concussion [68]. Though we did not utilize a direct measure of DHA in brain/neural cell membranes, it is reasonable to assume that 2 g•day^{-1} algal DHA would be adequate to increase DHA in neural tissues [217], as we found DHA incorporation at a systemic level (erythrocyte phospholipid cell membrane composition) in these athletes [142]. This finding may be of great benefit to sport dieticians, athletic trainers, and team physicians who administer DHA for post-concussion therapy. Further research is needed to determine whether or not this dosage will also provide improved lipid profiles, anti-inflammatory benefits, and neuroprotection.

Inflammation and DHA Supplementation

During preseason camp, Eotaxin and MCP-1 elevations were attenuated after supplementing with 2 g•day^{-1} DHA. RANTES was significantly elevated in both the DHA and Placebo groups. However, the percent change increase in the Placebo group was nearly two times that of the DHA group. In both groups, white blood cell counts decreased during preseason camp. With no change noted in several other cytokine markers, the effectiveness of the 2 g•day^{-1} DHA dosage as an anti-inflammatory agent for intense physical training is uncertain.

Lipoproteins and DHA Supplementation

Following supplementation, total cholesterol as well as lipoprotein cholesterols HDL-C and LDL-C were not affected (Post-camp not different from Summer) by training when supplemented with DHA. With increased training, both groups had increased in HDL_{2b}-C and HDL_{2a}-C at Pre-camp. Notably, preseason camp did decrease LDL-C in the Placebo group. LDL_3-C (at Pre-camp) and LDL_4-C (at Post-camp) increased in only the DHA group. Summer IDL-C was significantly ($P < 0.05$) different between groups, with the DHA group having higher initial concentrations compared to the Placebo group. Percent change VLDL number was significantly ($P < 0.05$) increased at Pre-camp and Post-camp in the Placebo group, compared to Summer. RLP-C increased from Summer baseline in only the Placebo group while the DHA remained relatively unchanged. RLP number did not change throughout the study and there was no difference between groups. LDL density decreased in the Placebo at Post-camp along with decreased HDL density at Pre-camp. The DHA group had no change in LDL density but did have decreased HDL density at both Pre-camp and Post-camp. Pre-camp percent change TG concentrations were significantly ($P < 0.05$) increased only in the Placebo group, as well as being significantly ($P < 0.05$) greater than the DHA group. Pre-camp percent change TG concentrations were significantly ($P < 0.05$) decreased from Summer in the DHA group and was significantly different from the Placebo group at Post-camp as well. There was no difference in lipoprotein-a (LP(a)) concentrations for either group throughout the study. Moreover, there was no difference between DHA and Placebo groups C-reactive protein concentrations. Insulin concentrations decreased

from Summer to Post-Camp in both groups, but the DHA group also had a significant percent change decrease from Summer at Post-camp. Homocysteine concentrations were only altered in the DHA group, where Pre-camp percent change was significantly higher than Summer.

Limitations

There are certain limitations that pertain to studying a collegiate football population. One such limitation is relying on the athletes to supplement themselves over the weekends. The athletes have the potential to return empty supplement envelopes without actually having ingested the supplement. Additionally, the extent to which the athlete will tolerate (digestion and absorption) a median dosage of DHA was out of our control. In our study, we only had one participant drop out from digestive issues with supplementation. This study used algal DHA for supplementation - a DHA source which can be refined and purified. There seems to be less incidence of gastro-intestinal distress with algal DHA, when compared to supplementation with fish oil. Ryan et. al. [165] noted less taste dissatisfaction and gastrointestinal upset with higher doses of algal DHA compared to fish oil. Athletes who want to supplement with omega-3 fatty acids could benefit from trying this form of omega-3 oil. Future studies might examine the use of algal DHA to investigate supplementation of higher doses of DHA with less chance of gastro-intestinal upset than could be tolerated with fish oil.

Additionally, consideration must always be taken with self-reported diets, as errors of quantity or omission of food can occur. For our study, having a certified dietician who regularly works with the athletes studied may have mitigated major

over/under-estimations. Reported diets may then be closer to what was actually consumed, when compared to an investigator interviewing a subject with whom he has no prior relationship.

The development of a college athlete varies from institution to institution. Differing training philosophies of individual strength coaches can impact athletes' training, rest, volume, and recovery. Dieticians for each team may have different recommendations or meal plans. Athletes from other sports may train differently, based on performance needs, and have different concentrations of baseline inflammation. Athletes of differing sizes, across a variety of sports, may experience differences in the incorporation and the utilization of DHA in the resolution phase of inflammation. The amount of stress placed on the muscle, in addition to the amount of adipose tissue, combined with IMTG containing DHA would influence the inflammatory outcome.

One additional consideration is the prior training experience of the athletic population. Pizza et. al. [159] noted a training effect on isolated myotubes following bouts of eccentric exercise where the first bouts were accompanied with higher concentrations of circulating neutrophils at 3, 6, and 9 hours post-exercise in comparison to the following exercise bouts. The football players in this study have years of training experience and could have previously adapted to the exercise immune response of fall training camp when compared to a sedentary population.

Delimitations

Certain delimitations were considered in the design of this study. The first delimitation is the population we investigated. This study focused on a collegiate

student athlete population (Placebo 20.1 ± 1.4 years, DHA 20.5 ± 1.6 years) under training conditions. Future studies would need to be conducted to determine whether DHA supplementation would affect lipid profiles, immune and inflammation markers in a younger or older population following similar training regimes. Supplementation in younger athletes could more efficiently aid in recovery and further enhance training adaptations. The aging athlete that wants to prolong their athletic career may seek supplementation to allow them to continue training and perform optimally for another season.

Caloric expenditure could also affect the systemic incorporation of DHA in each athlete. Using the mean anthropometrics of our subjects and an activity factor of 2.2, the predicted caloric expenditure, based on the Harris Benedict equation, [71] was 4,668 calories per day. The activity factor of 2.2 was based on discussions with coaching staff and observations of the athletes' daily activity during each training period. When examining caloric intake from dietary recall, all time points had lower intakes than the estimated expenditure [142]. This higher expendiure to intake ratio, coupled with the decreased total fat consumed by the athletes from Summer through preseason training [142], could have affected whether the ingested PUFAs were oxidized as fuel, or incorporated into tissue membranes. Nevertheless, there continued to be an increase in percent change of DHA content in plasma and erythrocyte cell membranes, regardless of lower fat intake and fewer calories consumed than expended [142]. We speculate that a higher caloric expenditure and lower fat intake might explain the signifcant drop in percent change of DHA of the Placebo group at Pre-camp in erythrocyte cell

membranes, as the placebo group might have been utilizing PUFAs stored in adipose tissue for fuel.

Future Studies

Future studies may aim to incorporate a performance outcome. Initially, this study included a vertical jump and triple jump measurement, but coaching staff was unable to execute the test with accuracy and reliability, and the performance measurement was eliminated. Future steps include having certified researchers establish performance tests at each of the supplementation time points. Strength, speed, agility, and endurance measurements would be recorded to determine increases, decreases, or the preservation of said attribute during the rigorous preseason training.

Further investigations are needed into other tissues, such as skeletal, cardiac (cell or animal model), neural (cell or animal model), and adipose tissues to see the impact of DHA supplementation on incorporation and inflammation. Muscle and adipose biopsies may shed light on potential storage and release of DHA, as well as remodeling factors following training. Moreover, measurements should occur at different times throughout the season, to determine if performance is maintained or enhanced with inflammation and recovery. Football players usually play over the weekend and complete at least two training sessions during the week, all while managing classes and travel to the next competition. The goal of training during the season is to maintain strength and speed for the upcoming game.

Additionally, suggestions for future studies involve addressing particular limitations in the above studies. First, this specific subject population's body stature and

performance obligations affect supplementation, diet, and exercise interventions. Ideally, a dose response for algal DHA would be utilized to determine if there is a regression that would fit a range of individual body sizes and compositions, for inflammatory and lipid markers. Controlling the exercise regime and exploring various intensities and volumes of activity, in conjunction with DHA supplementation, could provide additional information about lipoprotein, immune, and inflammatory responses. Second, follow up studies are needed examining the effects of DHA supplementation, with adequate fueling, so the DHA ingested is not potentially oxidized as fuel. Adequate incorporation could further be validated with the addition of an adipose tissue biopsy prior to the beginning of the playing season.

REFERENCES

1. NCAA College Sports Statistics and Records [Internet]: NCAA [cited 2013 12/02]. Available from: http://ncaa.org/wps/wcm/connect/public/ncaa/resources/stats/index.html.

2. Occupational Outlook Handbook (2014-2015 Edition): Athletes and Sports Competitors [Internet]: Bureau of Labor Statistics, U.S. Department of Labor [cited 2014 04/13]. Available from: http://www.bls.gov/ooh/entertainment-and-sports/athletes-and-sports-competitors.htm.

3. Albright J. Injury patterns in big ten conference football. *Am J Sports Med*. 2004; 32(6):1394-404.

4. Albright JP, Powell JW, Martindale A, Black R, Crowley E, Schmidt P, Monroe J, Locy D, Aggler T, Davis WR, Salvaterra G, Miller D, Helwig D, Soboroff S, Nivens J, Carpenter J, Kovan J, Arndt E, Sweeney H, Lombardo J, Sebastianelli WJ, Krauss M, Landry G. Injury Patterns in Big Ten Conference Football. *Am J Sport Med*. 2004; 32(6):1394-404.

5. Allard C, Alteresco M, Ferguson RJ, Chaniotis L, Choquette G, Skinner J. Changes in adipose tissue and increased serum cholesterol of coronary patients following training. *Can Med Assoc J*. 1973; 109(3):194-7.

6. Arab L. Biomarkers of fat and fatty acid intake. *J Nutr*. 2003; 133 Suppl 3:925S-32S.

7. Arner P, Kriegholm E, Engfeldt P, Bolinder J. Adrenergic regulation of lipolysis insitu at rest and during exercise. *J Clin Invest*. 1990; 85(3):893-8.

8. Ayre KJ, Phinney SD, Tang AB, Stern JS. Exercise training reduces skeletal muscle membrane arachidonate in the obese (fa/fa) Zucker rat. *J Appl Physiol*. 1998; 85(5):1898-902.

9. Ayre K. Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. *J Nutr*. 1996; 126(3):653.

10. Bailes J, Mills J. Docosahexaenoic acid reduces traumatic axonal injury in a rodent head injury model. *J Neurotrauma*. 2010; 27(9):1617.

11. Baron S, Rinsky R. *Health Hazard Evaluation Report, National Football League Players Mortality Study*. Atlanta, GA: HETA 88-085; 1994. Available From: Centers for Disease Control and Prevention, NIOSH.

12. Baron SL, Hein MJ, Lehman E, Gersic CM. Body Mass Index, Playing Position, Race, and the Cardiovascular Mortality of Retired Professional Football Players. *Am J Cardiol*. 2012; 109(6):889-96.

13. Bautmans I, Njemini R, Vasseur S, Chabert H, Moens L, Demanet C, Mets T. Biochemical changes in response to intensive resistance exercise training in the elderly. *Gerontology*. 2005; 51(4):253-65.

14. Benatti P, Peluso G, Nicolai R, Calvani M. Polyunsaturated fatty acids: Biochemical, nutritional and epigenetic properties. *J Am Coll Nutr*. 2004; 23(4):281-302.

15. Bistrian B, Askew W, Erdman J, Oria M. Nutrition and Traumatic Brain Injury: A Perspective From the Institute of Medicine Report. *J Parenter Enteral Nutr*. 2011; 35(5):556-9.

16. Bloomer R, Larson D, Fisher-Wellman K, Galpin A, Schilling B. Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: a randomized, placebo controlled, cross-over study. *Lipids Health Dis*. 2009; 8:36.

17. Bloomer R. The role of nutritional supplements in the prevention and treatment of resistance exercise-induced skeletal muscle injury. *Sports Med*. 2007; 37(6):519.

18. Boyden TW, Pamenter RW, Going SB, Lohman TG, Hall MC, Houtkooper LB, Bunt JC, Ritenbaugh C, Aickin M. Resistance exercise training is associated with decreases in serum low-density lipoportein cholesterol levels in premenopausal women. *Arch Intern Med*. 1993; 153(1):97-100.

19. Brenner I, Natale V, Vasiliou P, Moldoveanu A, Shek P, Shepard R. Impact of three different types of exercise on components of the inflammatory response. *Eur J Appl Physiol O*. 1999; 80(5):452.

20. Brilla LR, Landerholm TE. Effect of fish oil supplementation and exercise on serum lipids and aerobic fitness. *J Sports Sci Med Phys Fitness*. 1990; 30(2):173-80.

21. Brolinson PG, Elliott D. Exercise and the Immune System. *Clin Sports Med*. 2007; 26(3):311-9.

22. Brüünsgaard H, Pedersen BK. Age-related inflammatory cytokines and disease. *Immunol Allergy Clin*. 2003; 23(1):15-39.

23. Buckley JD, Burgess S, Murphy KJ, Howe PRC. DHA-rich fish oil lowers heart rate during submaximal exercise in elite Australian Rules footballers. *J Sci Med Sport*. 2009; 12(4):503-7.

24. Buell JL, Calland D, Hanks F, Johnston B, Pester B, Sweeney R, Thorne R. Presence of metabolic syndrome in football linemen. *J Athl Training*. 2008; 43(6):608-16.

25. Calder P. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr*. 2006; 83(6):1505S-19S.

26. Calder P, Lindley M, Burke L, Stear S, Castell L. A-Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance Part 14. *Brit J Sport Med*. 2010; 44(14):1065-7.

27. Calder P. Dietary modification of inflammation with lipids. *P Nutr Soc*. 2002; 61(3):345.

28. Calder P. Fatty acids and inflammation: The cutting edge between food and pharma. *Eur J Pharmacol*. 2011; 668:S50-8.

29. Calder P. Long-chain fatty acids and inflammation. *P Nutr Soc*. 2012; 71(02):284-9.

30. Calder P. Mechanisms of action of (n-3) fatty acids. *J Nutr*. 2012; 142(3):592S.

31. Calder P. Polyunsaturated fatty acids and inflammation. *Prosta Leukotr Ess*. 2006; 75(3):197-202.

32. Calder P. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie*. 2009; 91(6):791-5.

33. Calle MC, Fernandez ML. Effects of resistance training on the inflammatory response. *Nutr Res Pract*. 2010; 4(4):259-69.

34. Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and Clearance of Omega-3 Fatty Acids in Erythrocyte Membranes and Plasma Phospholipids. *Clin Chem*. 2006; 52(12):2265-72.

35. Centers for Disease Control and Prevention (CDC). Nonfatal Traumatic Brain Injuries from Sports and Recreation Activities--United States, 2001-2005. *Morb Mortal Wkly Rep*. 2007; 56(29):733-7.

36. Chapkin R, Kim W, Lupton J, McMurray D. Dietary docosahexaenoic and eicosapentaenoic acid: Emerging mediators of inflammation. *Prosta Leukotr Ess*. 2009; 81(2-3):187-91.

37. Charlton-Menys V, Durrington PN. Human cholesterol metabolism and therapeutic molecules. *Exp Physiol*. 2007; 93(1):27-42.

38. Chiu D, Kuypers F, Lubin B. Lipid peroxidation in human red cells. *Semin Hematol*. 1989; 26(4):257-76.

39. Choi KM, Kim JH, Cho GJ, Baik SH, Park HS, Kim SM. Effect of exercise training on plasma visfatin and eotaxin levels. *Eur J Endocrinol*. 2007; 157(4):437-42.

40. Clark M, Reed DB, Crouse SF, Armstrong RB. Pre-and Post-Season Dietary Intake, Body Composition, and Performance Indices of NCAA Division I Female Soccer Players. *Int J Sport Nutr Exe*. 2003; 13(3):303-19.

41. Colville-Nash PR, Gilroy DW, Willis D, Paul-Clark MJ, Moore AR, Willoughby DA. Prostaglandin F2alpha produced by inducible cyclooxygenase may contribute to the resolution of inflammation. *Inflammopharmacology*. 2005; 12(5-6):473,6;discussion477-80.

42. Crouse SF, O'Brien BC, Rochack JJ, Lowe RC, Green JS, Tolson H, Reed JL. Changes in serum lipids and apoproteins after exercise in men with high cholesterol: influence of intensity. *J Appl Physiol*. 1995; 79:279-86.

43. Davis PG, Bartoli WP, Durstine JL. Effects of acute exercise intensity on plasma lipids and apolipoproteins in trained runners. *J Appl Physiol*. 1992; 72:914-9.

44. De Caterina R, Cybulsky MI, Clinton SK, Gimbrone Jr MA, Libby P. The omega-3 fatty acid docosahexaenoate reduces cytokine induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler Thromb*. 1994; 14(11):1829-36.

45. De Caterina R, Cybulsky MA, Clinton SK, Gimbrone Jr MA, Libby P. Omega-3 fatty acids and endothelial leukocyte adhesion molecules. *Prosta Leukotr Ess*. 1995; 52(2-3):191-5.

46. DeFina L. Effects of omega-3 supplementation in combination with diet and exercise on weight loss and body composition. *Am J Clin Nutr*. 2011; 93(2):455-62.

47. DeMartini JK, Martschinske JL, Casa DJ, Lopez RM, Ganio MS, Walz SM, Coris EE. Physical demands of National Collegiate Athletic Association division I football players during preseason training in the heat. *J Strength Cond Res*. 2011; 25(11):2935-43.

48. Dick R, Ferrara M, Agel J, Courson R, Marshall S, Hanley M, Reifsteck F. Descriptive epidemiology of collegiate men's football injuries: National Collegiate Athletic Association Injury Surveillance System, 1988-1989 through 2003-2004. *J Athl Training*. 2007; 42(2):221.

49. Ditschuneit HH, Frier HI, Flechtner-Mors M. Lipoprotein responses to weight loss and weight maintenance in hish-risk obese subjects. *Eur J Clin Nutr*. 2002; 56(3):264-70.

50. Durstine JL, Haskell WL. Effect of exercise training on plasma lipids and lipoproteins. *Exerc Sport Sci R*. 1994:477-521.

51. Durstine JL, Grandjean PW, Cox CA, Thompson PD. Lipids, lipoproteins, and exercise. *J Cardiopulm Rehabil*. 2002; 22(6):385-98.

52. Egert S, Kannenberg F, Somoza V, Erbersdobler HF, Wahrburg U. Dietary alpha-linolenic acid, EPA, and DHA have differential effects on LDL fatty acid composition but similar effects on serum lipid profiles in normolipidemic humans. *J Nutr*. 2009; 139(5):861.

53. Ervin R, Wright J, Wang C, Kennedy-Stephenson J. Dietary intake of fats and fatty acids for the United States population: 1999-2000. *Adv Data*. 2004(348):1.

54. Eslick G. Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. *Int J Cardiol*. 2009; 136(1):4-16.

55. Febbraio M, Steensberg A, Keller C, Starkie R, Nielsen H, Krustrup P, Ott P, Secher N, Pedersen B. Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *J Physiol-London*. 2003; 549(Pt 2):607-12.

56. Feeley B, Kennelly S, Barnes R, Muller M, Kelly B, Rodeo S, Warren R. Epidemiology of National Football League training camp injuries from 1998 to 2007. *Am J Sport Med*. 2008; 36(8):1597-603.

57. Fehrenbach E, Schneider M. Trauma-induced systemic inflammatory response versus exercise-induced immunomodulatory effects. *Sports Med*. 2006; 36(5):373.

58. Ferguson MA, Alderson NL, Trost SG, Essig DA, Burke JR, Durstine JL. Effects of four different single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. *J Appl Physiol*. 1998; 85(3):1169-74.

59. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Laurentani F, Martin A, Andres-Lacueva C, Senin U, Guralnik J. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocr Metab*. 2006; 91(2):439.

60. Flachs P, Rossmeisl M, Bryhn M, Kopecky J. Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. *Clin Sci*. 2009; 116(1):1.

61. Frey I, Baumstark MW, Berg A. Acute and delayed effects of prolonged exercise on serum lipoproteins. I. Composition and distribution of high-intensity lipoprotein subfractions. *Eur J Appl Physiol*. 1993; 66:521-5.

62. Gallin JI, Snydderman R, Fearon DT, Haynes BF, Nathan C. *Inflammation: Basic Principles and Clinical Correlates.* Philadelphia: Lippincott Williams & Wilkins; 1999.

63. Garland M, Sacks FM, Colditz GA, Rimm EB, Sampson LA, Willett WC, Hunter DJ. The relation between dietary intake and adipose tissue composition of selected fatty acids in US women. *Am J Clin Nutr*. 1998; 67(1):25.

64. Garry JP, Mcshane JJ. Analysis of Lipoproteins and Body Mass Index in Professional Football Players. *Prev Cardiol*. 2001; 4(3):103-8.

65. Grundy SM. Cholesterol Metabolism in Man. *West J Med*. 1978; 128(1):13-25.

66. Grundy SM, Florentin L, Nix D, Whelan MF. Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. *Am J Clin Nutr*. 1988; 47:965-9.

67. Gupta AK, Ross EA, Myers JN, Kashyap ML. Increased reverse cholesterol transport in athletes. *Metabolis*. 1993; 42:684-90.

68. Guskiewicz K, McCrea M, Marshall S, Cantu R, Randolph C, Barr W, Onate J, Kelly J. Cumulative Effects Associated with Recurrent Concussion in Collegiate Football Players: The NCAA Concussion Study. *J Am Med Assoc*. 2003; 290(19):2549-55.

69. Halson S, Jeukendrup A. Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Med*. 2004; 34(14):967-81.

70. Harp JB, Hecht L. Obesity in the National Football League. *J Am Med Assoc*. 2005; 293(9):1061-2.

71. Harris JA, Benedict FG. A biometric study of the basal metabolism in man. *Proc Natl Acad Sci U S A*. 1918; 4(12):370-3.

72. Harris W, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med*. 2004; 39(1):212-20.

73. Harris W, Bulchandani D. Why do omega-3 fatty acids lower serum triglycerides? *Curr Opin Lipidol*. 2006; 17(4):387.

74. Harris W. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr*. 1997; 65(5):1645S.

75. Herbenick M, King J, Altobelli G, Nguyen B, Podesta L. Injury Patterns in Professional Arena Football. *Am J Sport Med*. 2008; 36(1):91.

76. Herzberg G, Skinner C. Differential accumulation and release of long-chain n-3 fatty acids from liver, muscle, and adipose tissue triacylglycerols. *Can J Physiol Pharmacol*. 1997; 75(8):945.

77. Hill JO, Peters JC, Swift LL, Yang D, Sharp T, Abumrad N, Greene HL. Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. *Journal of lipid research*. 1990; 31(3):407-16.

78. Hill A. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. *Am J Clin Nutr*. 2007; 85(5):1267.

79. Hill A. n -3 Fatty acid supplementation and regular moderate exercise: differential effects of a combined intervention on neutrophil function. *Brit J Nutr*. 2007; 98(02):300-9.

80. Hoffman JR, Kang J, Ratamess NA, Faigenbaum AD. Biochemical and Hormonal Responses during an Intercollegiate Football Season. *Med Sci Sport Exer*. 2005; 37(7):1237-41.

81. Holloszy J, Coyle E. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol Respir Environ Exerc Physiol*. 1984; 56(4):831-8.

82. Holway FE, Spriet LL. Sport-specific nutrition: Practical strategies for team sports. *J Sports Sci*. 2011; 29(1):S115-25.

83. Horowitz JF. Lipid metabolism during endurance exercise. *Am J Clin Nutr*. 2000; 72(2):558-63.

84. Horowitz J. Fatty acid mobilization from adipose tissue during exercise. *Trends Endocrinol Metab*. 2003; 14(8):386-92.

85. Houmard JA, Bruno NJ, Bruner RK, McCammon MR, Israel RG, Barakat HA. Effects of exercise training on the chemical composition plasma LDL. *Arterioscler Thromb*. 1994; 14:325-30.

86. Hubinger L. Acute effects of treadmill running on lipoprotein(a) levels in males and females. *Med Sci Sport Exer*. 1997; 29(4):436-42.

87. Hulbert A, Else P. Membranes as Possible Pacemakers of Metabolism. *J Theor Biol*. 1999; 199(3):257-74.

88. Innis S. Essential dietary lipids. In: Ziegler EE, Filer LJ, editors. *Present Knowledge in Nutrition*. Washington D.C.: ILSI Press; 1996, p. 58-66.

89. Iosia MF, Bishop PA. Analysis of Exercise-to-Rest Ratios During Division IA Televised Football Competition. *J Strength Cond Res*. 2008; 22(2):332-40.

90. Irick E. *Student-Athlete Participation 1981-1982-2011-12: NCAA Sports Sponsorship and Participation Rates Report*. Indianapolis, IN; 2012. 1 p. Available From: NCAA.

91. Jeukendrup AE. Fat metabolism during exercise: a review. Part I: fatty acid mobilization and muscle metabolism. *Int J Sports Med*. 1998; 19(4):231.

92. Jeukendrup AE, Gleeson M. *Sport Nutrition: An Introduction to Energy Production and Performance.* Champaign, IL: Human Kinetics; 2004. 411 p.

93. Jouris KB, McDaniel JL, Weiss EP. The effect of omega-3 fatty acid supplementation on the inflammatory response to eccentric strength exercise. *J Sports Sci Med*. 2011; 10(3):432-8.

94. Jump DB. The Biochemistry of n-3 Polyunsaturated Fatty Acids. *J Biol Chem*. 2002; 277(11):8755-8.

95. Kaiser GE, Womack JW, Green JS, Pollard B, Miller GS, Crouse SF. Morphological Profiles for First-Year National Collegiate Athletic Association Division I Football Players. *J Strength Cond Res*. 2008; 22(1):243-9.

96. Kamada T, Tokuda S, Aozaki S, Otsuji S. Higher levels of erythrocyte membrane fluidity in sprinters and long-distance runners. *J Appl Physiol*. 1993; 74(1):354-8.

97. Kang J, Weylandt K. Modulation of inflammatory cytokines by omega-3 fatty acids. *Subcell Biochem*. 2008; 49:133.

98. Kaplan L, Flanigan D, Norwig J, Jost P, Bradley J. Prevalence and variance of shoulder injuries in elite collegiate football players. *Am J Sport Med*. 2005; 33(8):1142-6.

99. Kasapis C, Thompson PD. The Effects of Physical Activity on Serum C-Reactive Protein and Inflammatory Markers. *J Am Coll Cardiol*. 2005; 45(10):1563-9.

100. Keller C, Steensberg A, Hansen A, Fischer C, Plomgaard P, Pedersen B. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. *J Appl Physiol*. 2005; 99(6):2075-9.

101. Kelley DS, Siegel D, Fedor DM, Adkins Y, Mackey BE. DHA supplementation decreases serum C-reactive protein and other markers of inflammation in hypertriglyceridemic men. *J Nutr*. 2009; 139(3):495-501.

102. Kelley DS, Taylor PC, Nelson GJ, Schmidt PC, Ferretti A, Erickson KL, Yu R, Chandra RK, Mackey B. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. *Lipids*. 1999; 34(4):317-24.

103. Kelley D, Siegel D, Vemuri M, Chung G, Mackey B. Docosahexaenoic acid supplementation decreases remnant-like particle-cholesterol and increases the (n-3) index in hypertriglyceridemic men. *J Nutr*. 2008; 138(1):30.

104. Kelley D, Siegel D, Vemuri M, Mackey B. Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am J Clin Nutr*. 2007; 86(2):324.

105. Kiens B, Lithell H. Lipoprotein metabolism influenced by training-induced changes in human skeletal muscle. *J Clin Invest*. 1989; 83:558-64.

106. Kiens B. Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiol Rev*. 2006; 86(1):205.

107. Kirwan RD, Kordick LK, McFarland S, Lancaster D, Clark K, Miles MP. Dietary, anthropometric, blood-lipid, and performance patterns of American College Football Players during 8 weeks of training. *Int J Sport Nutr Exerc Metab*. 2012; 22(6):444-51.

108. Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G. Cytokine Levels in the Serum of Healthy Subjects. *Mediat Inflamm*. 2013; 2013:1-6.

109. Koh T, Pizza F. Do inflammatory cells influence skeletal muscle hypertrophy? *Front Biosci*. 2009; 1:60.

110. Kokkinos PF, Hurley BF, Vaccaro P, Patterson JC, Gardner LB, Ostrove SM, Goldberg AP. Effects of low-repetition and high-repetition resitive training on lipoprotein-lipid profiles. *Med Sci Sport Exer*. 1988; 20:50-4.

111. Kopecky J, Rossmeisl M, Flachs P, Kuda O, Brauner P, Jilkova Z, Stankova B, Tvrzicka E, Bryhn M. n -3 PUFA: bioavailability and modulation of adipose tissue function. *P Nutr Soc*. 2009; 68(04):361-9.

112. Kraemer WJ, Torine JC, Silvestre R, French DN, Ratamess NA, Spiering BA, Hatfield DL, Vingren JL, Volek JS. Body size and composition of national football league players. *J Strength Cond Res*. 2005; 19(3):485-9.

113. Krauss RM. Regulation of high-density lipoprotein levels. *Med Clin N Am*. 1982; 66:403-30.

114. Kreider R, Fry A, O'Toole M. *Overtraining in Sport.* Champaign, IL: Human Kinetics; 1998.

115. Kris-Etherton P, Harris W, Appel L. American Heart Association Scientific Statement: Fish Consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 2002; 106:2747-57.

116. Lambert BS, Oliver JM, Katts GR, Green JS, Martin SE, Crouse SF. DEXA or BMI: Clinical Considerations for Evaluating Obesity in Collegiate Division I-A American Football Athletes. *Clin J Sport Med*. 2012; 22(5):436-8.

117. Lawrence T. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol*. 2002; 2(10):787-95.

118. Levy B, Clish C, Schmidt B, Gronert K, Serhan C. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol*. 2001; 2(7):612-9.

119. Lora K, Lewis N. Omega-3 and Omega-6 Fatty Acids. In: Driskell JA(A, editor. *Sports Nutrition : Fats and Proteins*. Boca Raton: Boca Raton : CRC Press; 2007.

120. MacIntyre DL. Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *Eur J Appl Physiol*. 2001; 84(3):180.

121. MacKinnon LT. Special feature for the Olympics: effects of exercise on the immune system: overtraining effects on immunity and performance in athletes. *Immunol Cell Biol*. 2000; 78(5):502-9.

122. Maki K, Van Elswyk M, McCarthy D, Hess S, Veith P, Bell M, Subbaiah P, Davidson M. Lipid responses to a dietary docosahexaenoic acid supplement in men and women with below average levels of high density lipoprotein cholesterol. *J Am Coll Nutr*. 2005; 24(3):189.

123. Marniemi J, Dahlstrom S, Kvist M, Seppanen A, Hietanen E. Dependence of serum lipid and lecithin: cholesterol acyltransferase levels on physical training of young men. *Eur J Appl Physiol*. 1982; 49:25-35.

124. Martorell M, Capó X, Sureda A, Tur JA, Pons A. Effects of docosahexaenoic acid diet supplementation, training, and acute exercise on oxidative balance in neutrophils. *Appl Physiol Nutr Metab*. 2013:1-12.

125. McDaniel J, Ahijevych K, Belury M. Effect of n-3 oral supplements on the n-6/n-3 ratio in young adults. *West J Nurs Res*. 2010; 32(1):64.

126. Meeusen R, Duclos M, Foster C, Fry A, Gleeson M, Nieman D, Raglin J, Rietjens G, Steinacker J, Urhausen A. Prevention, diagnosis and treatment of the overtraining syndrome: Joint consensus statement of the European College of Sport Science (ECSS) and the American College of Sports Medicine (ACSM). *Eur J Sport Sci*. 2013; 13(1):1-24.

127. Miller M, Beach V, Sorkin JD, Mangano C, Dobmeier C, Novacic D, Rhyne J, Vogel RA. Comparative effects of three popular diets on lipids, endothelial function, and C-reactive protein during weight maintenance. *J Am Diet Assoc*. 2009; 109(4):713-7.

128. Mitchell T. Exercise alters the profile of phospholipid molecular species in rat skeletal muscle. *J Appl Physiol*. 2004; 97(5):1823.

129. Mora S, Szklo M, Otvos J, Greenland P, Psaty B, Goff DJ, O'Leary D, Saad M, Tsai M, Sharrett A. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. 2007; 192(1):211-7.

130. Mori T, Watts G, Burke V, Hilme E, Puddey I, Beilin L. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation*. 2000; 102(11):1264.

131. Mori T, Bao D, Burke V, Puddey I, Beilin L. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension*. 1999; 34(2):253.

132. Mori T, Beilin L. Omega-3 fatty acids and inflammation. *Curr Atheroscler Rep*. 2004; 6(6):461.

133. Mori T, Burke V, Puddey I, Watts G, O'Neal D, Best J, Beilin L. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr*. 2000; 71(5):1085.

134. NCAA Academic and Membership Affairs Staff. *2012-2013 NCAA Division 1 Manual*. Indianapolis, Indiana; 2012. Available From: SO&SO Co., LLC.

135. Neff LM. Algal Docosahexaenoic Acid Affects Plasma Lipoprotein Particle Size Distribution in Overweight and Obese Adults. *J Nutr*. 2011; 141(2):207-13.

136. Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Schmitt RL, Bailey EM, Warren BJ, Utter A, Davis JM. Carbohydrate and the cytokine response to 2.5 h of running. *J Appl Physiol*. 1997; 82(5):1662-7.

137. Newsholme EA. Biochemical mechanisms to explain immunosuppression in well-trained and overtrained athletes. *Int J Sports Med*. 1994; 15 Suppl 3:S142-7.

138. Nicholas J, Rosenthal P, Gleim G. A historical perspective of injuries in professional football. Twenty-six years of game-related events. *J Am Med Assoc*. 1988; 260(7):939.

139. Nieman D, Henson D, Davis J, Dumke C, Gross S, Jenkins D, Murphy E, Carmichael M, Quindry J, McAnulty S, McAnulty L, Utter A, Mayer E. Quercetin ingestion does not alter cytokine changes in athletes competing in the Western States Endurance Run. *J Interferon Cytokine Res*. 2007; 27(12):1003.

140. Nieman D. Immune response to heavy exertion. *J Appl Physiol*. 1997; 82(5):1385-94.

141. Nikolaidis M, Mougios V. Effects of exercise on the fatty-acid composition of blood and tissue lipids. *Sports Med*. 2004; 34(15):1051-76.

142. Oliver JM, Dobson JP, Weir D, Bramhall JP, Tanguay J, Smith SB, Martin SE, Fluckey JD, Riechman SE, Crouse SF.
Algal Docosahexaenoic Acid Supplementation Affects Plasma and Erythrocyte Cell Membrane Fatty Acid Content in Division I Football Players During Heavy Physical Training. *Int J Sport Nutr*. in review.

143. Oliver J, Mardock M, Dobson J. Fat Needs of Athletes. In: Taylor L, editor. *Nutritional Guidelines for Athletic Performance*. Boca Raton, FL: CRC Press; 2012, p. 139-154.

144. Oostenbrug GS, Mensink RP, Hardeman MR, De Vries T, Brouns F, Hornstra G. Exercise performance, red blood cell deformability, and lipid peroxidation: effects of fish oil and vitamin E. *J Appl Physiol*. 1997; 83(3):746-52.

145. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *The Journal of Physiology*. 1999; 515(1):287-91.

146. Palacios-Pelaez R, Lukiw W, Bazan N. Omega-3 Essential Fatty Acids Modulate Initiation and Progression of Neurodegenerative Disease. *Mol Neurobiol*. 2010; 41(2-3):367-74.

147. Papanicolaou D. Exercise stimulates interleukin-6 secretion: Inhibition by glucocorticoids and correlation with catecholamines. *Am J Physiol-Endoc M*. 1996; 271(3):E601-5.

148. Park Y, Harris W. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. *J Lipid Res*. 2003; 44(3):455.

149. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw K, Wareham NJ, Forouhi NG. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. *Am J Clin Nutr*. 2010; 92(5):1214.

150. Pedersen B, Ostrowski K, Rohde T, Bruunsgaard H. The cytokine response to strenuous exercise. *Can J Physiol Pharmacol*. 1998; 76(5):505.

151. Pedersen BK. Exercise and cytokines. *Immunol Cell Biol*. 2000; 78(5):532-5.

152. Pedersen B, Toft A. Effects of exercise on lymphocytes and cytokines. *Brit J Sport Med*. 2000; 34(4):246-51.

153. Pedersen B, Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev*. 2000; 80(3):1055-81.

154. Peoples G. Dietary fish oil reduces skeletal muscle oxygen consumption, provides fatigue resistance and improves contractile recovery in the rat in vivo hindlimb. *Brit J Nutr*. 2010; 104(12):1771-9.

155. Peoples G. Fish oil reduces heart rate and oxygen consumption during exercise. *J Cardiovasc Pharm*. 2008; 52(6):540.

156. Petersen A, Pedersen B. The anti-inflammatory effect of exercise. *J Appl Physiol*. 2005; 98(4):1154.

157. Peterson JM, Trappe TA, Mylona E, White F, Lambert CP, Evans WJ, Pizza FX. Ibuprofen and acetaminophen: effect on muscle inflammation after eccentric exercise. *Med Sci Sport Exer*. 2003; 35(6):892-6.

158. Petraglia A, Winkler E, Bailes J. Stuck at the bench: Potential natural neuroprotective compounds for concussion. *Surg Neurol Int*. 2011; 2(1):146-.

159. Pizza FX, McLoughlin TJ, McGregor SJ, Calomeni EP, Gunning WT. Neutrophils injure cultured skeletal myotubes. *Am J Physiol Cell Physiol*. 2001; 281(1):C335-41.

160. Posner B, Cupples L, Franz M, Gagnon D. Diet and heart-disease risk-factors in adult American men and women - The Framingham Offspring-spouse Nutrition Studies. *Int J Epidemiol*. 1993; 22(6):1014-25.

161. Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J Clin Invest*. 2006; 116(12):3090-100.

162. Robinson L, Buchholz A, Mazurak V. Inflammation, obesity, and fatty acid metabolism: influence of n-3 polyunsaturated fatty acids on factors contributing to metabolic syndrome. *Appl Physiol Nutr Metab*. 2007; 32(6):1008.

163. Roche HM. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. *Am J Clin Nutr*. 2000; 71(1 Suppl):232S-7S.

164. Rupp H, Wagner D, Rupp T, Schulte L, Maisch B. Risk stratification by the "EPA+DHA level" and the "EPA/AA ratio" focus on anti-inflammatory and antiarrhythmogenic effects of long-chain omega-3 fatty acids. *Herz*. 2004; 29(7):673-85.

165. Ryan AS, Keske MA, Hoffman JP, Nelson EB. Clinical Overview of Algal-Docosahexaenoic Acid: Effects on Triglyceride Levels and Other Cardiovascular Risk Factors. *Am J Ther*. 2009; 16(2):183-92.

166. Savill J. Apoptosis in resolution of inflammation. *Kidney Blood Press Res*. 2000; 23(3-5):173-4.

167. Schaefer E. Lipoproteins, nutrition, and heart disease. (Special Article). *Am J Clin Nutr*. 2002; 75(2):191.

168. Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. *Prog Lipid Res*. 2008; 47(2):147.

169. Schwellenbach L. The triglyceride-lowering effects of a modest dose of docosahexaenoic acid alone versus in combination with low dose eicosapentaenoic acid

in patients with coronary artery disease and elevated triglycerides. *J Am Coll Nutr*. 2006; 25(6):480-5.

170. Schwingshackl L, Hoffmann G. Long-term effects of low-fat diets either low or high in protein on cardiovascular and metabolic risk factors: a systematic review and meta-analysis. *Nutr J*. 2013; 12(48):1-9.

171. Seip RL, Moulin P, Cocke T, Tall A, Kohrt WM, Mankowitz K, Semenkovich CF, Ostlund R, Schonfeld G. Exercise training decreases plasma cholesteryl ester transfer protein. *Arterioscler Thromb*. 1993; 13(9):1359-67.

172. Serhan C, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol*. 2005; 6(12):1191.

173. Shaikh PZ. Cytokines & their physiologic and pharmacologic functions in inflammation: A review. *Int J of Pharm & Life Sci*. 2011; 2(11):1247-63.

174. Sharp N, Koutedakis Y. Sport and the overtraining syndrome: immunological aspects. *Brit Med Bull*. 1992; 48(3):518-33.

175. Simonson S. The Immune Response to Resistance Exercise. *J Strength Cond Res*. 2001; 15(3):378.

176. Simopoulos A. Omega-3 fatty acids and athletics. *Curr Sports Med Rep*. 2007; 6(4):230.

177. Simopoulos A. *Trans*fatty acids. In: Spiller G, editor. *Handbook of Lipids in Human Nutrition*. Boca Raton: CRC Press; 1995c, p. 91-99.

178. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*. 2002; 56(8):365-79.

179. Slentz CA, Tanner CJ, Bateman LA, Durheim MT, Huffman KM, Houmard JA, Kraus WE. Effects of exercise training intensity on pancreatic B-cell function. *Diabetes Care*. 2009; 32:1807-11.

180. Smith L. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sport Exer*. 2000; 32(2):317.

181. Smith L. Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med Sci Sport Exer*. 1991; 23(5).

182. Smith L. Tissue trauma: the underlying cause of overtraining syndrome? *J Strength Cond Res*. 2004; 18(1):185.

183. Spector AA. Lipid metabolism: essential fatty acids. In: Stipanuk MH, editor. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia, PA: W.B. Saunders Company; 2000, p. 365-383.

184. Steensberg A. IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. *Am J Physiol-Endoc M*. 2002; 283(6):E1272.

185. Steinke J, Borish L. Cytokines and chemokines. *J Allergy Clin Immunol*. 2006; 117(2):S441-5.

186. Sumikawa K, Mu Z, Inoue T, Okochi T, Yoshida T, Adachi K. Changes in erythrocyte membrane phospholipid composition induced by physical training and physical exercise. *Eur J Appl Physiol O*. 1993; 67(2):132-7.

187. Sun Q, Ma J, Campos H, Hankinson S, Hu F. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *Am J Clin Nutr*. 2007; 86(1):74.

188. Superko HR. Exercise and lipoprotein metabolism. *J Cardiovasc Risk*. 1995; 2(4):310-5.

189. Superko HR. Exercise training, serum lipids and lipoprotein particles: is there a change threshold? *Med Sci Sports Exerc*. 1991; 23:677-85.

190. The Nation Federation of State High School Associations. *2012-2013 High School Athletics Participation Survey*. Indianapolis, IN; 2014. 52 p. Available From: NFHS.

191. Theobald H, Chowienczyk P, Whittall R, Humphries S, Sanders T. LDL cholesterol-raising effect of low-dose docosahexaenoic acid in middle-aged men and women. *Am J Clin Nutr*. 2004; 79(4):558.

192. Thomas TR, Adeniran SB, Iltis PW, Aquiar CA, Albers JJ. Effects of interval and continous running on HDL-cholesterol, apoproteins A-1 and B, and LCAT. *Can J Appl Sports Sci*. 1985; 10:52-9.

193. Thompson PD, Yurgalevitch SM, Flynn MM, Zmuda ZM, Spannausmartin D, Saritelli A, Bausserman L, Herbert PN. Effect of prolonged exercise training without weight loss on high-density lipoprotein metabolism in overweight men. *Metabolism*. 1997; 46:217-23.

194. Thurman D, Branche C, Sniezek J. The Epidemiology of Sports-Related Traumatic Brain Injuries in the United States: Recent Developments. *J Head Trauma Rehabil*. 1998; 13(2):1-8.

195. Tipton K. Nutrition for acute exercise-induced injuries. *Ann Nutr Metab*. 2010; 57 Suppl 2:43.

196. Tiryaki-Sönmez G, Schoenfeld B, Vatansever-Ozen S. Omega-3 fatty acids and exercise: a review of their combined effects on body composition and physical performance. *Biomed Hum Kinetics*. 2011; 3(-1):23-9.

197. Toumi H, Best TM. The inflammatory response: friend or enemy for muscle injury? *Brit J Sport Med*. 2003; 37(4):284-6.

198. Trappe TA, Fluckey JD, White F, Lambert CP, Evans WJ. Skeletal muscle PGF(2)(alpha) and PGE(2) in response to eccentric resistance exercise: influence of ibuprofen acetaminophen. *J Clin Endocr Metab*. 2001; 86(10):5067-70.

199. Troup JM. Method for analyzing blood for lipoprotein components. *United States Patent*. 2010; 11/611,497(US 7,856,323 B2).

200. Tucker AM, Vogel RA, Lincoln AE, Dunn RE, Ahrensfield DC, Allen TW, Castle LW, Heyer RA, Pellman EJ, Strollo PJ, Wilson PWF, Yates AP. Prevalence of Cardiovascular Disease Risk Factors Among National Football League Players. *J Am Med Assoc*. 2009; 301(20):2111-9.

201. Vihko V, Sarviharju PJ, Suominen H. Effect of endurance training on concentrations of individual plasma free fatty acids in young men at rest and after moderate bicycle ergometer exercise. *Ann Med Exp Biol Fenn*. 1973; 51(3):112-7.

202. Wagner D. Eating on the Road: Practical Nutrition Strategies for the Traveling Athlete. *Athl Ther Today*. 2009; 14(5):1-4.

203. Walser B. Omega-3 fatty acid supplementation enhances stroke volume and cardiac output during dynamic exercise. *Eur J Appl Physiol*. 2008; 104(3):455.

204. Walser B. Supplementation with omega-3 polyunsaturated fatty acids augments brachial artery dilation and blood flow during forearm contraction. *Eur J Appl Physiol*. 2006; 97(3):347.

205. Walsh NP. Chapter 8 - Exercise, nutrition and immune function I. Macronutrients and amino acids. In: Gleeson M, editor. *Immune Function in Sport and Exercise*. Edinburgh: Churchill Livingstone; 2006, p. 161-181.

206. Warner JGJ, Ullrich IH, Albrink MJ, Yeater RA. Combined effects of aerobic exercise and omega-3 fatty acids in hyperlipidemic persons. *Med Sci Sport Exer*. 1989; 21(5):498-505.

207. Welch A, Shakya-Shrestha S, Lentjes M, Wareham N, Khaw K. Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the product-precursor ratio corrected of α-linolenic acid to long-chain n-3 polyunsaturated fatty acids: results from the EPIC-Norfolk cohort. *Am J Clin Nutr*. 2010; 92(5):1040.

208. Weldon S, Mullen A, Loscher C, Hurley L, Roche H. Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J Nutr Biochem*. 2007; 18(4):250-8.

209. Weldon S, Mullen A, Loscher C, Hurley L, Roche H. Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J Nutr Biochem*. 2007; 18(4):250-8.

210. Williams PT, Krauss RM, Vranizan KM, Wood PD. Changes in lipoprotein subfractions during diet-induced and exercise-induced weight loss in moderately overweight men. *Circulation*. 1990; 81:1293-304.

211. Williams PT, Krauss RM, Vranizan KM, Albers JJ, Terry RB, Wood PD. Effects of exercise-induced weight loss on low-density lipoprotein subfractions in healthy men. *Arteriosclerosis*. 1989; 9:623-32.

212. Williams T, Peck M. Role of prostaglandin-mediated vasodilatation in inflammation. *Nature*. 1977; 270(5637):530-2.

213. Wirth A, Neermann G, Eckert W, Heuck C, Weicker H. Metabolic response to heavy physical exercise before and after a 3-month training period. *Eur J Appl Physiol O*. 1979; 41(1):51-9.

214. Wong H, Pfeiffer RM, Fears TR, Vermeulen R, Ji S, Rabkin CS. Reproducibility and Correlations of Multiplex Cytokine Levels in Asymptomatic Persons. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(12):3450-6.

215. Wooten JS, Biggerstaff KD, Ben-Ezra V. Responses of LDL and HDL particle size and distribution to omega-3 fatty acid supplementation and aerobic exercise. *J Appl Physiol*. 2009; 107(3):794-800.

216. Wu A, Ying Z, Gomez-Pinilla F. Exercise facilitates the action of dietary DHA on functional recovery after brain trauma. *Neuroscience*. 2013; 248:655-63.

217. Wu A, Ying Z, Gomez-Pinilla F. The salutary effects of DHA dietary supplementation on cognition, neuroplasticity, and membrane homeostasis after brain trauma. *J Neurotrauma*. 2011; 28(10):2113.

218. Ylä Herttuala S, Lipton BA, Rosenfeld ME, Särkioja T, Yoshimura T, Leonard EJ, Witztum JL, Steinberg D. Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci U S A*. 1991; 88(12):5252-6.

219. Zhao G, Etherton TD, Martin KR, Vanden Heuvel JP, Gillies PJ, West SG, Kris-Etherton PM. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. *Biochem Biophys Res Commun*. 2005; 336(3):909-17.

APPENDIX A

CONSENT FORM

Algal DHA Supplementation: Effects on Markers of Inflammation, Muscle Power, and Lipid CHD Risk in Collegiate Football Athletes During Sport Training

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. If you decide to participate in this study, this form will also be used to record your consent.

You have been asked to participate in a research project studying the effects of DHA (docosahexaenoic acid) during summer and fall football camp. DHA is an omega-3 fatty acid that is used in the body for making cell membranes. It is a very important fatty acid in nervous tissue, like the brain, but is also found in muscle. DHA is often obtained through eating certain fish, like tuna and salmon. The DHA in fish comes from the algae they eat as food. Since people usually don't get enough fish in their diet, DHA supplements can provide an extra source of this important nutrient. DHA supplements are usually found in capsule form, and come from fish oil or from algae. Taking DHA has been shown to reduce muscle inflammation, like the type that makes you sore and weak after a hard workout. Also, DHA has been shown to reduce the fats in the blood that can cause heart disease and high-blood pressure. No one has studied football players to find out if taking DHA can reduce muscle inflammation caused by hard workouts. You are being asked to participate in this study to determine if taking DHA supplements during your summer and fall training camps can help prevent some of the muscle inflammation that makes you sore, and causes you to lose muscle power. You will also find out if taking DHA can help change the fats in your blood to make you less likely to get heart disease when you get older.

You were selected to be a possible participant because you are an active student-athlete member of the Texas A&M University football team.

What will I be asked to do?

If you agree to participate in this study, you will be asked to provide three blood samples: 1) at the beginning of summer camp; 2) at the beginning of fall camp; and 3) at the end of fall camp. All of these blood samples will be taken from a vein in your arm where your elbow bends, early in the morning before you eat breakfast. You will be asked not eat or drink anything but water for 10 hours before the blood draw. We will use the blood to measure blood fats and also markers of muscle inflammation that make you sore. You will also be asked to take capsules daily containing either DHA or no DHA (placebo) for about 8 weeks starting in summer camp and continuing until the end

of fall camp. Along with the supplement, you will be asked to fill out three diet records, just like you normally complete with the TAMU performance nutritionist. On each day that a blood sample is taken, the football strength coaches will ask you to perform a vertical jump and horizontal jump test. These tests will allow the coaches to measure your muscle power. By agreeing to participate in this study, you are allowing us to have access to this information. None of the procedures you will be asked to do for this study will interfere with your normal football meetings and practices.

What are the risks involved in this study?
The risks associated with this study include possible soreness and bruising in the arm in which we take your blood. The supplements you take may have a "fishy" taste and could give you a slight stomach-ache, but this is rare. Since we are not doing any other tests other than those you perform for the coaching staff, there are no other risks associated with this study.

What are the possible benefits of this study?
The possible benefits of participation are you will be provided information regarding the current level of fat in your blood as well as your risk for heart disease. You will also receive information about whether or not DHA prevents soreness during summer and fall camp.

Do I have to participate?
No. You do not have to participate. You may decide not to participate or to withdraw at any time without your current or future relations with Texas A&M University being affected. If you decide to participate, you will not receive any benefits from the football coaching staff on or off the field. Additionally, if you decide not to participate, you will not be punished by the coaches on or off the field. The choice to participate or not is freely yours.

Who will know about my participation in this research study?
The records of this study will be kept private and confidential. No identifiers linking you to this study will be included in any sort of report that might be published or presented. Research records with all of your data will be stored securely and only the researchers involved will have access to the records.

Whom do I contact with questions about the research?
If you have questions regarding this study, you may contact the Jon Oliver at 979-845-3997 or joliver@hlkn.tamu.edu or Dr. Stephen Crouse at 979-845-3997 or s-crouse@tamu.edu.

Whom do I contact about my rights as a research participant?
This research study has been reviewed by the Human Subjects' Protection Program and/or the Institutional Review Board at Texas A&M University. For research-related

problems or questions regarding your rights as a research participant, you can contact these offices at (979)458-4067 or irb@tamu.edu.

Signature

Please be sure you have read the above information, asked questions and received answers to your satisfaction. You will be given a copy of the consent form for your records. By signing this document, you consent to participate in this study and understand that your participation is voluntary and will not result in any special treatment by coaches or football staff either on or off the field.

Signature of Participant: _____ Date: _____

Printed Name: _____

Signature of Person Obtaining Consent: _____ Date: _____

Printed Name: _____

APPENDIX B

ADVERSE EVENT REPORT

Texas A&M University
Protocol for Human Subjects in Research

Complete this form and submit with all appropriate documentation to the Institutional Review Board, General Services Complex, 750 Agronomy Rd, Suite 3501, College Station, Texas 77843 (MS 1186). You may contact
Ms. Melissa McIlhaney, Compliance Coordinator, at (979)458-4067. **NOTE**: You are still required to report such events to the sponsor and/or FDA as applicable to your research project.

IRB PROTOCOL #: _____

IRB Office Use Only

Investigator Information

Principal Investigator Name: _____
Faculty ☐ Staff ☐ Graduate Student ☐ Undergraduate Student ☐
Department: _____ College: _____ Mail Stop: _____
Phone: _____ Email: _____ Fax: _____

Co-Investigator Name: _____
Faculty ☐ Staff ☐ Graduate Student ☐ Undergraduate Student ☐
Department: _____ College: _____ Mail Stop: _____
Phone: _____ Email: _____ Fax: _____

Graduate Committee Chair/Faculty Advisor Name (if student): _____
Department: _____ College: _____ Mail Stop: _____
Phone: _____ Email: _____ Fax: _____

Project Title: _____

Funding Status: Funded: ☐ Not Funded ☐
Funding Agency: _____ Funding Amount: _____
Funding Administrator: RF ☐ TAES ☐ TEES ☐ TAMU ☐ TTI ☐

Adverse Event Information
Is this a follow-up report? Yes ☐ No ☐

1. Date of Event: _____

2. Describe the Adverse Event:

   _____

3. Attach a summary of all circumstances related to this event. All hospitalization and/or medical treatment must be reported. Include all notifications, correspondence, and other related materials of this adverse event from the study sponsor or study sites. Include a statement regarding this adverse event and its relation to the study at Texas A&M University.

Signature of PI: _____ Date: _____

Typed Name: _____

Signature of Faculty Advisor (if student): _____ Date: _____

Typed Name: _____

Signature of Department Head: _____ Date: _____

Typed Name: _____

The information provided will be reviewed by the Texas A&M University Institutional Review Board for compliance with federal regulations and the University's Institutional Federal Wide Assurance document approved by OHRP.

APPENDIX C

SUPPLEMENT ADHERENCE CHECKLIST (SAMPLE)

LNAME	FNAME	8/23/2010	8/24/2010	8/25/2010	8/26/2010	8/27/2010	8/28/2010	8/29/2010

APPENDIX D

24 HOUR DIETARY RECALL

Online Diet Recall

Name:	**Sex**:	**Average hours per day spent in class**:
	Height:	**Days per week you have class**:
Sport / Position:		
	Weight:	**Average hours per day spent studying**:
Age / Birth Date:	**Desired Weight**:	**Days per week that you study**:
Year of eligibility:		**Average hours sleeping (*weekday*)**:
i.e. Redshirt fr, soph, junior,etc.		**Average hours sleeping (*weekend*)**:
		Average hours per day spent training:
		Days per week you train:

Nutritional Goals (i.e. What would you like to get back from this record?):

DAY 1

Time:	**Time:**	**Time:**	**Time:**	**Time:**	**Time:**
Breakfast	**Snack / Recovery**	**Lunch**	**Snack / Recovery**	**Dinner**	**Snack / Desert**
Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:
How are you feeling?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?

DAY 2 ***Date:*** */ /*

Time:	**Time:**	**Time:**	**Time:**	**Time:**	**Time:**
Breakfast	**Snack / Recovery**	**Lunch**	**Snack / Recovery**	**Dinner**	**Snack / Desert**
Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:
How are you feeling?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?

DAY 3 ***Date:*** */ /*

Time:	**Time:**	**Time:**	**Time:**	**Time:**	**Time:**
Breakfast	**Snack / Recovery**	**Lunch**	**Snack / Recovery**	**Dinner**	**Snack / Desert**
Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:	Description: Amt.:
How are you feeling?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?	**How are you feeling**?

APPENDIX E

SUPPLEMENT ADHERENCE QUESTIONNAIRE

Supplement Adherence Questionnaire

Name: _____
Age: _____
Ethnicity: _____
Position: _____

Algal DHA Supplementation: Effects on Markers of Inflammation, Muscle Power, and Lipid CHD Risk in Collegiate Football Athletes During Sport Training

1. Have you taken all the supplements as prescribed? Yes □ No □
   a. If no, why not? _____
2. Have you had anything to eat or drink, except water, since 10:00 PM last night? Yes □ No □
   a. If yes, then what have you had to eat or drink? _____
3. How many servings of fish have you had in the last week? 1 2 3 4 5
   a. What kind of fish have you had to eat in the last week? _____
4. Have you taken any other supplements in the last week? Yes □ No □
   a. If yes, then what kind of supplements have you taken? _____
5. Have you had any injuries in the last week? Yes □ No □
   a. If yes, then what type of injury? How treated? _____
6. Have you had an illness in the last week? Yes □ No □
   a. If yes, what kind of illness did you have? How treated? _____
7. Were you excluded or limited from practice in the last week? Yes □ No □
   a. If yes, what for and for how long were you limited or excluded? _____
8. Have you taken ANY medications in the last week? Yes □ No □
   (These include antibiotics, any over the counter medicines, including aspirin, Advil, Aleve, BC powder, allergy medicine or any other medications that have not been listed.)
   a. If yes, what were the names of the medications? _____
9. About how long ago since you last exercised (circle one)? 12hr 24hr 48hr 72hr Longer

1. Supplement Adherence Questionnaire

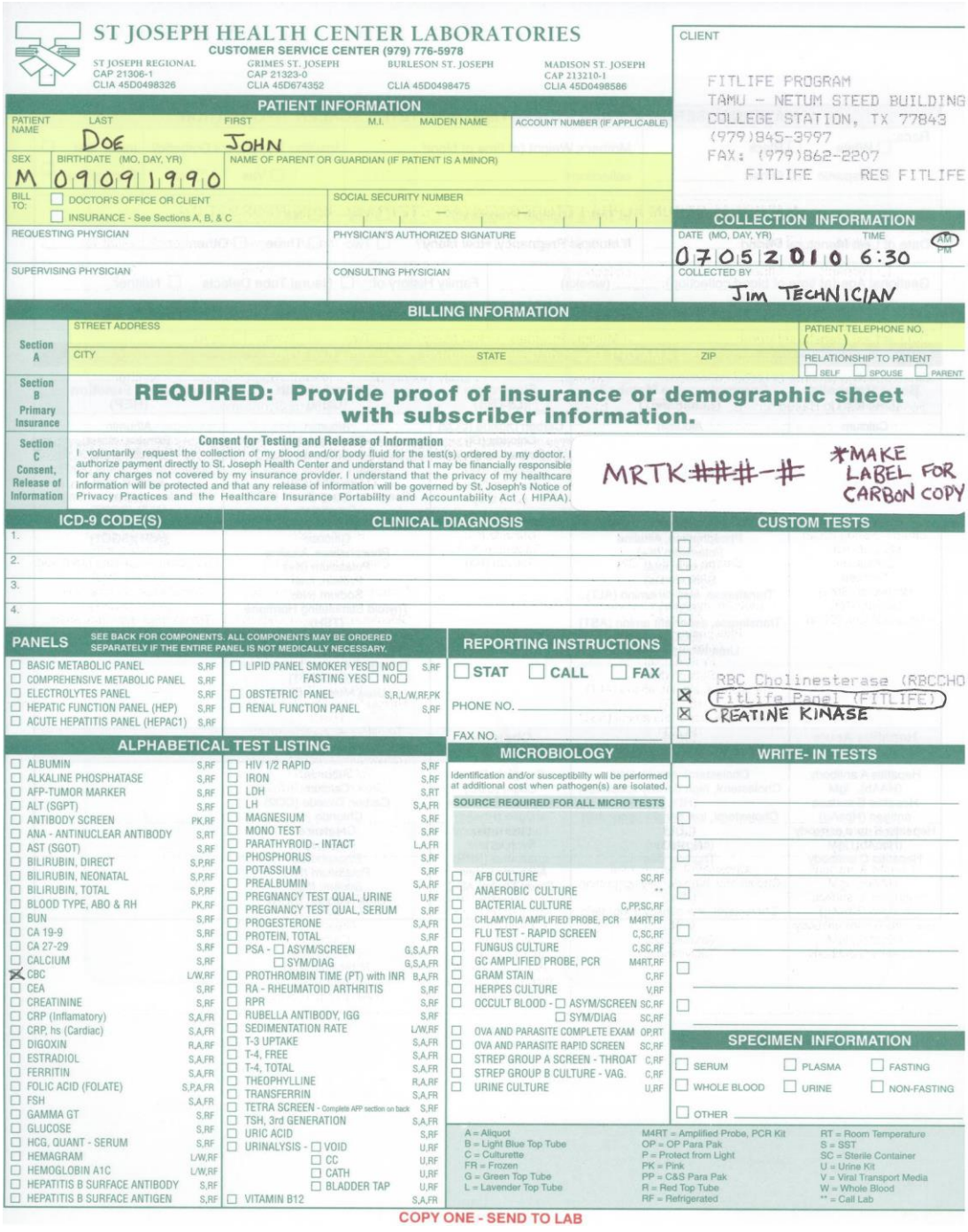
Supplement Adherence Questionnaire

Name: _____
Age: _____
Ethnicity: _____
Position: _____

10. Have you taken all the supplements as prescribed? Yes □ No □
    a. If no, why not? _____
11. Have you had anything to eat or drink, except water, since 10:00 PM last night? Yes □ No □
    a. If yes, then what have you had to eat or drink? _____
12. How many servings of fish have you had in the last week? 1 2 3 4 5
    a. What kind of fish have you had to eat in the last week? _____
13. Have you taken any other supplements in the last week? Yes □ No □
    a. If yes, then what kind of supplements have you taken? _____
14. Have you had any injuries in the last week? Yes □ No □
    a. If yes, then what type of injury? How treated? _____
15. Have you had an illness in the last week? Yes □ No □
    a. If yes, what kind of illness did you have? How treated? _____
16. Were you excluded or limited from practice in the last week? Yes □ No □
    a. If yes, what for and for how long were you limited or excluded? _____
17. Have you taken ANY medications in the last week? Yes □ No □
    (These include antibiotics, any over the counter medicines, including aspirin, Advil, Aleve, BC powder, allergy medicine or any other medications that have not been listed.)
    a. If yes, what were the names of the medications? _____
18. About how long ago since you last exercised (circle one)? 12hr 24hr 48hr 72hr Longer

APPENDIX F

ST. JOSEPH HEALTH CENTER FORM



ST JOSEPH HEALTH CENTER LABORATORIES
CUSTOMER SERVICE CENTER (979) 776-5978
ST JOSEPH REGIONAL CAP 21306-1 CLIA 45D0498326
GRIMES ST. JOSEPH CAP 21323-0 CLIA 45D674352
BURLESON ST. JOSEPH CLIA 45D0498475
MADISON ST. JOSEPH CAP 213210-1 CLIA 45D0498586

CLIENT
FITLIFE PROGRAM
TAMU - NETUM STEED BUILDING
COLLEGE STATION, TX 77843
(979)845-3997
FAX: (979)862-2207
FITLIFE RES FITLIFE

PATIENT INFORMATION
PATIENT NAME LAST: DOE FIRST: JOHN M.I. MAIDEN NAME ACCOUNT NUMBER (IF APPLICABLE)
SEX: M BIRTHDATE (MO, DAY, YR): 09 09 1990 NAME OF PARENT OR GUARDIAN (IF PATIENT IS A MINOR)
BILL TO: ☐ DOCTOR'S OFFICE OR CLIENT ☐ INSURANCE - See Sections A, B, & C SOCIAL SECURITY NUMBER
REQUESTING PHYSICIAN PHYSICIAN'S AUTHORIZED SIGNATURE
SUPERVISING PHYSICIAN CONSULTING PHYSICIAN

COLLECTION INFORMATION
DATE (MO, DAY, YR): 07 05 2010 TIME: 6:30 AM PM
COLLECTED BY: JIM TECHNICIAN

BILLING INFORMATION
Section A: STREET ADDRESS CITY STATE ZIP PATIENT TELEPHONE NO. ( ) RELATIONSHIP TO PATIENT ☐ SELF ☐ SPOUSE ☐ PARENT
Section B Primary Insurance: REQUIRED: Provide proof of insurance or demographic sheet with subscriber information.
Section C Consent, Release of Information: Consent for Testing and Release of Information
I voluntarily request the collection of my blood and/or body fluid for the test(s) ordered by my doctor. I authorize payment directly to St. Joseph Health Center and understand that I may be financially responsible for any charges not covered by my insurance provider. I understand that the privacy of my healthcare information will be protected and that any release of information will be governed by St. Joseph's Notice of Privacy Practices and the Healthcare Insurance Portability and Accountability Act (HIPAA).

MRTK###-# *MAKE LABEL FOR CARBON COPY

ICD-9 CODE(S) 1. 2. 3. 4.
CLINICAL DIAGNOSIS

CUSTOM TESTS
☐ RBC Cholinesterase (RBCCHO
☒ FitLife Panel (FITLIFE)
☒ CREATINE KINASE

PANELS — SEE BACK FOR COMPONENTS. ALL COMPONENTS MAY BE ORDERED SEPARATELY IF THE ENTIRE PANEL IS NOT MEDICALLY NECESSARY.
☐ BASIC METABOLIC PANEL S,RF
☐ COMPREHENSIVE METABOLIC PANEL S,RF
☐ ELECTROLYTES PANEL S,RF
☐ HEPATIC FUNCTION PANEL (HEP) S,RF
☐ ACUTE HEPATITIS PANEL (HEPAC1) S,RF
☐ LIPID PANEL SMOKER YES☐ NO☐ FASTING YES☐ NO☐ S,RF
☐ OBSTETRIC PANEL S,R,L/W,RF,PK
☐ RENAL FUNCTION PANEL S,RF

REPORTING INSTRUCTIONS
☐ STAT ☐ CALL ☐ FAX
PHONE NO. _____
FAX NO. _____

ALPHABETICAL TEST LISTING
☐ ALBUMIN S,RF
☐ ALKALINE PHOSPHATASE S,RF
☐ AFP-TUMOR MARKER S,RF
☐ ALT (SGPT) S,RF
☐ ANTIBODY SCREEN PK,RF
☐ ANA - ANTINUCLEAR ANTIBODY S,RT
☐ AST (SGOT) S,RF
☐ BILIRUBIN, DIRECT S,P,RF
☐ BILIRUBIN, NEONATAL S,P,RF
☐ BILIRUBIN, TOTAL S,P,RF
☐ BLOOD TYPE, ABO & RH PK,RF
☐ BUN S,RF
☐ CA 19-9 S,RF
☐ CA 27-29 S,RF
☐ CALCIUM S,RF
☒ CBC L/W,RF
☐ CEA S,RF
☐ CREATININE S,RF
☐ CRP (Inflamatory) S,A,FR
☐ CRP, hs (Cardiac) S,A,FR
☐ DIGOXIN R,A,RF
☐ ESTRADIOL S,A,FR
☐ FERRITIN S,A,FR
☐ FOLIC ACID (FOLATE) S,P,A,FR
☐ FSH S,A,FR
☐ GAMMA GT S,RF
☐ GLUCOSE S,RF
☐ HCG, QUANT - SERUM S,RF
☐ HEMAGRAM L/W,RF
☐ HEMOGLOBIN A1C L/W,RF
☐ HEPATITIS B SURFACE ANTIBODY S,RF
☐ HEPATITIS B SURFACE ANTIGEN S,RF
☐ HIV 1/2 RAPID S,RF
☐ IRON S,RF
☐ LDH S,RT
☐ LH S,A,FR
☐ MAGNESIUM S,RF
☐ MONO TEST S,RF
☐ PARATHYROID - INTACT L,A,FR
☐ PHOSPHORUS S,RF
☐ POTASSIUM S,RF
☐ PREALBUMIN S,A,RF
☐ PREGNANCY TEST QUAL, URINE U,RF
☐ PREGNANCY TEST QUAL, SERUM S,RF
☐ PROGESTERONE S,A,FR
☐ PROTEIN, TOTAL S,RF
☐ PSA - ☐ ASYM/SCREEN G,S,A,FR ☐ SYM/DIAG G,S,A,FR
☐ PROTHROMBIN TIME (PT) with INR B,A,FR
☐ RA - RHEUMATOID ARTHRITIS S,RF
☐ RPR S,RF
☐ RUBELLA ANTIBODY, IGG S,RF
☐ SEDIMENTATION RATE L/W,RF
☐ T-3 UPTAKE S,A,FR
☐ T-4, FREE S,A,FR
☐ T-4, TOTAL S,A,FR
☐ THEOPHYLLINE R,A,RF
☐ TRANSFERRIN S,A,FR
☐ TETRA SCREEN - Complete AFP section on back S,RF
☐ TSH, 3rd GENERATION S,A,FR
☐ URIC ACID S,RF
☐ URINALYSIS - ☐ VOID U,RF ☐ CC U,RF ☐ CATH U,RF ☐ BLADDER TAP U,RF
☐ VITAMIN B12 S,A,FR

MICROBIOLOGY
Identification and/or susceptibility will be performed at additional cost when pathogen(s) are isolated.
SOURCE REQUIRED FOR ALL MICRO TESTS
☐ AFB CULTURE SC,RF
☐ ANAEROBIC CULTURE **
☐ BACTERIAL CULTURE C,PP,SC,RF
☐ CHLAMYDIA AMPLIFIED PROBE, PCR M4RT,RF
☐ FLU TEST - RAPID SCREEN C,SC,RF
☐ FUNGUS CULTURE C,SC,RF
☐ GC AMPLIFIED PROBE, PCR M4RT,RF
☐ GRAM STAIN C,RF
☐ HERPES CULTURE V,RF
☐ OCCULT BLOOD - ☐ ASYM/SCREEN SC,RF ☐ SYM/DIAG SC,RF
☐ OVA AND PARASITE COMPLETE EXAM OP,RT
☐ OVA AND PARASITE RAPID SCREEN SC,RF
☐ STREP GROUP A SCREEN - THROAT C,RF
☐ STREP GROUP B CULTURE - VAG. C,RF
☐ URINE CULTURE U,RF

WRITE- IN TESTS

SPECIMEN INFORMATION
☐ SERUM ☐ PLASMA ☐ FASTING
☐ WHOLE BLOOD ☐ URINE ☐ NON-FASTING
☐ OTHER _____

A = Aliquot
B = Light Blue Top Tube
C = Culturette
FR = Frozen
G = Green Top Tube
L = Lavender Top Tube
M4RT = Amplified Probe, PCR Kit
OP = OP Para Pak
P = Protect from Light
PK = Pink
PP = C&S Para Pak
R = Red Top Tube
RF = Refrigerated
RT = Room Temperature
S = SST
SC = Sterile Container
U = Urine Kit
V = Viral Transport Media
W = Whole Blood
** = Call Lab

COPY ONE - SEND TO LAB

APPENDIX G

RANDOMIZATION DATA AND DEMOGRAPHICS

OL		DL		LB	
MRTK002	4	MRTK009	4	MRTK001	3
MRTK016	2	MRTK011	4	MRTK005	4
MRTK017	2	MRTK023	3	MRTK008	4
MRTK026	3	MRTK056	3	MRTK010	2
MRTK036	4			MRTK018	3
MRTK049	2			MRTK020	4
				MRTK027	3
				MRTK037	2
				MRTK043	1
				MRTK058	1
DB		QBR		OB	
MRTK003	3	MRTK004	2	MRTK021	1
MRTK007	4	MRTK006	4	MRTK024	1
MRTK019	2	MRTK013	2	MRTK031	2
MRTK025	2	MRTK014	2	MRTK032	1
MRTK030	2	MRTK028	4	MRTK033	4
MRTK035	1	MRTK029	3	MRTK034	2
MRTK038	1	MRTK040	4	MRTK051	3
MRTK041	2	MRTK042	3		
MRTK044	4	MRTK050	2	K	
MRTK060	4	MRTK052	2	MRTK012	2
		MRTK055	1	MRTK015	2
		MRTK057	4	MRTK022	2
				MRTK039	3

Group #	Capsules	Color
3	placebo	Burgundy
2	placebo	Silver
1	active	Blue
4	active	White

Assignments

SID	Ethnicity	Position	COLOR	TREATMENT
MRTK001	B	LB	Burgundy	Placebo
MRTK002	W	OL	White	DHA
MRTK003	B	DB	Burgundy	Placebo

MRTK004	W	QBR	Silver	Placebo
MRTK005	B	LB	White	DHA
MRTK006	W	QBR	White	DHA
MRTK007	B	DB	White	DHA
MRTK008	B	LB	White	DHA
MRTK009	B	DL	White	DHA
MRTK010	B	LB	Silver	Placebo
MRTK011	B	DL	White	DHA
MRTK012	H	DS	Silver	Placebo
MRTK013	B	QBR	Silver	Placebo
MRTK014	B	QBR	Silver	Placebo
MRTK015	W	K	Silver	Placebo
MRTK016	W	OL	Silver	Placebo
MRTK017	W	OL	Silver	Placebo
MRTK018	B	LB	Burgundy	Placebo
MRTK019	W	DB	Silver	Placebo
MRTK020	W	LB	White	DHA
MRTK021	B	OB	Blue	DHA
MRTK022	H	K	Silver	Placebo
MRTK023	B	DL	Burgundy	Placebo
MRTK024	B	OB	Blue	DHA
MRTK025	B	DB	Silver	Placebo
MRTK026	H	OL	Burgundy	Placebo
MRTK027	B	LB	Burgundy	Placebo
MRTK028	W	QBR	White	DHA
MRTK029	W	QBR	Burgundy	Placebo
MRTK030	W	DB	Silver	Placebo
MRTK031	B	OB	Silver	Placebo
MRTK032	W	OB	Blue	DHA
MRTK033	B	OB	White	DHA
MRTK034	B	OB	Silver	Placebo
MRTK035	B	DB	Blue	DHA
MRTK036	W	OL	White	DHA
MRTK037	B	LB	Silver	Placebo
MRTK038	W	DB	Blue	DHA
MRTK039	W	K	Burgundy	Placebo
MRTK040	W	QBR	White	DHA
MRTK041	B	DB	Silver	Placebo
MRTK042	B	QBR	Burgundy	Placebo
MRTK043	B	LB	Blue	DHA

MRTK044	B	DB	White	DHA
MRTK045	W	K	White	DHA
MRTK046	W	QB	Blue	DHA
MRTK048	H	FB	Burgundy	Placebo
MRTK049	B	OL	Silver	Placebo
MRTK050	W	QBR	Silver	Placebo
MRTK051	B	OB	Burgundy	Placebo
MRTK052	B	QBR	Silver	Placebo
MRTK053	W	LB	White	DHA
MRTK054	W	TE	White	DHA
MRTK055	B	QBR	Blue	DHA
MRTK056	B	DL	Burgundy	Placebo
MRTK057	B	QBR	White	DHA
MRTK058	W	LB	Blue	DHA
MRTK059	W	K	Burgundy	Placebo
MRTK060	W	DB	White	DHA
MRTK062	B	OL	Blue	DHA

Demographics

SID	TREATMENT	AGE	HEIGHT	WEIGHT	BMI
MRTK002	DHA	21	191.008	140.1599	38.41676
MRTK005	DHA	19	186.182	97.52228	28.1338
MRTK007	DHA	19	179.832	76.65705	23.70381
MRTK008	DHA	21	189.23	109.7693	30.65496
MRTK009	DHA	20	193.04	136.5312	36.63846
MRTK011	DHA	21	193.04	124.2842	33.35196
MRTK020	DHA	24	182.88	101.151	30.2439
MRTK024	DHA	19	189.23	91.62558	25.58803
MRTK028	DHA	23	184.912	80.73938	23.61322
MRTK033	DHA	18	193.04	107.0477	28.7265
MRTK035	DHA	19	180.594	84.36811	25.86853
MRTK036	DHA	21	195.58	138.7992	36.2859
MRTK038	DHA	22	195.58	119.7483	31.30548
MRTK040	DHA	19	185.674	87.99685	25.52495
MRTK043	DHA	22	177.8	90.7184	28.69669
MRTK044	DHA	23	185.42	87.99685	25.59493
MRTK045	DHA	20	193.04	117.9339	31.64784
MRTK053	DHA	21	187.96	105.6869	29.9151
MRTK057	DHA	20	193.04	99.79024	26.77894
MRTK058	DHA	21	187.706	102.0582	28.9662
MRTK060	DHA	20	191.008	120.2019	32.94641
MRTK062	DHA	19	182.88	139.7063	41.77185

MRTK001	Placebo	18	177.8	102.5118	32.42726
MRTK004	Placebo	22	192.278	98.42946	26.62355
MRTK010	Placebo	19	184.404	108.8621	32.01371
MRTK016	Placebo	21	196.85	138.3456	35.70215
MRTK018	Placebo	21	187.96	105.6869	29.9151
MRTK019	Placebo	23	187.96	95.25432	26.96211
MRTK023	Placebo	20	189.23	117.9339	32.93508
MRTK025	Placebo	19	172.72	73.02831	24.4797
MRTK026	Placebo	20	195.58	129.2737	33.79569
MRTK027	Placebo	20	192.278	100.2438	27.1143
MRTK029	Placebo	20	170.434	84.36811	29.04463
MRTK030	Placebo	18	185.166	89.35762	26.06208
MRTK034	Placebo	23	187.96	102.0582	28.88797
MRTK037	Placebo	20	186.944	95.25432	27.25597
MRTK039	Placebo	20	186.436	87.54326	25.18623
MRTK041	Placebo	18	191.77	112.4908	30.58833
MRTK042	Placebo	19	191.77	100.2438	27.25815
MRTK049	Placebo	20	190.5	141.5207	38.99689
MRTK051	Placebo	21	178.816	88.45044	27.66223
MRTK052	Placebo	20	182.626	87.08966	26.11208
MRTK056	Placebo	20	189.484	125.1914	34.86819

APPENDIX H

CHAPTER 2: CYTOKINE RAW DATA

SID	TREATMENT	Eotaxin 1	Eotaxin 2	Eotaxin 3	IFN-g 1	IFN-g 2	IFN-g 3	IL-6 1	IL-6 2	IL-6 3
MRTK002	DHA	77.93	56.99	71.89	6.16	10.88	7.9	42.12	41.82	30.19
MRTK005	DHA	49.56	55.38	42.16	33.12	4.39	0.73	13.56	0.355	0.355
MRTK007	DHA	56.46	38.91	40.1	0.73	0.73	1.55	0.355	0.355	0.355
MRTK008	DHA	53.33	59.35	58.9	4.51	4.6	5.06	14.11	15.72	20.37
MRTK009	DHA	52.03	62.44	65.05	66.97	20.06	23.72	2.71	0.355	0.355
MRTK020	DHA	48.81	84.32	63.96	28.25	35.66	117	1.38	1.38	1.38
MRTK028	DHA	29.69	46.29	54.84	1.39	2.11	1.88	1.01	8.86	11.45
MRTK036	DHA	61.19	72.01	51.09	15.6	11.3	17.92	146	0.76	0.76
MRTK038	DHA	43	54.56	28.16	304	93.16	85.21	178	146	115
MRTK040	DHA	47.69	55.7	44.43	12.47	7.16	6.07	0.76	0.76	0.76
MRTK044	DHA	24.85	26.27	29.81	27.84	45.64	80.29	0.76	0.76	0.76
MRTK045	DHA	52.64	77.66	85.54	6.77	16.05	20.36	4.51	11.25	19.25
MRTK053	DHA	79.62	91.31	95.18	204	163	117	43.77	51	18.79
MRTK057	DHA	111	119	76.18	0.71	2.05	2.32	0.76	11.25	6.18
MRTK058	DHA	48.81	47.26	30.8	28.4	17.8	15.48	30.79	14.14	19.14
MRTK011	DHA	47.02	48.07	60.92	97.35	60.36	77.26	31.77	22.71	11.01
MRTK024	DHA	46.17	28.05	66.41	47.89	16.01	150			
MRTK033	DHA	107	93.29	94.29	122	231	236	29.34	43.36	54.88
MRTK035	DHA	80.08	98.58	65	17.84	6.03	7.47	272	163	111
MRTK043	DHA	14.9	10.94	28.59	39.54	51.25	89.23	14.21	9.06	69.29
MRTK060	DHA	45.82	28.36	27.11	8.22	44.76	27.51	0.76	6.88	3.21

SID	TREATMENT	Eotaxin 1	Eotaxin 2	Eotaxin 3	IFN-g 1	IFN-g 2	IFN-g 3	IL-6 1	IL-6 2	IL-6 3
MRTK062	DHA	40.22	36.98	46.55	10.14	15.48	32.24	0.76	0.76	0.76
MRTK001	Placebo	91.98	78.34	59.95	116	66.65	91.94	61.16	28.09	60.75
MRTK004	Placebo	29.94	48.53	40.02	0.73	1.61	0.73	0.355	0.355	0.355
MRTK010	Placebo	59.99	49.25	50.6	189	112	95.79	66.16	26.18	24.17
MRTK016	Placebo	16.53	45.22	28.83	4.55	4.24	2.76	1.38	1.38	1.38
MRTK018	Placebo	26.34	40.44	53.92	6.7	14.59	19.77	1.38	18.92	24.81
MRTK019	Placebo	28.63	163	104	16.8	347	187	7.15	198	119
MRTK023	Placebo	27.05	25.45	30.44	12.23	17.76	5.69	0.76	0.76	0.76
MRTK025	Placebo	30.72	49.92	44.19	9.76	539	450			
MRTK026	Placebo	30.72	39	55.52	18.58	16.81	21.95			
MRTK027	Placebo	139	129	160	46.43	74.3	239	111	65.44	121
MRTK029	Placebo	21.05	60.35	55.97	42.55	8.07	39.12			
MRTK030	Placebo	36.13	63.86	42.23	28.76	113	129			
MRTK034	Placebo	46.05	31.66	37.27	2.32	1.52	1.86	9.86	9.61	4.74
MRTK037	Placebo	13.92	27.17	35.07	3.89	6.03	9.89	0.76	0.76	5.45
MRTK039	Placebo	46.26	59.51	53.14	4.97	3.42	3.28	18.15	28.47	13.73
MRTK041	Placebo	68.92	53.76	45.9	178	61.65	97.85	86.48	40.52	92.59
MRTK042	Placebo	29.89	26.27	32.46	35.01	26.98	24.51	37.39	13.19	12.38
MRTK049	Placebo	28.36	32.74	41.63	1.99	4.37	4.33	0.76	0.76	0.76
MRTK051	Placebo	28.74	31.61	31.2	0.71	0.71	0.71	0.76	0.76	0.76
MRTK052	Placebo	31.39	37.66	30	50.23	19.75	34.33	55.48	56.84	60.3
MRTK056	Placebo	28.72	34.82	23.35	0.71	0.71	0.71	0.76	0.76	0.76

SID	TREATMENT	IL-8 1	IL-8 2	IL-8 3	IL-17 1	IL-17 2	IL-17 3	MCP-1	MCP-2	MCP-3
MRTK002	DHA	17.65	22.11	19.98	23.09	45.26	30.67	375	250	346
MRTK005	DHA	14.56	15.38	12.94	36.34	2.87	1.49	232	273	199
MRTK007	DHA	5.93	6.5	9.67	0.325	0.325	0.325	362	270	343
MRTK008	DHA	11.68	17.53	15.07	1.14	0.88	1.21	214	277	227
MRTK009	DHA	5.35	3.98	1.2	9.02	3.15	6.6	150	162	182
MRTK020	DHA	3.87	6.12	15.62	0.585	2.27	14.34	179	240	210
MRTK028	DHA	4.46	7.39	9.65	1.53	2.82	3.59	149	197	203
MRTK036	DHA	33.17	9.74	8.13	4.86	4.34	6.52	255	366	273
MRTK038	DHA	122	38.6	47.19	120	36.47	31.47	84.08	279	129
MRTK040	DHA	6.34	4.57	2.82	3.65	1.13	1.13	213	270	184
MRTK044	DHA	0.495	2.57	5.9	5.31	8.24	12.14	243	217	198
MRTK045	DHA	9.8	16.6	21.78	7.08	9.13	10.97	124	206	159
MRTK053	DHA	52.78	49.58	40.09	49.12	46.22	27.79	276	354	362
MRTK057	DHA	7.85	7.89	7.36	1.13	1.13	1.13	348	302	237
MRTK058	DHA	23.32	10.89	18.93	5.97	3.19	2.67	282	227	242
MRTK011	DHA	18.02	10.26	8.57	25.61	16.25	6.28	265	276	254
MRTK024	DHA	78.95	62.52	69.28	51.61	36.41	84.25	169	181	212
MRTK033	DHA	20.5	36.03	32.93	22.51	42.37	37.65	797	923	258
MRTK035	DHA	70.03	64.67	43.09	5.61	2.93	3.2	278	272	178
MRTK043	DHA	90.63	32.26	65.04	102	14.45	27.03	177	285	345
MRTK060	DHA	3.32	5.59	6.68	2.44	8.01	7.13	382	328	240

SID	TREATMENT	IL-8 1	IL-8 2	IL-8 3	IL-17 1	IL-17 2	IL-17 3	MCP-1	MCP-2	MCP-3
MRTK062	DHA	7.65	6.82	15.01	1.13	2.4	2.81	216	251	277
MRTK001	Placebo	105	77.15	71.5	255	167	141	368	448	199
MRTK004	Placebo	7.74	11.55	10.4	2.38	2.34	2.54	226	423	267
MRTK010	Placebo	30.46	25.95	33.76	60.16	65.51	57.07	240	309	279
MRTK016	Placebo	2.45	3.2	1.82	0.585	0.585	0.585	210	514	328
MRTK018	Placebo	3.92	13.2	16.79	2.46	9.65	11.75	125	203	231
MRTK019	Placebo	9.43	59.9	37.23	9.29	143	88.19	137	470	327
MRTK023	Placebo	4.24	3.96	0.495	4.24	5.61	3.38			
MRTK025	Placebo	7.71	119	108	1.74	50.76	48.65	77.33	200	145
MRTK026	Placebo	10.78	13.98	2.49	7.5	8.92	10.57	192	231	308
MRTK027	Placebo	51.86	41.95	64.47	39.8	31.07	74.35	143	295	217
MRTK029	Placebo	21.3	7.19	14.73	16.28	5.12	18.89	112	420	270
MRTK030	Placebo	7.35	23.18	20.68	3.75	19.5	20.24	193	395	261
MRTK034	Placebo	11.94	8.48	10.95	2.35	1.13	1.13	368	329	245
MRTK037	Placebo	2.98	5.66	9.39	1.13	3.17	4.81	176	312	410
MRTK039	Placebo	14.95	22.55	19.72	1.13	3.58	1.13	117	248	168
MRTK041	Placebo	36.29	18.6	26.34	15.82	4.84	14.06	289	249	168
MRTK042	Placebo	221	310	263	230	544	322	144	187	181
MRTK049	Placebo	5.27	6.52	8.65	1.13	1.13	1.13	218	291	301
MRTK051	Placebo	0.495	1.81	2.77	1.13	1.13	1.13	166	167	166
MRTK052	Placebo	61.35	67.48	71.82	54.64	42.54	63.07	142	144	139
MRTK056	Placebo	3.18	4.55	7.54	1.13	1.13	1.13	171	182	172

SID	TREATMENT	RANTES 1	RANTES 2	RANTES 3	TNF-a 1	TNF-a 2	TNF-a 3
MRTK002	DHA	1897	1607	1522	30.98	19.34	18.06
MRTK005	DHA	837	1515	1338	7.76	9.06	7.14
MRTK007	DHA	1859	3134	2480	8.51	8.83	8.84
MRTK008	DHA	2562	3078	2972	6.31	8.74	6.87
MRTK009	DHA	1579	1642	2025	9.54	10.59	13.77
MRTK020	DHA	433	451	432	7.17	6.98	8.73
MRTK028	DHA	1627	4244	4423	4.94	7.34	7.26
MRTK036	DHA	5737	5768	6214	6.94	6.77	6.98
MRTK038	DHA	953	3963	2937	8.4	10.57	5.75
MRTK040	DHA	3863	4210	5376	6.26	6.05	5.37
MRTK044	DHA	5104	5078	5987	3.8	2.69	4.12
MRTK045	DHA	0.945	5.43	5.12	4.05	5.51	5.51
MRTK053	DHA	5858	4658	3877	4.26	3.81	3.91
MRTK057	DHA	5002	5381	5730	5.11	6.71	4.93
MRTK058	DHA	3027	1577	3423	6.16	3.61	5.8
MRTK011	DHA	3232	5787	4830	5.35	5.85	7.08
MRTK024	DHA	3643	3840	5045	7.36	5.54	13.52
MRTK033	DHA	6505	5340	1282	11.45	13.79	11.85
MRTK035	DHA	7219	5072	5298	8.93	5.02	4.01
MRTK043	DHA	3757	6335	7068	3.68	5.33	10.12
MRTK060	DHA	1748	2481	1721	7.28	6.35	4.86

SID	TREATMENT	RANTES 1	RANTES 2	RANTES 3	TNF-a 1	TNF-a 2	TNF-a 3
MRTK062	DHA	4991	4355	5212	4.15	4.19	6.44
MRTK001	Placebo	9068	8207	3413	14.28	8	6.2
MRTK004	Placebo	1680	3164	2369	5.85	6.34	6.4
MRTK010	Placebo	823	5577	6419	5.32	4.93	6.26
MRTK016	Placebo	1401	1624	1732	4.48	6.08	5.97
MRTK018	Placebo	4724	9877	9633	2.09	2.79	5.72
MRTK019	Placebo	861	2093	937	5.85	25.8	15.36
MRTK023	Placebo				2.18	2.57	0.655
MRTK025	Placebo	472	2124	1920	1.74	7.78	8.05
MRTK026	Placebo	2646	3005	3932	5.52	6.25	9.05
MRTK027	Placebo	2809	5216	4430	44.5	36.21	67.88
MRTK029	Placebo	657	2617	1650	5.25	8.15	5.16
MRTK030	Placebo	1296	2305	2303	6	7.09	6.48
MRTK034	Placebo	4202	4487	3665	6.26	5.16	6.03
MRTK037	Placebo	2119	2872	4518	4.1	5.55	9.02
MRTK039	Placebo	2519	5603	2842	4.31	6.39	4.52
MRTK041	Placebo	6517	3077	4197	5.7	4.17	3.71
MRTK042	Placebo	6888	6271	7282	5.96	3.08	4.03
MRTK049	Placebo	2171	5215	4854	5.84	6.69	7.75
MRTK051	Placebo	1300	4142	3764	4.64	4.01	5.04
MRTK052	Placebo	2322	5200	4535	4.31	4.32	6.79
MRTK056	Placebo	6919	7400	7651	5.75	6.59	2.95

APPENDIX I

CHAPTER 2: WBC RAW DATA

SID	TREATMENT	WBC S	WBC PR	WBC PO	RBC S	RBC PR	RBC PO
1	DHA	4.7	5.4	4.6	4.89	4.83	5.19
3	DHA	7.2	8.8	5.5	4.98	4.93	5.43
4	DHA	4.1	4.7	4.3	5.63	4.92	4.94
10	DHA	4.2	4.6	4.0	5.41	5.74	4.68
12	DHA	6.4	7.8	5.8	4.80	4.39	5.67
13	DHA	8.2	6.2	7.2	6.10	5.64	4.95
14	DHA	5.7	6.9	5.9	5.58	5.29	4.86
15	DHA	6.1	5.9	5.9	5.14	4.27	4.87
16	DHA	8.4	10.1	7.4	5.00	4.80	5.32
17	DHA	5.6	6.3	5.4	5.40	5.98	4.48
18	DHA	6.1	5.8	5.7	4.58	.	5.01
19	DHA	5.1	4.4	5.1	4.79	4.79	4.53
23	DHA	7.4	8.2	4.3	4.78	4.74	5.18
25	DHA	4.8	6.6	4.4	6.31	4.15	4.87
26	DHA	6.5	5.7	4.5	4.61	4.84	4.86
27	DHA	9.6	6.5	5.4	4.55	5.05	5.98
29	DHA	7.6	7.5	6.0	5.15	5.13	5.29
30	DHA	5.4	6.3	4.4	5.06	5.35	4.89
31	DHA	6.9	8.1	5.6	5.50	.	5.39
34	DHA	4.7	5.7	4.5	4.98	5.40	4.76
37	DHA	8.5	10.0	7.5	.	5.93	4.46
39	DHA	5.3	6.4	4.6	4.66	5.34	4.24
41	DHA	3.7	4.5	3.1	4.85	4.72	4.58
42	DHA	3.3	5.1	3.4	4.91	4.17	.
48	DHA				4.44	4.46	5.43

SID	TREATMENT	WBC S	WBC PR	WBC PO	RBC S	RBC PR	RBC PO
49	DHA	4.7	5.6	4.1	.	4.78	5.01
50	DHA				5.35	5.24	4.99
51	DHA	5.4	5.5	5.6	4.92	5.22	4.55
52	DHA	7.0	6.9	6.3	5.05	.	5.35
56	DHA	5.9	9.6	6.0	5.39	4.18	4.76
2	Placebo	7.4	7.4	5.8	5.00	4.65	5.19
5	Placebo	6.1	5.7	4.8	6.24	4.34	5.14
6	Placebo				4.79	5.01	5.06
7	Placebo	5.0	5.2	3.7	4.57	4.91	6.28
8	Placebo	5.2	5.5	4.1	4.64	4.86	5.74
9	Placebo	5.6	6.2	5.5	5.15	5.55	5.04
20	Placebo	3.6	3.3	3.1	5.00	5.02	4.89
21	Placebo	5.4	5.1	4.3	4.95	4.96	5.11
28	Placebo	5.6	6.0	4.4	4.81	4.97	5.01
32	Placebo				4.61	4.24	5.30
36	Placebo	6.2	5.9	5.5			5.39
38	Placebo	8.0	7.3	6.8	4.67	4.84	
40	Placebo	5.5	7.4	6.3		5.11	4.81
44	Placebo	6.3	5.9	5.3	5.22	4.13	4.75
45	Placebo	4.9	6.7	4.3	5.78	4.00	4.80
46	Placebo				4.34	4.72	4.76
53	Placebo	4.9	6.1	5.0	4.54	4.08	4.85
54	Placebo				5.43	5.08	4.75
55	Placebo				4.77	5.47	4.26
57	Placebo	6.5	8.6	6.4			4.87
58	Placebo	6.5	6.1	6.3		5.41	4.50

SID	TREATMENT	PNEU S	PNEU PR	PNEU PO	PLYM S	PLYM PR	PLYM PO
1	DHA	37.0	65.3	46.6	46.0	27.6	44.9
3	DHA	40.4	26.0	52.1	46.0	67.0	37.2
4	DHA	58.9	50.0	47.0	27.7	41.1	43.1
10	DHA	51.4	58.9	37.4	33.9	32.7	48.7
12	DHA	45.8	55.1	50.5	43.1	29.4	42.1
13	DHA	56.2	47.8	41.4	33.0	39.0	42.7
14	DHA	30.0	44.0	41.0	55.0	42.8	49.0
15	DHA	54.2	37.5	42.5	36.8	49.3	43.6
16	DHA	59.3	50.9	52.2	28.0	38.0	38.0
17	DHA	45.3	41.7	46.8	42.8	44.5	36.7
18	DHA	47.4	.	45.4	41.2	.	40.0
19	DHA	51.0	52.8	61.2	36.9	39.4	24.8
23	DHA	31.0	43.7	54.4	55.0	45.5	33.3
25	DHA	39.0	45.7	59.2	43.0	42.8	25.5
26	DHA	52.0	52.0	64.0	43.3	37.4	29.6
27	DHA	52.8	41.0	46.1	34.7	43.9	37.9
29	DHA	16.0	44.9	55.8	69.0	42.1	35.2
30	DHA	55.4	43.1	28.0	34.2	49.2	60.0
31	DHA	55.1	.	47.3	32.3	.	38.5
34	DHA	39.0	42.2	37.6	47.2	44.8	49.4
37	DHA	.	54.0	42.0	.	34.7	41.1
39	DHA	35.0	55.8	59.2	49.0	37.2	27.7
41	DHA	21.0	64.0	55.1	63.0	30.6	37.9
42	DHA	49.7	58.4	.	39.0	27.6	.
48	DHA	48.7	40.6	37.0	35.5	42.7	52.0

SID	TREATMENT	PNEU S	PNEU PR	PNEU PO	PLYM S	PLYM PR	PLYM PO
49	DHA	.	30.0	44.9	.	52.0	42.2
50	DHA	48.7	50.5	59.9	36.0	35.9	29.6
51	DHA	44.6	53.2	39.8	43.5	38.5	47.2
52	DHA	54.7	.	69.6	31.5	.	20.7
56	DHA	57.5	44.0	43.2	30.7	34.2	45.2
2	Placebo	40.7	58.5	61.8	45.9	27.5	28.8
5	Placebo	47.0	48.0	48.5	46.0	41.1	36.1
6	Placebo	63.9	52.9	48.5	27.2	34.7	40.2
7	Placebo	37.6	25.0	28.0	47.4	60.0	67.0
8	Placebo	35.0	40.6	39.0	45.0	42.5	47.3
9	Placebo	27.0	43.9	60.4	59.0	42.2	25.4
20	Placebo	41.6	56.2	34.0	43.9	30.9	53.0
21	Placebo	21.0	62.3	49.0	72.0	32.4	44.7
28	Placebo	42.4	43.7	12.0	45.3	41.6	73.0
32	Placebo	52.5	50.8	57.8	35.3	36.1	33.3
36	Placebo			49.0			42.8
38	Placebo	49.6	7.0		39.9	70.0	
40	Placebo		55.4	36.0		33.4	56.0
44	Placebo	56.0	58.6	45.5	29.7	31.6	43.1
45	Placebo	50.6	51.6	66.1	37.5	36.9	20.8
46	Placebo	63.5	56.1	62.1	28.2	29.0	28.1
53	Placebo	48.8	60.4	50.8	38.4	23.4	40.6
54	Placebo	43.3	48.8	45.5	35.7	38.4	42.6
55	Placebo	40.1	48.1	38.2	38.4	38.3	45.5
57	Placebo			47.2		.	43.0
58	Placebo		56.0	53.1		23.0	28.2

SID	TREATMENT	PMON S	PMON PR	PMON PO	PEOS S	PEOS PR	PEOS PO
1	DHA	8.0	5.6	6.8	2.0	1.3	1.4
3	DHA	10.0	4.0	7.0	3.2	2.0	3.3
4	DHA	9.2	6.9	8.2	3.8	1.7	1.4
10	DHA	11.5	6.0	6.6	2.6	2.1	6.9
12	DHA	7.8	9.2	6.0	2.9	6.0	1.2
13	DHA	6.4	9.5	12.5	4.1	3.3	3.0
14	DHA	11.0	9.3	9.0	2.0	3.3	1.0
15	DHA	5.2	7.0	11.6	3.3	5.5	1.9
16	DHA	10.6	9.9	9.1	1.8	0.9	0.0
17	DHA	9.8	11.0	9.4	1.8	2.4	6.5
18	DHA	8.7	.	11.7	2.2	.	2.6
19	DHA	6.4	6.0	8.2	5.3	1.3	4.7
23	DHA	11.0	4.7	9.7	2.0	5.8	2.1
25	DHA	13.0	8.0	9.4	1.0	3.1	5.4
26	DHA	2.8	7.4	3.9	1.5	2.8	2.0
27	DHA	7.3	11.9	13.5	4.5	2.6	2.2
29	DHA	6.0	10.5	6.9	2.0	2.2	1.7
30	DHA	8.8	5.2	5.0	1.0	2.1	6.0
31	DHA	6.8	.	10.7	5.4	.	3.1
34	DHA	7.8	9.9	10.4	5.5	2.5	2.3
37	DHA	.	9.9	9.3	.	0.9	7.2
39	DHA	1.0	5.4	8.2	3.0	1.2	4.5
41	DHA	11.0	4.1	5.4	3.0	0.9	1.2
42	DHA	9.3	11.6	.	1.4	1.7	.
48	DHA	7.1	13.3	11.0	8.0	2.6	.

SID	TREATMENT	PMON S	PMON PR	PMON PO	PEOS S	PEOS PR	PEOS PO
49	DHA	.	15.0	8.1	.	.	4.3
50	DHA	12.4	10.6	9.2	2.3	2.8	0.9
51	DHA	9.5	5.7	6.4	1.7	2.2	6.2
52	DHA	10.3	.	7.7	3.2	.	2.0
56	DHA	7.1	9.5	9.2	4.2	11.3	2.0
2	Placebo	11.5	10.7	6.5	1.6	2.8	2.5
5	Placebo	5.7	7.9	9.5	0.7	2.6	5.6
6	Placebo	7.0	8.6	6.3	1.7	3.5	4.3
7	Placebo	10.4	6.0	3.0	4.1	5.0	1.0
8	Placebo	9.0	12.8	8.4	2.0	3.4	4.9
9	Placebo	8.0	11.1	13.3	2.0	2.7	0.5
20	Placebo	12.6	7.6	8.0	1.5	4.8	5.0
21	Placebo	3.0	4.0	4.6	2.0	0.9	1.4
28	Placebo	9.8	12.8	9.0	2.1	1.4	2.0
32	Placebo	9.0	6.0	7.7	2.7	6.5	0.9
36	Placebo			7.8		.	0.0
38	Placebo	8.6	8.0		1.3	8.0	.
40	Placebo		7.7	3.0		3.2	2.0
44	Placebo	10.3	6.8	9.4	3.6	2.7	1.6
45	Placebo	10.4	8.9	10.0	1.1	2.1	2.7
46	Placebo	6.3	11.4	6.9	1.8	3.2	2.5
53	Placebo	9.2	6.9	7.4	3.0	8.7	0.8
54	Placebo	6.5	7.0	8.2	13.9	5.1	3.3
55	Placebo	8.8	10.6	14.3	12.0	2.5	1.1
57	Placebo		.	9.1			0.0
58	Placebo		11.0	11.4		5.0	6.8

SID	TREATMENT	PBAS S	PBAS PR	PBAS PR
1	DHA	.	0.2	0.3
3	DHA	0.4	.	0.4
4	DHA	0.4	0.3	0.3
10	DHA	0.6	0.3	0.4
12	DHA	0.4	0.3	0.2
13	DHA	0.3	0.4	0.4
14	DHA	.	0.6	.
15	DHA	0.5	0.7	0.4
16	DHA	0.3	0.3	0.7
17	DHA	0.3	0.4	0.6
18	DHA	0.5	.	0.3
19	DHA	0.4	0.5	1.1
23	DHA	.	0.3	0.5
25	DHA	.	0.4	0.5
26	DHA	0.4	0.4	0.5
27	DHA	0.7	0.6	0.3
29	DHA	2.0	0.3	0.4
30	DHA	0.6	0.4	.
31	DHA	0.4	.	0.4
34	DHA	0.5	0.6	0.3
37	DHA	.	0.5	0.4
39	DHA	2.0	0.4	0.4
41	DHA	.	0.4	0.4
42	DHA	0.6	0.7	.
48	DHA	0.7	0.8	.

SID	TREATMENT	PBAS S	PBAS PR	PBAS PR
49	DHA	.	.	0.5
50	DHA	0.6	0.2	0.4
51	DHA	0.7	0.4	0.4
52	DHA	0.3	.	0.0
56	DHA	0.5	1.0	0.4
2	Placebo	0.3	0.5	0.4
5	Placebo	0.6	0.4	0.3
6	Placebo	0.2	0.3	0.7
7	Placebo	0.5	.	.
8	Placebo	1.0	0.7	0.4
9	Placebo	.	0.1	0.4
20	Placebo	0.4	0.5	.
21	Placebo	1.0	0.4	0.3
28	Placebo	0.4	0.5	1.0
32	Placebo	0.5	0.6	0.3
36	Placebo	.	.	0.4
38	Placebo	0.6	.	.
40	Placebo	.	0.3	.
44	Placebo	0.4	0.3	0.4
45	Placebo	0.4	0.5	0.4
46	Placebo	0.2	0.3	0.4
53	Placebo	0.6	0.6	0.4
54	Placebo	0.6	0.7	0.4
55	Placebo	0.7	0.5	0.9
57	Placebo	.	.	0.7
58	Placebo	.	1.0	0.5

APPENDIX J

CHAPTER 3: ST. JOSEPH'S LIPID PANEL RAW DATA

SID	TREATMENT	CHOL S	CHOL PR	CHOL PO	TRI S	TRI PR	TRI PO
MRTK002	DHA	205	208	208	133	119	124
MRTK005	DHA	219	221	211	94	107	76
MRTK007	DHA	147	147	173	76	91	59
MRTK008	DHA	129	144	112	63	88	48
MRTK009	DHA	175	171	158	45	57	47
MRTK011	DHA	175	182	151	76	75	59
MRTK020	DHA	162	153	142	171	86	85
MRTK021	DHA	132	102	121	210	105	58
MRTK028	DHA	198	228	218	45	74	44
MRTK033	DHA	162	188	198	93	227	70
MRTK035	DHA	113	137	137	90	89	77
MRTK036	DHA	175	167	149	155	158	110
MRTK038	DHA	237	216	223	108	211	195
MRTK040	DHA	226	218	192	143	135	77
MRTK043	DHA	154	144	158	86	71	100
MRTK044	DHA	152	220	228	138	75	79
MRTK045	DHA	189	194	165	123	127	88
MRTK053	DHA	192	229	180	88	90	59
MRTK057	DHA	199	225	190	230	118	108
MRTK058	DHA	174	160	159	102	61	70
MRTK060	DHA	178	205	168	200	178	168
MRTK062	DHA	145	146	153	68	91	73

SID	TREATMENT	CHOL S	CHOL PR	CHOL PO	TRI S	TRI PR	TRI PO
MRTK001	Placebo	140	145	145	71	86	53
MRTK003	Placebo	226	243	217	37	51	44
MRTK004	Placebo	145	133	167	63	97	91
MRTK010	Placebo	153	131	142	58	46	50
MRTK012	Placebo	216	198	204	188	172	212
MRTK013	Placebo	134	125	115	89	97	70
MRTK014	Placebo	174	160	153	61	159	47
MRTK015	Placebo	163	147	164	57	87	59
MRTK017	Placebo	175	150	146	163	194	84
MRTK018	Placebo	180	162	167	73	92	75
MRTK019	Placebo	166	182	173	60	65	64
MRTK023	Placebo	214	176	250	142	176	169
MRTK025	Placebo	168	185	199	54	138	80
MRTK026	Placebo	175	176	179	128	163	150
MRTK027	Placebo	160	206	173	120	162	106
MRTK029	Placebo	213	193	179	121	186	138
MRTK030	Placebo	142	168	158	86	99	54
MRTK031	Placebo	141	151	145	82	119	74
MRTK034	Placebo	183	158	162	210	181	121
MRTK037	Placebo	219	195	222	136	129	125
MRTK039	Placebo	146	150	141	83	87	65
MRTK041	Placebo	176	184	189	68	116	63
MRTK049	Placebo	189	224	207	197	186	186
MRTK051	Placebo	212	212	200	47	47	54
MRTK056	Placebo	163	158	187	110	269	146

SID	TREATMENT	HDL S	HDL PR	HDL PO	LDL S	LDL PR	LDL PO
MRTK002	DHA	31	37	33	147	147	150
MRTK005	DHA	50	52	38	150	148	158
MRTK007	DHA	49	46	52	83	83	109
MRTK008	DHA	46	36	41	70	90	61
MRTK009	DHA	55	43	45	111	117	104
MRTK011	DHA	68	62	66	92	105	73
MRTK020	DHA	46	44	39	82	92	86
MRTK021	DHA	37	39	48	53	42	61
MRTK028	DHA	56	72	56	133	141	153
MRTK033	DHA	40	49	52	103	94	132
MRTK035	DHA	36	38	37	59	81	85
MRTK036	DHA	39	39	38	105	96	89
MRTK038	DHA	52	44	43	163	130	141
MRTK040	DHA	50	56	49	147	135	128
MRTK043	DHA	56	58	58	81	72	80
MRTK044	DHA	35	51	58	89	154	154
MRTK045	DHA	49	64	51	115	105	96
MRTK053	DHA	57	59	51	117	152	117
MRTK057	DHA	61	88	65	92	113	103
MRTK058	DHA	46	41	38	108	107	107
MRTK060	DHA	55	53	42	83	116	92
MRTK062	DHA	39	38	38	92	90	100

SID	TREATMENT	HDL S	HDL PR	HDL PO	LDL S	LDL PR	LDL PO
MRTK001	Placebo	57	61	62	69	67	72
MRTK003	Placebo	137	155	109	82	78	99
MRTK004	Placebo	46	45	47	86	69	102
MRTK010	Placebo	56	47	53	85	75	79
MRTK012	Placebo	52	39	40	126	128	122
MRTK013	Placebo	34	34	36	82	72	65
MRTK014	Placebo	56	48	46	106	80	98
MRTK015	Placebo	69	58	60	83	72	92
MRTK017	Placebo	54	46	45	88	65	84
MRTK018	Placebo	55	53	46	110	91	106
MRTK019	Placebo	47	48	45	107	121	115
MRTK023	Placebo	71	54	54	115	87	162
MRTK025	Placebo	46	46	49	111	111	134
MRTK026	Placebo	37	37	40	112	106	109
MRTK027	Placebo	37	59	45	99	115	107
MRTK029	Placebo	31	26	28	158	130	123
MRTK030	Placebo	49	60	49	76	88	98
MRTK031	Placebo	51	48	46	74	79	84
MRTK034	Placebo	54	52	45	87	70	93
MRTK037	Placebo	70	61	64	122	108	133
MRTK039	Placebo	44	49	47	85	84	81
MRTK041	Placebo	58	54	55	104	107	121
MRTK049	Placebo	41	48	45	109	139	125
MRTK051	Placebo	75	76	72	128	127	117
MRTK056	Placebo	35	29	45	106	75	113

APPENDIX K

CHAPTER 3: SPECTRACELL'S LIPID PANEL RAW DATA

		SPECTC			SPECLDL			SPECHDL		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	161	199	188	109	145	136	37	39	31
MRTK005	DHA	157	193	192	99	124	130	51	57	49
MRTK007	DHA	95	125	142	48	73	87	42	46	49
MRTK008	DHA	81	133	83	44	84	41	34	36	36
MRTK009	DHA	142	141	120	91	95	77	49	39	38
MRTK011	DHA	166	177	138	93	109	69	68	60	62
MRTK020	DHA	135	122	132	72	72	78	42	38	37
MRTK021	DHA	114	93	94	54	47	50	45	34	37
MRTK024	DHA	151	133	127	77	63	66	62	56	52
MRTK028	DHA	208	203	159	116	121	105	84	74	46
MRTK033	DHA	153	187	145	99	110	94	45	57	43
MRTK035	DHA	125	141	140	76	89	94	41	43	35
MRTK036	DHA	164	132	138	94	70	91	44	41	32
MRTK038	DHA	206	198	224	146	114	149	45	53	43
MRTK040	DHA	203	175	173	132	100	115	53	63	46
MRTK043	DHA	135	116	153	66	56	87	58	54	52
MRTK044	DHA	151	173	217	86	114	157	44	52	49
MRTK045	DHA	170	160	155	97	84	91	48	64	50
MRTK053	DHA	177	205	188	110	131	118	54	60	58
MRTK057	DHA	181	229	195	100	125	111	63	89	68
MRTK058	DHA	148	129	157	86	71	95	54	49	48
MRTK060	DHA	174	196	146	100	109	88	57	59	35
MRTK062	DHA	138	144	151	90	87	101	38	48	40
MRTK001	Placebo	117	139	113	57	68	53	52	63	52
MRTK003	Placebo	173	231	166	65	102	68	101	121	90
MRTK004	Placebo	104	101	116	55	53	68	43	37	36
MRTK010	Placebo	111	114	121	60	63	64	47	43	51
MRTK012	Placebo	176	193	202	106	124	120	45	41	49
MRTK013	Placebo	113	98	96	64	55	50	39	35	36
MRTK014	Placebo	127	149	137	70	90	79	53	52	54
MRTK015	Placebo	133	128	128	61	58	62	67	63	62
MRTK016	Placebo	138	178	169	80	89	72	29	43	40
MRTK017	Placebo	143	138	125	73	75	69	54	46	45
MRTK018	Placebo	129	144	143	69	82	86	52	50	46
MRTK019	Placebo	115	131	136	65	83	87	43	41	41
MRTK023	Placebo	162	187	235	92	111	160	62	59	51
MRTK025	Placebo	114	156	165	64	99	116	42	45	39
MRTK026	Placebo	138	154	130	82	94	81	40	39	36
MRTK027	Placebo	119	190	153	68	112	92	37	61	39
MRTK029	Placebo	182	192	116	124	126	72	40	37	32
MRTK030	Placebo	127	151	100	68	85	56	46	56	36
MRTK031	Placebo	107	127	113	57	69	64	43	48	40
MRTK034	Placebo	183	161	153	92	66	89	67	65	50
MRTK037	Placebo	177	171	164	100	84	100	59	67	50
MRTK039	Placebo	115	117	117	63	62	64	43	49	45

MRTK041	Placebo	160	165	158	93	87	96	60	66	53
MRTK042	Placebo	117	126	134	59	62	74	51	60	52
MRTK049	Placebo	203	209	201	123	127	132	51	56	43
MRTK051	Placebo	175	168	191	105	84	111	63	75	71
MRTK052	Placebo	147	170	187	76	89	112	64	73	64
MRTK056	Placebo	141	162	199	91	88	117	37	44	50

		SPECTRI			SPEC_CRP			SPECINS		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	139	116	123	1.01	0.18	0.41	14.4	9.3	12.8
MRTK005	DHA	96	106	79	0.32	0.10	0.34	11.9	9.6	7.3
MRTK007	DHA	74	93	53	0.02	0.02	0.04	6.2	5.8	3.9
MRTK008	DHA	52	85	43	0.08	0.06	0.24	4.2	3.9	3.9
MRTK009	DHA	46	53	49	0.21	0.20	0.17	10.6	11.5	7.5
MRTK011	DHA	80	71	57	0.08	0.04	0.25	5.5	4.1	3.9
MRTK020	DHA	167	85	84	0.11	0.05	1.01	10.2	10.5	5.5
MRTK021	DHA	197	111	62	0.31	1.01	0.42	24.1	5.5	7.9
MRTK024	DHA	110	191	56	0.07	0.02	0.06	8.7	4.8	5.8
MRTK028	DHA	85	79	46	0.03	0.02	0.02	7.9	7.2	3.9
MRTK033	DHA	88	217	68	0.05	0.06	0.29	26.0	20.7	5.5
MRTK035	DHA	93	90	76	0.21	0.01	0.07	37.1	3.9	3.9
MRTK036	DHA	152	147	109	0.17	0.10	0.23	5.3	5.3	6.7
MRTK038	DHA	106	214	204	0.07	0.06	0.09	9.7	8.6	12.0
MRTK040	DHA	143	139	73	0.02	0.01	0.02	8.7	4.2	7.3
MRTK043	DHA	84	76	98	0.01	0.05	0.16	10.0	4.7	8.8
MRTK044	DHA	140	71	81	0.31	0.11	0.10	3.9	4.1	7.9
MRTK045	DHA	120	122	83	0.26	0.15	0.17	8.3	13.6	7.8
MRTK053	DHA	91	91	64	0.04	0.04	0.14	6.7	16.9	3.9
MRTK057	DHA	246	118	110	0.06	0.02	0.17	8.1	4.4	3.9
MRTK058	DHA	103	61	71	0.02	0.02	0.24	4.7	3.9	5.1
MRTK060	DHA	186	177	179	0.13	0.07	0.23	10.6	9.4	9.5
MRTK062	DHA	69	94	74	0.06	0.15	0.52	9.8	7.5	5.8
MRTK001	Placebo	74	89	46	0.05	0.02	0.07	6.9	4.2	3.9
MRTK003	Placebo	52	48	37	0.04	0.05	0.11	13.5	5.8	8.7
MRTK004	Placebo	64	93	94	0.09	0.07	0.03	12.8	4.8	3.9
MRTK010	Placebo	56	46	46	0.12	0.04	0.15	14.9	7.0	9.6
MRTK012	Placebo	187	169	202	0.10	0.05	0.10	14.7	16.1	9.3
MRTK013	Placebo	89	90	64	0.06	0.11	1.01	12.0	12.9	11.8
MRTK014	Placebo	64	168	44	0.05	0.05	0.10	4.5	17.1	7.9
MRTK015	Placebo	52	87	53	0.01	0.02	0.26	6.1	10.3	10.3
MRTK016	Placebo	327	782	319	0.07	0.06	0.08	22.3	39.3	12.4
MRTK017	Placebo	162	187	78	0.15	0.05	0.32	5.1	24.0	4.0
MRTK018	Placebo	72	95	70	0.04	0.05	0.25	6.6	6.9	6.7
MRTK019	Placebo	60	61	61	0.13	0.11	0.23	7.8	4.7	4.8
MRTK023	Placebo	153	169	167	0.38	0.07	0.14	19.9	13.0	13.5
MRTK025	Placebo	53	122	75	0.03	0.03	0.07	3.9	4.9	3.9
MRTK026	Placebo	144	158	143	0.04	0.04	0.15	5.9	8.9	12.4
MRTK027	Placebo	114	143	96	1.01	0.03	0.11	8.6	3.9	3.9
MRTK029	Placebo	123	182	136	0.07	0.07	0.09	7.2	4.6	7.8
MRTK030	Placebo	97	94	48	0.02	0.01	0.02	6.1	8.9	6.5
MRTK031	Placebo	80	116	70	0.06	0.09	0.34	6.9	3.9	8.9
MRTK034	Placebo	225	181	116	0.04	0.04	0.14	19.1	7.7	13.0

MRTK037	Placebo	123	121	112	0.16	0.07	0.13	18.7	8.3	9.0
MRTK039	Placebo	85	88	62	0.15	0.07	0.07	8.2	7.4	6.4
MRTK041	Placebo	77	119	74	0.02	0.07	0.08	11.8	6.8	6.3
MRTK042	Placebo	52	71	42	0.01	0.02	0.08	8.1	5.4	3.9
MRTK049	Placebo	199	180	155	0.28	0.14	0.16	33.1	13.4	10.4
MRTK051	Placebo	47	51	52	0.02	0.03	0.15	3.9	3.9	3.9
MRTK052	Placebo	125	77	72	0.37	0.05	0.32	13.7	15.3	6.0
MRTK056	Placebo	112	259	142	0.15	0.08	0.26	16.4	21.2	15.2

		SPECHOMO			SPECVLDL			SPECLDLT		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	11.7	9.3	10.0	58	56	84	820	1012	988
MRTK005	DHA	8.0	9.1	8.9	27	44	49	707	822	897
MRTK007	DHA	7.5	6.9	6.8	18	24	26	353	530	605
MRTK008	DHA	9.2	12.9	10.7	12	50	22	325	581	292
MRTK009	DHA	8.8	10.4	9.4	11	26	20	629	634	557
MRTK011	DHA	7.4	6.1	5.8	21	34	26	598	717	465
MRTK020	DHA	24.9	51.4	40.7	81	44	67	502	501	545
MRTK021	DHA	8.7	8.1	7.4	61	47	31	356	323	349
MRTK024	DHA	6.7	7.7	6.4	42	56	32	552	464	452
MRTK028	DHA	9.7	11.0	7.8	29	30	30	845	852	765
MRTK033	DHA	6.8	7.7	7.4	31	78	31	666	773	651
MRTK035	DHA	5.7	6.6	6.1	32	36	46	498	601	624
MRTK036	DHA	8.2	9.4	6.8	96	78	57	644	546	634
MRTK038	DHA	7.7	7.9	7.3	54	118	125	990	851	1028
MRTK040	DHA	8.1	9.2	7.6	71	46	44	899	732	781
MRTK043	DHA	8.2	9.7	10.7	42	21	51	464	408	597
MRTK044	DHA	9.8	11.2	8.5	80	27	42	618	820	1067
MRTK045	DHA	10.3	10.1	9.3	97	47	56	650	609	616
MRTK053	DHA	7.4	8.1	9.2	48	55	44	785	908	841
MRTK057	DHA	7.2	7.6	6.3	65	56	63	681	886	776
MRTK058	DHA	9.2	8.7	9.4	30	33	55	593	542	675
MRTK060	DHA	7.1	7.4	5.7	66	110	88	653	841	643
MRTK062	DHA	9.9	11.6	9.4	41	33	36	618	609	676
MRTK001	Placebo	9.4	8.6	9.0	29	33	29	417	483	380
MRTK003	Placebo	8.0	8.5	7.3	29	31	29	487	765	509
MRTK004	Placebo	6.6	7.3	7.0	26	43	44	391	390	450
MRTK010	Placebo	7.4	8.4	7.5	16	26	24	427	444	433
MRTK012	Placebo	8.0	8.9	6.9	94	105	129	765	856	857
MRTK013	Placebo	10.8	10.4	11.4	42	30	40	458	104	338
MRTK014	Placebo	7.1	8.1	7.4	18	26	15	494	626	545
MRTK015	Placebo	7.0	7.0	6.3	19	25	16	428	432	445
MRTK016	Placebo	8.0	3.6	8.3	113	178	215	561	635	506
MRTK017	Placebo	8.2	9.7	9.2	62	68	39	517	526	491
MRTK018	Placebo	9.2	9.7	9.6	30	49	44	489	580	593
MRTK019	Placebo	9.0	7.7	8.5	27	30	31	464	569	599
MRTK023	Placebo	9.4	9.1	7.9	30	67	94	653	760	1080
MRTK025	Placebo	5.7	6.8	5.8	29	44	38	463	687	804
MRTK026	Placebo	9.5	11.0	8.8	59	80	49	599	640	560
MRTK027	Placebo	7.1	8.9	8.6	53	66	83	502	761	635
MRTK029	Placebo	6.3	6.4	6.8	72	109	47	906	879	541

MRTK030	Placebo	6.4	6.9	5.9	49	42	30	493	596	407
MRTK031	Placebo	8.1	8.0	7.1	27	37	37	417	480	434
MRTK034	Placebo	8.8	8.3	11.1	94	114	55	627	453	600
MRTK037	Placebo	11.2	12.1	11.2	71	76	55	679	599	661
MRTK039	Placebo	6.1	3.7	6.5	37	22	34	439	443	452
MRTK041	Placebo	9.6	8.3	8.3	26	46	32	623	593	666
MRTK042	Placebo	11.4	12.4	9.1	26	13	31	430	463	530
MRTK049	Placebo	10.4	9.7	8.9	111	101	102	863	961	888
MRTK051	Placebo	75.4	76.2	74.3	26	33	36	720	625	752
MRTK052	Placebo	7.5	7.6	7.5	26	33	40	555	655	814
MRTK056	Placebo	7.7	8.0	7.5	52	113	125	598	584	771

		SPECRLIPO			SPECDLDLIII			SPECDLDLIV		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	109	129	144	300	286	342	116	89	134
MRTK005	DHA	77	128	129	199	165	209	88	65	96
MRTK007	DHA	37	47	62	115	159	150	42	65	59
MRTK008	DHA	29	86	45	106	125	75	46	65	39
MRTK009	DHA	69	88	53	158	131	160	68	58	65
MRTK011	DHA	133	117	62	95	123	91	67	68	45
MRTK020	DHA	94	85	90	138	119	144	63	62	64
MRTK021	DHA	91	82	55	51	51	64	62	62	64
MRTK024	DHA	68	58	70	150	137	80	63	66	64
MRTK028	DHA	59	69	40	223	193	208	108	93	88
MRTK033	DHA	98	97	74	134	224	147	63	65	64
MRTK035	DHA	95	95	121	61	144	103	63	46	67
MRTK036	DHA	124	71	101	167	236	181	63	89	67
MRTK038	DHA	140	114	184	225	314	326	79	115	88
MRTK040	DHA	134	69	98	200	238	149	76	83	76
MRTK043	DHA	76	49	124	127	131	138	56	52	73
MRTK044	DHA	81	98	147	193	246	244	73	101	100
MRTK045	DHA	164	83	107	152	211	124	59	69	65
MRTK053	DHA	77	93	72	217	211	206	82	77	96
MRTK057	DHA	125	125	122	151	270	200	67	86	100
MRTK058	DHA	74	41	93	121	188	180	65	77	88
MRTK060	DHA	141	124	120	127	389	243	62	107	91
MRTK062	DHA	95	84	124	138	155	136	70	69	74
MRTK001	Placebo	45	54	48	111	101	91	60	68	59
MRTK003	Placebo	37	49	38	146	175	151	74	144	71
MRTK004	Placebo	51	49	99	115	110	63	46	54	53
MRTK010	Placebo	46	61	68	117	107	90	52	58	39
MRTK012	Placebo	125	126	157	253	214	255	101	78	100
MRTK013	Placebo	55	48	74	135	112	77	55	54	39
MRTK014	Placebo	43	64	57	142	138	118	46	68	63
MRTK015	Placebo	46	43	45	116	131	111	46	61	63
MRTK016	Placebo	128	176	154	188	178	142	64	106	78
MRTK017	Placebo	60	74	59	132	133	130	62	61	58
MRTK018	Placebo	60	86	97	17	146	155	62	66	59
MRTK019	Placebo	55	68	77	120	110	147	63	62	64
MRTK023	Placebo	92	122	181	165	155	260	70	74	76
MRTK025	Placebo	48	100	81	123	172	194	63	74	72
MRTK026	Placebo	82	129	83	210	163	131	63	66	64
MRTK027	Placebo	83	132	124	162	163	147	82	81	76

MRTK029	Placebo	128	142	52	303	268	205	123	81	64
MRTK030	Placebo	63	72	33	146	143	86	63	66	64
MRTK031	Placebo	41	64	72	110	127	70	63	45	64
MRTK034	Placebo	122	113	129	143	124	113	75	45	67
MRTK037	Placebo	126	103	126	143	186	113	71	64	67
MRTK039	Placebo	71	64	59	91	133	97	63	52	67
MRTK041	Placebo	112	111	86	124	125	151	79	71	75
MRTK042	Placebo	40	32	56	129	161	126	56	53	67
MRTK049	Placebo	149	147	162	264	412	218	92	120	73
MRTK051	Placebo	75	43	97	147	194	156	69	73	77
MRTK052	Placebo	59	69	87	132	161	206	109	116	130
MRTK056	Placebo	140	143	194	113	112	165	56	69	65

		SPECHDLT			SPECBHDL			SPECLDLMS		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	7661	7844	5999	839	1045	836	19.88	20.04	19.90
MRTK005	DHA	10480	11006	9964	1004	1806	1400	20.08	20.25	20.16
MRTK007	DHA	8727	9165	9593	945	1258	1440	19.97	20.01	20.16
MRTK008	DHA	7000	6685	6980	899	1260	1104	19.96	20.19	20.08
MRTK009	DHA	9919	7424	7310	1286	1235	1211	20.14	20.24	20.13
MRTK011	DHA	11379	10096	10343	2806	2495	2603	20.36	20.38	20.33
MRTK020	DHA	8709	7727	7526	1021	1011	897	20.03	20.12	20.12
MRTK021	DHA	8584	6561	7249	1388	1067	1012	20.33	20.21	20.30
MRTK024	DHA	11083	10156	8639	2157	2022	2345	20.09	19.99	20.34
MRTK028	DHA	15821	13778	9110	2649	2474	1360	20.13	20.28	20.08
MRTK033	DHA	9238	10592	8678	1149	1699	1098	20.32	20.03	20.20
MRTK035	DHA	7950	7605	6622	1262	1511	1098	20.50	20.16	20.29
MRTK036	DHA	9261	8091	6000	977	1149	948	20.08	19.80	20.10
MRTK038	DHA	9486	10120	8312	1035	1560	1285	20.13	19.88	19.95
MRTK040	DHA	10376	11231	9256	1487	2199	1252	20.19	19.98	20.28
MRTK043	DHA	10974	9994	10209	1821	1773	1615	20.06	19.96	20.11
MRTK044	DHA	8535	9605	9080	1391	1735	1782	19.97	19.99	20.18
MRTK045	DHA	9051	11954	9350	1583	2026	1726	20.09	19.92	20.21
MRTK053	DHA	10095	11337	11129	1784	1962	1838	20.11	20.20	20.17
MRTK057	DHA	11245	14292	11172	2296	3833	3010	20.16	20.03	20.08
MRTK058	DHA	10802	9812	9632	1535	1370	1282	20.24	19.94	20.03
MRTK060	DHA	10968	11532	6890	1702	1560	958	20.21	19.78	19.83
MRTK062	DHA	6632	9365	7762	1396	1463	1225	20.17	20.12	20.20
MRTK001	Placebo	9836	11199	9674	1620	2148	1671	20.09	20.21	20.12
MRTK003	Placebo	14961	16996	13583	5412	6825	4612	20.05	20.17	20.06
MRTK004	Placebo	8659	7274	6510	1082	1012	1198	20.00	20.01	20.42
MRTK010	Placebo	9204	8571	9360	1367	1160	1711	20.09	20.13	20.22
MRTK012	Placebo	9490	8587	10025	990	963	1252	19.92	20.08	19.96
MRTK013	Placebo	7721	7193	7297	1117	864	1023	20.00	20.02	20.11
MRTK014	Placebo	9667	9783	10754	1835	1727	1629	20.10	20.21	20.22
MRTK015	Placebo	11421	11281	11508	2726	2272	2061	20.11	19.99	20.13
MRTK016	Placebo	5463	8125	8095	850	1434	1037	19.88	19.92	19.90
MRTK017	Placebo	10779	8812	9244	1473	1406	1156	20.07	20.12	20.11
MRTK018	Placebo	10774	9475	9350	1275	1404	1157	20.23	20.06	20.09
MRTK019	Placebo	8875	8224	8647	1049	1096	897	20.15	20.27	20.18
MRTK023	Placebo	10810	10833	9220	2270	1968	1735	20.15	20.22	20.24
MRTK025	Placebo	8928	8797	7803	908	1320	1039	20.14	20.12	20.12
MRTK026	Placebo	8486	7481	7279	881	1124	897	19.92	20.08	20.09

MRTK027	Placebo	7390	11515	7756	937	1855	1011	19.94	20.18	20.12
MRTK029	Placebo	8295	7111	6269	893	1127	897	19.92	19.98	19.90
MRTK030	Placebo	9356	10716	7357	1235	1659	897	20.09	20.16	20.3
MRTK031	Placebo	8846	9237	8061	976	1361	982	20.14	20.06	20.37
MRTK034	Placebo	12584	12012	9679	2155	2122	1496	20.13	20.01	20.29
MRTK037	Placebo	11426	12734	10002	1751	1898	1373	20.18	20.00	20.28
MRTK039	Placebo	8598	9474	9280	1149	1447	1008	20.18	19.98	20.27
MRTK041	Placebo	10289	11362	9062	2470	2512	2188	20.20	20.17	20.18
MRTK042	Placebo	9656	10449	9685	1701	2187	1741	20.05	19.96	20.16
MRTK049	Placebo	10255	11356	8919	1390	1373	975	19.96	19.81	20.13
MRTK051	Placebo	10896	12733	12350	2568	3148	2923	20.32	20.01	20.23
MRTK052	Placebo	11680	12514	12092	2302	2898	2285	20.12	20.11	20.11
MRTK056	Placebo	7776	8709	10036	791	1121	1253	20.29	20.26	20.23

		SPECLPA			SPECVLDLC			SPECLDLCT		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	15.3	12.7	11.0	15.0	14.7	21.9	109.3	144.7	135.6
MRTK005	DHA	111.1	88.1	88.3	7.0	11.6	12.9	98.9	123.8	130.1
MRTK007	DHA	44.6	44.2	50.4	4.8	6.3	6.8	48.0	72.6	86.6
MRTK008	DHA	11.3	14.7	15.4	3.0	12.9	5.9	43.7	83.9	41.3
MRTK009	DHA	20.2	23.4	14.3	2.9	6.9	5.1	90.6	94.6	77.4
MRTK011	DHA	9.8	10.7	10.1	5.6	8.8	6.9	93.2	108.6	68.9
MRTK020	DHA	7.3	4.9	6.9	21.2	11.6	17.4	71.7	71.7	77.6
MRTK021	DHA	16.3	23.8	22.5	15.8	12.2	8.0	54.0	46.6	49.6
MRTK024	DHA	77.2	73.0	61.3	11.1	14.7	8.4	77.4	62.6	65.7
MRTK028	DHA	92.7	88.6	68.2	7.5	7.8	7.7	116.2	121.8	104.6
MRTK033	DHA	64.3	61.9	78.3	8.0	20.3	8.0	99.2	110.2	94.0
MRTK035	DHA	12.7	22.6	14.1	8.3	9.3	11.9	75.5	88.7	93.5
MRTK036	DHA	4.9	4.9	4.9	25.0	20.4	15.0	94.4	70.1	91.1
MRTK038	DHA	15.5	9.9	9.8	14.1	30.7	32.6	146.2	113.8	148.6
MRTK040	DHA	100.5	66.1	64.4	18.4	12.0	11.6	132.4	100.4	114.9
MRTK043	DHA	30.6	31.5	23.0	10.9	5.4	13.3	65.7	56.0	87.1
MRTK044	DHA	38.2	63.2	67.9	20.8	7.1	10.9	85.7	114.4	156.9
MRTK045	DHA	65.9	74.4	71.4	25.2	12.1	14.6	96.9	83.6	90.5
MRTK053	DHA	9.4	10.8	8.3	12.6	14.2	11.5	109.9	130.8	118.4
MRTK057	DHA	14.2	21.0	21.7	17.0	14.6	16.4	100.3	125.3	110.7
MRTK058	DHA	89.4	79.5	78.2	7.8	8.7	14.3	86.1	71.1	94.9
MRTK060	DHA	4.9	4.9	4.9	17.1	28.8	23.0	99.5	108.6	87.9
MRTK062	DHA	33.2	34.8	34.7	10.6	8.7	9.3	90.0	87.1	100.9
MRTK001	Placebo	25.4	27.4	38.0	7.5	8.6	7.5	57.2	68.1	52.8
MRTK003	Placebo	33.7	42.2	53.4	7.5	8.2	7.5	64.7	101.9	68.1
MRTK004	Placebo	5.5	5.3	5.0	6.7	11.3	11.5	54.7	53.4	68.4
MRTK010	Placebo	39.7	43.4	53.0	4.2	6.9	6.2	60.1	63.3	64.4
MRTK012	Placebo	12.1	12.5	8.10	24.6	27.5	33.8	106.2	123.8	119.9
MRTK013	Placebo	42.9	50.4	56.6	10.9	7.7	10.4	63.5	55.0	49.8
MRTK014	Placebo	52.8	61.2	58.3	4.6	6.9	3.9	69.6	89.9	78.6
MRTK015	Placebo	86.4	77.7	80.4	5.0	6.6	4.2	60.8	58.3	62.0
MRTK016	Placebo	7.7	5.7	5.2	29.6	46.5	56.1	79.6	89.1	75.5
MRTK017	Placebo	19.2	19.7	22.7	16.1	17.9	10.1	73.0	74.5	69.5
MRTK018	Placebo	13.2	13.5	13.4	7.9	12.8	11.4	69.2	81.7	85.5
MRTK019	Placebo	49.2	65.2	50.5	7.0	7.8	8.0	64.8	82.6	86.6
MRTK023	Placebo	52.9	46.7	60.3	7.9	17.5	24.5	92.0	111.1	159.7
MRTK025	Placebo	25.0	18.8	28.0	7.6	11.6	9.9	63.8	98.6	115.6

MRTK026	Placebo	5.3	7.7	7.9	15.5	21.0	12.9	82.5	93.9	81.1
MRTK027	Placebo	22.9	17.7	19.90	13.9	17.2	21.6	67.9	111.7	92.4
MRTK029	Placebo	5.8	5.5	4.9	18.7	28.5	12.2	124.0	126.1	71.8
MRTK030	Placebo	4.9	4.9	4.90	12.8	11.0	7.7	67.5	84.5	56.0
MRTK031	Placebo	23.7	28.7	30.6	7.0	9.7	9.7	57.0	69.1	63.8
MRTK034	Placebo	92.8	79.7	85.20	24.6	29.7	14.3	91.8	65.7	89.4
MRTK037	Placebo	85.6	69.9	72.4	18.6	19.7	14.3	100.2	84.2	99.6
MRTK039	Placebo	45.0	40.4	43.8	9.6	5.8	8.8	62.7	62.4	63.9
MRTK041	Placebo	95.2	84.0	90.8	6.7	12.0	8.4	93.2	87.4	96.3
MRTK042	Placebo	51.0	59.7	66.0	6.7	3.5	8.2	58.7	62.3	74.5
MRTK049	Placebo	10.5	12.8	13.1	28.8	26.4	26.7	123.1	127.0	131.8
MRTK051	Placebo	101.7	94.2	84.8	6.7	8.7	9.3	105.5	84.3	110.7
MRTK052	Placebo	181.5	158.8	140.0	6.7	8.7	10.5	75.8	89.2	112.2
MRTK056	Placebo	28.6	31.7	26.7	13.6	29.5	32.6	90.9	88.1	117.2

		SPECRLPC			SPECIDLC			SPECDLDLIIID		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	25.6	30.4	33.9	21.4	26.7	30.3	31.6	30.1	36.0
MRTK005	DHA	18.0	30.3	30.3	16.3	27.5	26.7	21.0	17.3	22.1
MRTK007	DHA	8.6	11.0	14.7	7.2	9.4	13.1	12.1	16.8	15.8
MRTK008	DHA	6.7	20.4	10.7	5.8	17.1	9.2	11.2	13.2	8.0
MRTK009	DHA	16.3	20.6	12.6	15.4	19.3	11.0	16.6	13.8	16.8
MRTK011	DHA	31.5	27.5	14.7	29.8	25.3	13.4	10.0	12.9	9.6
MRTK020	DHA	22.1	20.0	21.3	19.3	16.9	18.0	14.5	12.5	15.1
MRTK021	DHA	21.5	19.4	12.9	19.6	16.0	11.3	5.4	5.3	6.8
MRTK024	DHA	16.1	13.8	16.4	13.6	10.6	14.2	15.8	14.4	8.4
MRTK028	DHA	13.8	16.3	9.3	11.7	14.4	7.4	23.5	20.3	21.9
MRTK033	DHA	23.0	22.8	17.4	21.1	19.9	15.1	14.1	23.6	15.5
MRTK035	DHA	22.4	22.4	28.6	19.8	19.6	24.5	6.4	15.2	10.9
MRTK036	DHA	29.1	16.8	23.8	25.6	14.3	21.4	17.6	24.9	19.0
MRTK038	DHA	33.0	26.8	43.2	29.4	22.5	38.8	23.7	33.1	34.3
MRTK040	DHA	31.5	16.3	23.1	27.5	14.2	20.4	21.1	25.0	15.6
MRTK043	DHA	17.9	11.5	29.1	15.2	10.0	25.1	13.3	13.8	14.6
MRTK044	DHA	19.0	23.2	34.7	16.3	20.8	31.6	20.3	25.9	25.7
MRTK045	DHA	38.6	19.4	25.1	32.5	16.7	21.4	16.0	22.2	13.0
MRTK053	DHA	18.2	21.9	17.1	15.6	18.7	14.9	22.8	22.2	21.7
MRTK057	DHA	29.5	29.5	28.8	25.3	25.7	25.4	15.9	28.5	21.1
MRTK058	DHA	17.4	9.7	22.0	15.2	7.6	18.9	12.8	19.8	18.9
MRTK060	DHA	33.3	29.1	28.3	29.9	23.9	25.0	13.4	40.9	25.6
MRTK062	DHA	22.5	19.8	29.3	19.9	17.4	26.3	14.6	16.3	14.3
MRTK001	Placebo	10.5	12.7	11.4	8.7	10.6	9.6	11.7	10.6	9.6
MRTK003	Placebo	8.7	11.6	9.0	6.9	8.9	6.9	15.4	18.5	15.9
MRTK004	Placebo	12.1	11.6	23.2	10.4	9.4	20.0	12.1	11.6	6.7
MRTK010	Placebo	10.9	14.3	16.1	9.9	12.7	14.3	12.3	11.3	9.5
MRTK012	Placebo	29.5	29.7	36.9	25.9	25.3	30.2	26.7	22.6	26.9
MRTK013	Placebo	13.0	11.3	17.4	10.9	9.1	15.0	14.2	11.8	8.1
MRTK014	Placebo	10.2	15.1	13.4	9.1	13.5	12.3	15.0	14.6	12.5
MRTK015	Placebo	10.9	10.2	10.5	10.0	8.3	9.3	12.2	13.8	11.7
MRTK016	Placebo	30.2	41.3	36.2	25.9	33.8	30.2	19.8	18.7	15.0
MRTK017	Placebo	14.2	17.3	13.8	12.3	14.6	12.2	13.9	14.0	13.7
MRTK018	Placebo	14.2	20.3	23.0	12.3	16.3	19.8	12.3	15.3	16.4
MRTK019	Placebo	13.0	16.0	18.0	1.1	13.8	16.4	12.6	11.6	15.5
MRTK023	Placebo	14.5	28.8	42.5	12.3	23.8	36.7	17.4	16.3	27.4

MRTK025	Placebo	11.4	23.5	19.0	9.5	20.3	16.7	13.0	18.2	20.4
MRTK026	Placebo	19.3	30.4	19.6	16.4	26.6	17.4	22.1	17.2	13.8
MRTK027	Placebo	19.6	31.0	29.3	16.4	26.9	25.1	17.1	17.2	15.5
MRTK029	Placebo	30.1	33.5	12.2	26.2	29.7	10.0	31.9	28.2	21.6
MRTK030	Placebo	14.7	16.9	7.7	11.8	14.1	6.1	15.4	15.0	9.0
MRTK031	Placebo	9.6	15.0	17.1	8.0	13.2	15.1	11.5	13.3	7.4
MRTK034	Placebo	28.8	26.7	30.3	25.3	22.0	25.8	15.0	13.1	11.9
MRTK037	Placebo	29.8	24.2	29.6	25.6	20.4	25.8	15.0	19.6	11.9
MRTK039	Placebo	16.6	15.2	13.9	14.1	13.3	11.9	9.6	14.0	10.2
MRTK041	Placebo	26.4	26.1	20.3	24.8	23.1	18.3	13.1	13.2	15.9
MRTK042	Placebo	9.3	7.6	13.3	8.0	6.7	11.2	13.6	17.0	13.3
MRTK049	Placebo	35.0	34.7	38.1	31.0	29.1	33.8	27.8	43.4	22.9
MRTK051	Placebo	17.6	10.1	22.9	16.3	8.0	21.1	15.5	20.5	16.4
MRTK052	Placebo	13.9	16.3	20.5	12.5	13.9	18.0	13.9	17.0	21.7
MRTK056	Placebo	32.9	33.7	45.6	28.7	29.5	39.4	11.9	11.8	17.4

		SPECDLDLIVC			SPECHDLCT			SPECBHDL2B		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	9.3	7.2	10.8	37.0	39.3	30.6	9.3	11.6	9.3
MRTK005	DHA	7.1	5.2	7.8	51.1	57.2	49.2	11.2	20.1	15.6
MRTK007	DHA	3.4	5.2	4.8	41.8	45.7	48.8	10.5	14.0	16.1
MRTK008	DHA	3.7	5.2	3.2	34.3	36.3	35.7	10.0	14.0	12.3
MRTK009	DHA	5.5	4.7	5.3	48.9	39.1	37.8	14.4	13.8	13.5
MRTK011	DHA	5.5	5.5	3.6	67.7	59.7	61.8	31.2	27.8	29.0
MRTK020	DHA	5.1	5.0	5.2	42.3	38.5	37.0	11.4	11.3	10.0
MRTK021	DHA	5.1	5.0	5.2	44.6	34.4	36.7	15.5	11.9	11.3
MRTK024	DHA	5.1	5.3	5.2	62.3	56.0	52.5	24.0	22.5	26.1
MRTK028	DHA	8.7	7.5	7.1	84.0	73.9	46.4	29.5	27.5	15.1
MRTK033	DHA	5.1	5.3	5.2	45.4	56.7	42.8	12.8	18.9	12.2
MRTK035	DHA	5.1	3.8	5.4	41.3	42.7	35.0	14.1	16.8	12.2
MRTK036	DHA	5.1	7.2	5.4	44.2	41.1	31.6	10.9	12.8	10.5
MRTK038	DHA	6.4	9.3	7.1	45.4	53.4	42.8	11.5	17.4	14.3
MRTK040	DHA	6.1	6.7	6.1	52.6	62.8	46.2	16.6	24.5	13.9
MRTK043	DHA	4.5	4.2	5.9	57.9	54.1	52.4	20.3	19.7	18.0
MRTK044	DHA	5.9	8.2	8.1	44.3	52.0	49.3	15.5	19.3	19.8
MRTK045	DHA	4.8	5.6	5.3	47.9	64.2	49.9	17.6	22.6	19.2
MRTK053	DHA	6.6	6.2	7.8	54.3	60.0	57.7	19.9	21.9	20.5
MRTK057	DHA	5.4	6.9	8.1	63.3	89.2	67.6	25.6	42.7	33.5
MRTK058	DHA	5.3	6.2	7.1	54.1	49.3	47.7	17.1	15.3	14.3
MRTK060	DHA	5.0	8.7	7.3	56.9	59.0	35.0	19.0	17.4	10.7
MRTK062	DHA	5.6	5.6	6.0	37.7	48.2	40.3	15.6	16.3	13.7
MRTK001	Placebo	4.8	5.5	4.8	52.4	62.6	52.2	18.1	23.9	18.6
MRTK003	Placebo	6.0	11.6	5.7	101.1	121.1	90.3	60.2	75.9	51.3
MRTK004	Placebo	3.7	4.4	4.3	42.5	36.6	35.8	12.1	11.3	13.3
MRTK010	Placebo	4.2	4.7	3.2	46.9	43.5	50.6	15.2	12.9	19.1
MRTK012	Placebo	8.1	6.3	8.1	44.8	41.3	48.8	11.0	10.7	14.0
MRTK013	Placebo	4.4	4.4	3.2	38.7	35.2	36.3	12.4	9.6	11.4
MRTK014	Placebo	3.7	5.5	5.1	53.1	52.0	54.5	20.4	19.3	18.2
MRTK015	Placebo	3.7	5.0	5.1	67.3	63.0	61.8	30.4	25.3	23.0
MRTK016	Placebo	5.2	8.5	6.3	28.7	42.9	40.2	9.5	16.0	11.6
MRTK017	Placebo	5.1	5.0	4.7	53.7	45.9	45.2	16.4	15.7	12.9
MRTK018	Placebo	5.1	5.3	4.8	52.1	49.8	45.9	14.2	15.6	12.9
MRTK019	Placebo	5.1	5.0	5.2	43.3	41.0	41.2	11.7	12.2	10.0

MRTK023	Placebo	5.7	5.9	6.1	61.9	58.8	50.9	25.3	21.9	19.3
MRTK025	Placebo	5.1	5.9	5.8	42.3	45.4	39.1	10.1	14.7	11.6
MRTK026	Placebo	5.1	5.3	5.2	40.4	39.1	36.1	9.8	12.5	10.0
MRTK027	Placebo	6.6	6.6	6.1	36.7	60.7	38.6	10.4	20.7	11.3
MRTK029	Placebo	9.9	6.6	5.2	39.7	36.9	32.2	9.9	12.5	10.0
MRTK030	Placebo	5.1	5.3	5.2	46.4	56.0	36.4	13.8	18.5	10.0
MRTK031	Placebo	5.1	3.6	5.2	42.6	47.8	39.6	10.9	15.2	10.9
MRTK034	Placebo	6.1	3.6	5.4	66.9	65.5	49.6	24.0	23.6	16.7
MRTK037	Placebo	5.8	5.1	5.4	58.6	66.9	50.0	19.5	21.1	15.3
MRTK039	Placebo	5.1	4.2	5.4	42.9	48.9	44.5	12.8	16.1	11.2
MRTK041	Placebo	6.4	5.7	6.1	60.3	66.0	53.1	27.5	27.9	24.3
MRTK042	Placebo	4.5	4.3	5.4	51.3	60.0	51.7	19.0	24.3	19.4
MRTK049	Placebo	7.5	9.7	5.9	51.3	55.5	42.8	15.5	15.3	10.9
MRTK051	Placebo	5.6	5.9	6.2	62.7	75.3	70.7	28.6	35.0	32.6
MRTK052	Placebo	8.8	9.4	10.5	64.1	72.5	64.5	25.6	32.3	25.4
MRTK056	Placebo	4.5	5.6	5.3	36.9	44.4	49.6	8.8	12.5	14.0

		SPECHDL2AC			SPECHDL3C			SPECTCHDL		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	4.8	5.5	4.8	22.9	22.2	16.5	4.36	5.05	6.15
MRTK005	DHA	10.9	6.1	2.4	29.0	31.1	31.2	3.07	3.37	3.91
MRTK007	DHA	4.3	4.4	4.5	26.9	27.2	28.2	2.26	2.73	2.91
MRTK008	DHA	2.8	4.7	2.9	21.5	17.6	20.6	2.36	3.67	2.32
MRTK009	DHA	4.4	5.0	3.0	30.1	20.4	21.3	2.91	3.60	3.18
MRTK011	DHA	10.9	8.8	9.8	25.5	23.1	23.1	2.46	2.97	2.23
MRTK020	DHA	5.1	5.0	5.2	25.9	22.2	21.9	3.19	3.16	3.6
MRTK021	DHA	5.1	5.0	5.2	24.0	17.5	20.3	2.57	2.71	2.75
MRTK024	DHA	12.6	7.5	7.4	25.6	26.0	19.0	2.42	2.38	2.41
MRTK028	DHA	12.3	9.4	5.2	42.1	36.9	26.1	2.47	2.75	3.42
MRTK033	DHA	5.1	11.4	5.2	27.5	26.4	25.4	3.36	3.30	3.38
MRTK035	DHA	5.1	7.6	5.4	22.1	18.2	17.3	3.03	3.30	4.01
MRTK036	DHA	5.1	5.4	5.4	28.2	22.9	15.6	3.70	3.20	4.35
MRTK038	DHA	5.1	9.9	5.4	28.8	26.1	23.1	4.53	3.71	5.23
MRTK040	DHA	6.1	11.4	5.4	29.9	27.0	26.9	3.87	2.79	3.74
MRTK043	DHA	8.0	8.6	5.0	29.6	25.7	29.5	2.32	2.14	2.92
MRTK044	DHA	4.8	7.6	5.0	24.0	25.0	24.5	3.40	3.34	4.40
MRTK045	DHA	5.3	11.1	5.0	24.9	30.5	25.7	3.55	2.49	3.11
MRTK053	DHA	8.3	7.3	5.3	26.1	30.9	31.9	3.26	3.42	3.25
MRTK057	DHA	10.8	19.8	9.0	27.0	26.7	25.1	2.85	2.57	2.88
MRTK058	DHA	5.0	5.6	5.0	32.0	28.5	28.5	2.74	2.62	3.29
MRTK060	DHA	7.8	10.8	5.3	30.2	30.9	19.0	3.05	3.33	4.17
MRTK062	DHA	7.0	5.6	5.3	15.2	26.4	21.3	3.67	2.99	3.74
MRTK001	Placebo	9.0	12.3	9.3	25.3	26.3	24.3	2.24	2.22	2.16
MRTK003	Placebo	13.5	19.2	14.4	27.4	26.0	24.6	1.71	1.91	1.84
MRTK004	Placebo	4.4	4.4	6.7	26.0	20.9	15.8	2.44	2.77	3.23
MRTK010	Placebo	4.8	6.9	6.8	26.8	23.7	24.8	2.37	2.61	2.39
MRTK012	Placebo	3.7	4.4	3.6	30.1	26.1	31.2	3.92	4.67	4.15
MRTK013	Placebo	3.0	4.4	3.0	23.2	21.2	21.9	2.92	2.78	2.66
MRTK014	Placebo	8.0	6.1	4.1	24.6	26.7	32.3	2.40	2.86	2.52
MRTK015	Placebo	11.0	9.9	8.1	25.9	27.8	30.8	1.98	2.03	2.07
MRTK016	Placebo	4.9	4.4	4.8	14.3	22.6	23.9	4.81	4.16	4.20
MRTK017	Placebo	5.4	6.1	3.9	31.9	24.2	28.4	2.66	3.01	2.76
MRTK018	Placebo	5.1	10.0	5.0	32.9	24.1	28.1	2.48	2.90	3.11

MRTK019	Placebo	5.4	5.0	5.2	26.2	23.8	26.1	2.66	3.21	3.30
MRTK023	Placebo	12.3	9.4	9.3	24.3	27.5	22.2	2.61	3.19	4.62
MRTK025	Placebo	5.1	6.6	5.4	27.2	24.1	22.1	2.69	3.43	4.21
MRTK026	Placebo	5.1	7.2	5.2	25.6	19.4	20.9	3.42	3.94	3.61
MRTK027	Placebo	5.1	9.7	5.2	21.2	30.4	22.2	3.23	3.12	3.95
MRTK029	Placebo	4.8	5.0	5.2	25.0	19.4	17.1	4.59	5.19	3.61
MRTK030	Placebo	5.1	9.1	5.2	27.5	28.5	21.3	2.73	2.70	2.75
MRTK031	Placebo	5.1	7.5	5.2	26.6	25.2	23.5	2.50	2.65	2.85
MRTK034	Placebo	9.3	11.8	5.4	33.6	30.0	27.5	2.74	2.46	3.09
MRTK037	Placebo	6.4	12.8	5.4	32.6	32.9	29.2	3.03	2.56	3.28
MRTK039	Placebo	5.1	6.3	5.4	25.0	26.5	27.9	2.69	2.39	2.63
MRTK041	Placebo	9.1	12.6	7.8	23.8	25.4	21.0	2.65	2.51	2.97
MRTK042	Placebo	5.6	12.4	6.1	26.4	23.4	26.2	2.28	2.09	2.60
MRTK049	Placebo	5.9	6.6	5.0	29.9	33.7	27.0	3.96	3.76	4.70
MRTK051	Placebo	7.2	11.1	6.5	27.0	29.1	31.6	2.79	2.24	2.7
MRTK052	Placebo	8.0	10.8	5.0	30.4	29.5	34.1	2.29	2.35	2.90
MRTK056	Placebo	4.5	9.0	6.5	23.6	22.9	29.1	3.83	3.65	4.02

		SPECLDLP			SPECLDLMDPH			SPECHDLMD		
SID	TREAT	SUM	PRE	POST	SUM	PRE	POST	SUM	PRE	POST
MRTK002	DHA	437	391	501	1.032	1.030	1.032	1.105	1.099	1.099
MRTK005	DHA	303	241	324	1.030	1.029	1.030	1.104	1.098	1.106
MRTK007	DHA	165	236	220	1.031	1.031	1.030	1.108	1.102	1.103
MRTK008	DHA	160	202	122	1.032	1.030	1.031	1.105	1.096	1.101
MRTK009	DHA	238	199	237	1.030	1.029	1.031	1.106	1.098	1.100
MRTK011	DHA	175	203	144	1.029	1.028	1.028	1.091	1.089	1.089
MRTK020	DHA	212	192	220	1.031	1.030	1.031	1.102	1.100	1.103
MRTK021	DHA	125	124	140	1.030	1.031	1.031	1.097	1.094	1.098
MRTK024	DHA	224	215	155	1.030	1.032	1.029	1.091	1.093	1.085
MRTK028	DHA	351	303	312	1.030	1.029	1.030	1.098	1.097	1.098
MRTK033	DHA	209	302	222	1.029	1.030	1.029	1.105	1.097	1.101
MRTK035	DHA	136	199	183	1.028	1.029	1.029	1.098	1.094	1.093
MRTK036	DHA	242	342	261	1.030	1.033	1.031	1.104	1.102	1.096
MRTK038	DHA	319	451	431	1.030	1.032	1.031	1.104	1.098	1.096
MRTK040	DHA	290	336	238	1.029	1.031	1.029	1.102	1.094	1.100
MRTK043	DHA	193	192	225	1.030	1.032	1.030	1.098	1.097	1.099
MRTK044	DHA	279	366	363	1.031	1.031	1.029	1.098	1.097	1.094
MRTK045	DHA	221	292	201	1.030	1.032	1.029	1.097	1.097	1.095
MRTK053	DHA	314	303	320	1.030	1.029	1.030	1.095	1.097	1.099
MRTK057	DHA	230	372	319	1.030	1.030	1.030	1.093	1.085	1.087
MRTK058	DHA	199	280	284	1.029	1.032	1.031	1.101	1.100	1.101
MRTK060	DHA	200	516	351	1.029	1.033	1.033	1.099	1.099	1.096
MRTK062	DHA	221	236	224	1.030	1.030	1.030	1.088	1.097	1.096
MRTK001	Placebo	182	181	162	1.031	1.030	1.031	1.095	1.093	1.094
MRTK003	Placebo	234	346	235	1.031	1.031	1.030	1.081	1.077	1.081
MRTK004	Placebo	169	174	126	1.031	1.031	1.028	1.106	1.099	1.092
MRTK010	Placebo	179	176	136	1.030	1.030	1.029	1.103	1.099	1.097
MRTK012	Placebo	373	307	374	1.032	1.030	1.032	1.110	1.103	1.107
MRTK013	Placebo	200	177	123	1.031	1.031	1.030	1.104	1.102	1.103
MRTK014	Placebo	196	219	193	1.030	1.029	1.030	1.097	1.096	1.104
MRTK015	Placebo	170	203	186	1.030	1.031	1.030	1.091	1.093	1.098
MRTK016	Placebo	264	303	235	1.032	1.032	1.033	1.096	1.096	1.105
MRTK017	Placebo	206	206	198	1.031	1.030	1.030	1.102	1.098	1.106

MRTK018	Placebo	191	224	226	1.030	1.031	1.030	1.104	1.097	1.105
MRTK019	Placebo	194	183	223	1.031	1.029	1.030	1.102	1.099	1.103
MRTK023	Placebo	249	242	350	1.030	1.029	1.029	1.090	1.094	1.091
MRTK025	Placebo	197	260	279	1.031	1.030	1.030	1.104	1.098	1.098
MRTK026	Placebo	284	242	207	1.031	1.030	1.030	1.103	1.096	1.102
MRTK027	Placebo	259	260	237	1.032	1.030	1.030	1.099	1.096	1.099
MRTK029	Placebo	449	364	281	1.032	1.031	1.032	1.103	1.095	1.099
MRTK030	Placebo	221	221	161	1.031	1.030	1.030	1.101	1.097	1.103
MRTK031	Placebo	185	180	146	1.031	1.030	1.029	1.103	1.101	1.101
MRTK034	Placebo	232	177	193	1.030	1.031	1.030	1.096	1.097	1.098
MRTK037	Placebo	227	262	193	1.030	1.031	1.029	1.100	1.099	1.100
MRTK039	Placebo	166	195	177	1.030	1.031	1.030	1.100	1.101	1.103
MRTK041	Placebo	218	209	240	1.030	1.030	1.030	1.089	1.091	1.087
MRTK042	Placebo	196	224	206	1.031	1.031	1.030	1.097	1.092	1.095
MRTK049	Placebo	373	555	304	1.031	1.033	1.030	1.101	1.102	1.103
MRTK051	Placebo	229	281	247	1.028	1.031	1.029	1.091	1.089	1.092
MRTK052	Placebo	261	299	361	1.031	1.031	1.031	1.094	1.090	1.096
MRTK056	Placebo	180	194	242	1.029	1.030	1.029	1.103	1.098	1.101

APPENDIX L

SAMPLE OF EXCEL STATISTICS FORMULAS

Statistics in Microsoft Excel 2010

=Average function ; =stdev(of average function) ;

SEE = (stdev value)/sqrt(count of group);

t-value = mean/SEE; one sample ttest and treatment vs treatment =ttest (treatment, zero range; ,2 tail, type 2);

time point to time point comparison t-value = mean/SEE; one sample ttest =ttest (treatment, zero range; ,2 tail, type 1);

effect size = mean/stdev; CI CoEff =CONFIDENCE(0.05, SD,n)
Lower Bound = mean - CI CoEff; Upper Bound = mean + CI CoEff

Example:

	Pre-Camp	Post-Camp
DHA	Eotaxin_CPR	Eotaxin_CPO
mean	6.71	8.41
n	22	22
SD	29.39	38.19
SEE	6.27	8.14
t-value (mean/see)	1.07	1.03
one sample ttest	0.296384346	0.313713341
Effect Size	0.23	0.22
CI CoEff	12.28	15.96
Lower Bound	-5.57	-7.55
Upper Bound	18.99	24.36
Placebo		
mean	56.70	43.61
n	21	21
SD	111.42	75.03
SEE	24.31	16.37
t-value (mean/see)	2.33	2.66
one sample ttest	0.030266854	0.01492606
Effect Size	0.51	0.58
CI CoEff	47.65	32.09
Lower Bound	9.05	11.52
Upper Bound	104.35	75.70