THE EFFECTS OF DHA SUPPLEMENTATION ON MARKERS OF INFLAMMATION AND MUSCLE DAMAGE FOLLOWING AN ACUTE ECCENTRIC EXERCISE BOUT

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ABSTRACT

Aim: The purpose of this study was to investigate the influence of docosahexaenoic acid (DHA) on muscle damage and inflammation following an acute eccentric exercise bout. **Methods**: A double-blind placebo-controlled, study was performed using 41 healthy, untrained males aged 18-28 y who consumed either 2 g/d DHA or placebo (PL, corn oil) for 32 days. Supplements were consumed for 28 days prior to exercise. Participants completed an eccentric exercise procedure of the elbow flexors at 140% of 1-RM (6 sets x 10 repetitions). The time under tension (TUT) for each set of eccentric contractions was recorded manually from the investigators voice commands. Fasted blood samples for prostaglandin E2 (PGE2), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL1-ra), C-reactive protein and creatine kinase (CK) were assessed on days 1, 2 and 4. Fasted serum DHA was measured at baseline (day -28) and on day 1. Peak isometric strength of the elbow flexors, delayed-onset muscle soreness, and range of motion were measured on day 1 prior to exercise and days 2, 3, and 4 following exercise. **Results**: DHA significantly reduced natural log of CK ($p<0.05$) response over 4 d. Additionally, IL-6 area under the curve (AUC) was reduced for DHA compared to PL (3.6 ± 2.5 pg/mL vs. 5.3 ± 2.7 pg/mL) ($p<0.05$). TUT/set was higher in the DHA group compared to placebo ($p<0.05$). There were no other significant differences between treatments. **Conclusion**: DHA supplementation produced lower indicators of muscle damage (CK) and inflammation (IL-6 AUC). DHA supplementation resulted in greater TUT/set.

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Chapter 1: INTRODUCTION

Introduction:

Various government agencies, health organizations and fitness associations provide exercise guidelines to the general public. The obesity epidemic that has gripped the United States is one of the factors influencing these aggressive campaigns. One of these organizations, the American College of Sports Medicine (ACSM) recommends resistance to improve bone strength, muscular strength and prevention of weight regain after weight loss (44). Some of the benefits associated with resistance training include increased fat free mass (133), improved nutrient partitioning (144, 130), reduced blood pressure, and improved bone mineral density (145). Arguably the most advantageous reasons to begin a resistance training program are the apparent positive correlations between muscular strength and lifespan (127).

While the majority of benefits associated with resistance training and aerobic exercise are commonly known by the majority of the population, few still engage in physical activity (23). As of 2004, only 22% of men and 18% of women took part in strength training exercises on two or more days per week according to data from the Centers for Disease Control (23). An obstacle that may prevent the continuation of exercise after one begins is delayed onset muscle soreness (DOMS). DOMS is the result of micro-trauma to muscle tissue as a consequence of strenuous exercise which triggers an acute inflammatory response (30). Those individuals who are relatively untrained and unaccustomed to particular exercises are going to experience greater amounts of soreness than individuals who participate in regular training and exercise due to an absence of muscular adaptation (90). The repair of muscle tissue following an exercise bout increases one's tolerance to future damaging muscular exercises and is known as the repeated bout effect (26). However, the extent of discomfort one experiences in the 24-72 hours following strenuous exercise could deter a person from continuing their exercise program so they never

reach a point of improved muscular adaptation (i.e. resistance of muscle fiber degradation to a familiar stimulus). Some individuals may use anti-inflammatory medications to reduce the pain following damaging exercise. However, some research suggests that these drugs may have detrimental effects on gastrointestinal integrity (81), interfere with subsequent muscle adaptation (167), and potentially increase the inflammatory response to exercise (98). As an alternative with fewer side effects, natural dietary supplements could be used to reduce muscular damage following initiation of resistance exercise to improve compliance with exercise recommendations.

Long chain polyunsaturated omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are vital nutrients for human health. Increased public awareness demonstrates the importance of omega-3 (n-3) fatty acid consumption, especially in relation to omega-6 (n-6) fatty acids. In support of this evidence, the American Heart Association (AHA) recommends that patients with documented coronary heart disease (CHD) consume 1g combined (DHA + EPA) per day and that n-3 fatty acids can significantly reduce cardiovascular disease (CVD) in those with coronary artery disease (72). Furthermore, the AHA suggests that omega-3 act by decreasing heart arrhythmias, slowing the rate of atherosclerotic plaque formation, improving endothelial function, decreasing the number of circulating triglycerides, reducing the risk for thrombosis and lowering inflammatory responses (72). Simopoulos noted that consumption of a ratio of n-6:n-3 fatty acid of 4:1 reduced all-cause mortality by 70% in patients with CVD (138). Additionally, women with a lower ratio of n-6:n-3 had a decreased risk for breast cancer, while a ratio of 2.5:1 in colorectal cancer patients reduced cell proliferation and rheumatoid arthritis patients with a lower ratio also experienced relief (138). However, people consuming a western style diet typically have an estimated ratio of omega-6 to omega-3 of

almost 10:1 or greater, with the majority of n-3 fatty acids coming from α-linolenic acid (ALA), a precursor to DHA and EPA (Table 1) (73,137). In total, the average American consumes 1.1-1.6 grams of omega-3 fatty acids each day, however only 0.1-0.2 g/day come from sources of preformed EPA and DHA (73). This disparity in polyunsaturated fatty acid (PUFA) intake is partly due to the prevalence of inexpensive, omega-6 rich oils that play an integral role in the current food supply. Examples of such omega-6 rich oils are corn, cottonseed, grapeseed, safflower, soybean, and sunflower oils.

Table 1: Common Omega-6 and Omega-3 Fatty Acids and their Abbreviations

Fatty Acid	**Abbreviation**	**Chemical Structure (Carbon Chain Length:Double Bonds)**
α-linolenic acid	ALA	18:3(n-3)
Arachidonic acid	AA	20:4(n-6)
Docosahexaenoic acid	DHA	22:6(n-3)
Eicosapentaenoic acid	EPA	20:5(n-3)
Linoleic Acid	LA	18:2(n-6)

Another reason why the proportion of n-6:n-3 consumption is so high lies in the reliance on grain to expedite the slaughter of livestock. Feeding livestock on pasture slows the rate of weight gain in the animals compared to grain feeding and results in an animal with less body fat. Duckett et al, observed that omega-3 fatty acid content of cattle was diminished each day they were on a feedlot (grain-based diet) (47). Additionally, cattle fed a pasture-based (non-grain) diet over the winter months had an omega-6:omega-3 ratio of 1.65:1, while traditional grain feeding

yielded a ratio of 4.84:1 (46). This evidence demonstrates the significant increase in the proportion of n-6:n-3 fatty acid ratio in cattle fed grain (the method of feeding for the majority of commercially available beef). Replacing grass fed beef with grain fed beef is another example of how American diets are heavily omega-6 based.

Therefore, one should take into account the amount of EPA and DHA when selecting food and supplemental sources of n-3 PUFA. A standardized serving of grass-fed beef (113 g) would provide approximately 88.5 mg of n-3 PUFA (2). In comparison, raw Coho salmon contains 1560 mg of EPA+DHA per 113 g serving while farmed and wild caught Atlantic salmon contain 1910 mg and 1410 mg per serving respectively (48). Other species of salmon such as Red and Pink provide less EPA+DHA at 69 mg per 100 g of raw fish (48). Sources of EPA and DHA on average are higher in fish sources compared to other foods.

Omega-3 fatty acids could benefit strength training athletes because of their anti-inflammatory properties. Some evidence suggests that pharmaceutical interventions which target cyclooxygenase (COX) enzymes reduce muscle soreness after novel eccentric exercise (113) while other evidence shows no difference (148). COX enzymes catalyze reactions for pro- and anti-inflammatory mediators and inhibition of these enzymes may relieve inflammation and pain. Unlike pharmaceuticals, omega-3 fatty acids promote the formation of anti-inflammatory compounds when they bind with COX enzymes as opposed to inhibiting the enzymes.

Omega-3 fatty acids may provide an effective remedy to reduce the inflammatory response and subsequent muscular pain that is experienced at the onset of a new strength training protocol. Research indicates that omega-3 fatty acids can mitigate the inflammatory response that often results from over consumption of omega-6 fatty acids, notably arachidonic acid (154). Omega-3 fatty acids are thought to alter various transcription factors that are responsible for

controlling a myriad of cytokine signaling pathways which influence the inflammatory process. A more specific example is that n-3 PUFA can inhibit nuclear factor-kB (NF-kB) signaling pathways by modifying the transcription of proinflammatory cytokines (154, 15). Additionally, eicosanoid synthesis can be influenced by the proportion of n-3 fatty acids contained within the cell (154, 54, 162). The eicosanoids derived from n-3 PUFA's (3-series prostaglandins and thromboxanes and the 5-series leukotrienes amongst others), are considered anti-inflammatory or mildly inflammatory compared to those produced by the n-6 PUFA, archadionic acid (2-series prostaglandins and thromboxanes and the 4-series leukotrienes) (154, 8, 123). Furthermore, n-3's act at the level of gene expression by influencing transcription factors such as NF-kB and ligand binding peroxisome proliferator-activator receptors (PPARs) (13). NF-kB is responsible for transcribing inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). Studies have shown that the presence of the n-3 fatty acid EPA slows the degradation of IkB, the inhibitory subunit of NF-kB in vitro (154, 82, 105, 166). Furthermore NF-kB can be inhibited by PPARs that bind with eicosanoids derived from n-3 PUFA (154). Therefore, increasing omega-3 status could potentially decrease the production of inflammatory cytokines.

Table 2. Inflammatory Markers

Cytokine	Abbreviation
C-Reactive Protein	CRP
Interleukin-6	IL-6
Interleukin-1 Receptor Antagonist	IL-1ra
Prostaglandin E2	PGE2
Tumor Necrosis Factor Alpha	TNF-α

Statement of Problem:

Muscle soreness and inflammation that results after novel eccentric exercise can be a painful and bothersome process when beginning a resistance training program. A nutritional supplement, like DHA, could be beneficial if it attenuated the soreness and damage induced by damaging resistance exercise. Pharmaceutical interventions like COX inhibitors have shown mixed effectiveness in this regard, and they are often accompanied by undesirable effects (gastrointestinal discomfort, potential negative effects on protein synthesis and potential adverse effects on organ systems). DHA exhibits properties which theoretically may relieve discomfort associated with resistance training as well as provide systemic benefits to decrease inflammation and benefit cardiac health.

Purpose and Significance:

The purpose of this study was to determine whether the consumption of omega-3 fatty acids attenuates either muscle damage indicators or the inflammatory processes which are responsible for the DOMS response that is experienced by untrained individuals beginning a resistance training exercise program. If the intervention is successful, it could potentially provide evidence for the value of DHA to increase adherence to novel resistance training.

Hypotheses:

We hypothesize that ingestion of DHA compared to placebo will reduce markers of inflammation (serum TNFα, IL-6, IL-1ra, PGE2, CRP) and muscle damage (serum CK, muscle strength reduction, muscle soreness) that occur within four days after an acute, novel eccentric resistance exercise bout.

Limitations:

- Subjects were free- living, i.e. may not have followed our directions explicitly

- Blood draws occurred only on the mornings of days -28, 1, 2 and 4.
- No blood was collected in the hours immediately following exercise
- No muscle biopsies or MRI imaging were conducted on the exercised limb to verify extent of muscle damage
- The smallest increments for 1-RM testing were 1.25 lbs
- Eccentric testing used ~140% of 1-RM but this amount varied slightly due to limitations in weight increments
- Subjects did not adhere to a standardized diet but were instead instructed to maintain their typical diet
- Data regarding typical dietary intakes were not collected
- Results can only be applied to subjects who are similar to the population studied
- Time under tension was manually measured
- Time under tension was measured by different study assistants

Delimitations:

- Subjects were relatively untrained (i.e. no resistance training for >6 months)
- Subjects were males, healthy, non-smokers between 18-28 years of age
- Subjects were randomly assigned treatment or placebo
- Investigators were blinded to subject treatment assignment
- The exercise test was 6x10 eccentric-only repetitions and administered by the same investigator
- Subjects were free of physical limitation or illness which would affect exercise performance

- Subjects were instructed to discontinue medication or supplementation which would confound results
- The independent variable for the DHA group was consumption of two grams of docosahexaenoic acid for 32 consecutive days (28 days prior to beginning eccentric exercise)
- The independent variable for the Placebo group was consumption of two grams of corn oil for 32 consecutive days (28 days prior to beginning eccentric exercise)
- The dependent variables were time under tension , muscle soreness, range of motion in the exercised limb, peak isometric strength (ISO), serum CK, CRP, IL-6, IL-1ra, TNF-α, PGE2 and DHA content

Basic Assumptions:

- Subjects adhered to dietary restrictions, such as the cessation of dietary supplements, vitamins, omega-3 fatty acids and anti-inflammatory medications
- Subjects returned the actual number of supplements that had not been consumed
- Subjects provided maximum effort during each test session
- Subjects fasted for a minimum of 10 hours prior to blood collections
- Subjects were honest in completing their exit survey
- Spectrophotometer, isokinetic dynamometer and blood assays operated and were performed correctly

Abbreviations and Definitions of Terms:

- 1-RM – 1-repetition maximum, the maximum load that can be lifted in one attempt of a given exercise
- BF – body fat percentage, the percentage of fat mass in proportion to total body mass

- BMI – body mass index, an anthropometric measurement calculated by dividing a subject's mass in kilograms by the square of their height in meters (kg/m^2)
- CK – creatine kinase, an enzyme used to indirectly assess the degree of muscle damage
- CNS – central nervous system
- COX – cyclooxygenase, an enzyme that catalyzes the formation of biological mediators (prostaglandins, thromboxanes)
- CRP- C-reactive protein, an inflammatory protein synthesized in the liver. Blood levels positively correlate with the amount of body inflammation
- Cytokine – protein signaling molecules used in intracellular communication
- DHA - docosahexaenoic acid, an omega-3 fatty acid that serves structural roles in the brain and retina and is suggested to have anti-inflammatory benefits
- DOMS – delayed onset muscle soreness, the pain and discomfort that is experienced in muscle tissue following unaccustomed exercise
- EPA - eicosapentaenoic acid, an omega-3 fatty acid that is suggested to have anti-inflammatory benefits
- IGF-1 – insulin-like growth factor 1, an anabolic hormone primarily synthesized in the liver
- IL-6 – interleukin-6, a cytokine that can exert anti-inflammatory or pro-inflammatory effects
- IL-1B –interleukin 1 beta, a pro-inflammatory cytokine
- IL1-ra – interleukin-1 receptor antagonist, a protein which binds to the interleukin-1 receptor preventing the binding of interleukin-1

- ISO – peak isometric strength, the maximum force generated by a particular muscle during a static contraction
- LOX – lipooxygenase, an enzyme responsible for the oxidation of polyunsaturated fatty acids
- Myofibril – One of the longitudinal fibrils in a skeletal muscle fiber
- MVC – maximum voluntary contraction, the maximum amount of force that is generated by a muscle during a voluntary isometric contraction
- n-3: a polyunsaturated omega-3 fatty acid
- n-6: a polyunsaturated omega-6 fatty acid
- NF-kB – nuclear factor kappa B, a protein complex found in most animal cells that controls the transcription of DNA
- PGE2 – prostaglandin E2, is a lipid compound released by blood vessels in response to inflammation or infection
- Prostaglandin – lipid compounds that serve as mediators with a wide array of cellular effects
- PUFA – polyunsaturated fatty acid
- ROM – range of motion. In this instance, the distance a limb can rotate about a joint; or, the flexibility of a joint
- ROS – reactive oxygen species, chemically reactive molecules with one unpaired electron that can be damaging to cell structure and function
- Smith squat – preforming a barbell squat on a Smith machine. A Smith machine has a barbell that is fixed (often at a slight angle) to two vertical rails which slide up and down
- Thromboxane – a lipid signaling molecule with vasoconstrictive properties

- TNF-α – Tumor Necrosis Factor-alpha, a pro-inflammatory cytokine involved in the regulation of immune cells
- Training Volume – the number of sets x repetitions x intensity
- TUT – time under tension, the amount of time during which an exercised muscle is contracting
- VAS – visual analog scale, a common indicator to assess the degree of muscle soreness following exercise
- VO_2Max – The maximal ability of one's body to transport and use oxygen during exertion.

Chapter 2: REVIEW OF LITERATURE

Introduction:

Eccentric exercise is a part of almost any resistance training program or activity and research has demonstrated that an emphasized eccentric component helps induce muscle hypertrophy and helps facilitate increases in muscle strength. However, unaccustomed or novel exercise results in muscle damage and soreness that is a result of many factors. Dietary supplements have been used as a means to increase muscle recovery and improve performance. Docosahexaenoic acid (DHA) could act as a potential ergogenic aid in attenuating muscle soreness and damage under anaerobic conditions and has yet to be fully investigated as a treatment. This literature review will cover muscle damage, inflammation, acute eccentric exercise protocols and current nutritional supplements designed to alleviate muscle damage. Lastly, it will introduce DHA and its potential benefits in improving exercise performance.

Muscle Damage:

Delayed onset muscle soreness (DOMS) is the pain associated with muscle tissue damage that peaks approximately 24-72 hours following bouts of intense or unaccustomed exercise that include eccentric or lengthening muscle contractions (30). The magnitude of DOMS can be assessed using several different parameters including prolonged reduction in muscular strength, muscle soreness, increases in inflammatory markers in muscle tissue and blood as well as an increased concentration of muscle proteins found in the blood. Additionally, muscle biopsies can reveal sarcomeric disruption along with z-line streaming which are both indicative of muscle breakdown (51). Although muscle damage is best confirmed using direct observation of muscle tissue, this is often impractical. Typically, less invasive studies rely on the indirect markers of muscle damage that include functional impairment (e.g., reduced strength;

soreness/tenderness; and reductions in the range of motion in the exercised limb) and blood markers of damage (30).

One of the indirect subcellular parameters used to confirm muscle damage is the circulating level of the enzyme, creatine kinase (CK). After undergoing eccentric exercise, the muscle sarcolemma can become stretched and damaged allowing release of CK from the muscle cytosol and mitochondria into the blood stream (20). Therefore, increased circulating levels of CK can be indicative of muscle degradation or injury, which has been exhibited during eccentric exercise bouts where a significant strength decline is present (132, 27, 76). Furthermore, increased CK levels appear to run concurrently with muscle fiber disruption (30). Nosaka, et al. reported muscle damage assessed by magnetic resonance imaging (MRI) positively correlates with circulating CK activity for the majority of subjects (r=0.90-0.94) (103). Therefore, individuals who exhibit the greatest muscle damage will most likely have the highest CK activity. However, one of the shortcomings of using CK activity as a marker of muscle damage is due to the large variability between subjects (103). For example, Nosaka et al. reported a peak CK activity for ten male subjects ranging from 236-25,244 IU/L. It is not currently understood why there is considerable variability between subject CK responses to exercise (30). Thus, it is preferable to include several measures of muscle damage in experiments.

The time course of peak CK activity also varies by the type of exercise activity that is performed (30). Bouts of high force eccentric exercise can produce peak CK activity 3-6 days following exercise (158, 66, 112, 84, 30) compared to instances of downhill running which demonstrate peak CK activity occurring between 12-24 hours following exercise bouts (106, 30). The CK activity of subjects performing Smith squats also had earlier peak CK levels (6-24 hours) after exercise (151, 42, 120). Moreover, it appears that the longer time course to peak CK

activity correlates with a larger increase in CK activity as well (30). For example, damaging high force eccentric exercise increased CK activity from 236-98,725 IU/L with an average CK activity of 18,129 ± 31,382 IU/l in one study (84), while other investigations have also observed substantial changes in CK (66, 103).

In research settings, visual analog scales (VAS) of soreness perception and reductions in the range of motion (ROM) of exercised limbs are also used to assess potential damage to muscle tissue. VAS are subjective measures completed by subjects after participating in damaging exercise in which a horizontal line (Figure 1) is anchored by a phrase representing the two opposing outcomes. Such as “no soreness” and “unbearable soreness” (two commonly used scales for this are 1-10 or 0-100, measured in centimeters or millimeters, respectively). Indications of soreness using such scales coincide with reductions in strength and elevations in circulating CK activity (30). Similarly, a reduction in range of motion is also evident after eccentric exercise (27, 76). The reduction in range of motion is the result of an accumulation of fluid in the damaged tissue as a part of the inflammatory response which restricts the movement of an exercised limb following a damaging bout.

Disruption in myofibrils and changes in peripheral nociceptor sensitivity are thought to be largely responsible for the perception of pain that is endured during DOMS (88). Specifically, it is thought that several compounds like prostaglandins and histamines, which are increased after strenuous activity, signal nerve receptors to send pain signals to the central nervous system (CNS) from the damaged muscle tissue (30). In addition, DOMS seems to cause the CNS to be more susceptible to muscular pain (88, 15). One of the consequences of DOMS is the reduced ability of the exercised muscles to function optimally. This experience is likely to dissuade some individuals from continuing or adhering to an exercise program. If exercise recovery could be

ameliorated by eliminating or severely reducing the DOMS response following resistance training bouts, it is possible that this will lead to an increased level of exercise tolerance by participants.

Inflammation:

Another result of damaging eccentric exercise is the development of inflammation, the body's natural response to injury and an essential component of the healing process. After bouts of strenuous exercise there will be an aggregation of inflammatory markers as a result of an accumulation of immune cells and interstitial fluids inside muscle tissue. Specifically, after exercise induced muscle damage, adhesion molecules expressed by the endothelial cells attract neutrophils and monocytes which migrate to the damaged tissue, with concentrations peaking at approximately 24 hours after initial injury (29, 21). Along with the neutrophils, pro-inflammatory cytokines, like tumor necrosis factor alpha (TNF-α) and Interleukin-1 beta, are released by monocyte-derived macrophages and begin phagocytosis of the damaged muscle tissue while inducing up-regulation of adhesion molecules through positive feedback (29, 21). A second wave of macrophages accumulate in the tissue roughly 2-4 days after injury and secrete anti-inflammatory cytokines and other factors which promote muscle growth and cease the localized inflammation process (29). One proposed mechanism that may promote muscle growth involves acute increases of insulin like growth factor – 1 (IGF-1) by elevated growth hormone levels following exercise, thereby contributing to increased rates of protein synthesis (21).

Cytokine synthesis can be regulated by stress hormones, reactive oxygen species and mechanical stress due to exertion-related muscle damage and can occur in several different cell types with the ability to produce overlapping cytokines (21). Consequently, these cytokines can be indirectly measured to gauge the muscle inflammatory response. In particular, circulating

concentrations of interleukin-6 (IL-6) become considerably elevated during prolonged periods of muscle contraction, more so than any other cytokine (142, 115). Additionally, IL-6 is responsible for signaling the transcription of other inflammatory cytokines as well as C-reactive protein (CRP) (165). Furthermore, IL-6 along with IL-1 up-regulates gene expression of the pro-inflammatory cytokine TNF-α (80). Chronic elevation in these pro-inflammatory cytokines has been associated with several negative health outcomes (24, 62). As examples, increased CRP levels have been positively correlated with cardiovascular disease and chronic inflammation is associated with obesity (24, 62). However, acute elevations of IL-6 have been linked with enhanced levels of fat oxidation, and it may even play a role in restricting the inflammatory process by stimulating the production of IL-1 receptor antagonist (IL-1ra) and halting the production of TNF-α (114, 161). IL-1ra can inhibit the inflammatory actions of interleukin-1 alpha and beta by binding to the IL-1 receptor. The exact role of IL-6 in the inflammatory process is still not fully understood; to this point, it is believed that it mostly acts in an anti-inflammatory capacity when elevated in response to an acute event (like novel anaerobic and aerobic exercise) because it is produced locally in exercising skeletal muscle, can be elevated by exercise even in the absence of muscle damage, and exhibits growth factor capabilities (115). However, as mentioned previously, chronic elevations of IL-6 seem to be pro-inflammatory and associated with negative consequences such as elevated CRP and TNF-α.

An acute inflammatory response is necessary to assist in the reparation of damaged tissue, while an augmented inflammatory response is catabolic and can lead to sarcopenia (92, 3). For instance, TNF-α can induce catabolism by selectively degrading myosin heavy chain protein as well as reduce anabolism by inhibiting IGF-1 signaling through NF-kB interference of IGF-1 receptor level phosphorylation (21, 100). Similarly, IGF-1 receptor activity and

subsequently IGF-1 can be reduced by IL-1 (100). Furthermore chronic expression of IL-6 has been linked with reductions in levels of myofibrillar protein by increasing the transcription of genes which suppress cytokine signaling (100). In rats, chronic inflammation results in muscle atrophy and a reduction in the rate of muscle protein synthesis by 23% (93). This reduction appears to be due to a translational inefficiency in the synthesis of myofibrillar and sarcoplasmic proteins leading to the reduction of muscle tissue as a result of leucine resistance (75). As a result, chronic inflammation is not conducive to muscle remodeling, thereby reducing the capacity of an individual to respond to exercise training and limiting their physical performance. Impaired muscle remodeling can be further evidenced by the elevations of prostaglandins. Prostaglandins are compounds derived from fatty acids that serve important roles inside the body. In particular, prostaglandin E2 (PGE2) has been shown to modulate protein turnover by increasing rates of protein degradation in vitro (125). PGE2 synthesis rates are increased in response to tissue injury, like those that would be experienced following damaging exercise (71). Furthermore, increases in serum PGE2 concentrations are used as an indicator of muscle breakdown following exercise (41).

Acute Eccentric Exercise Protocols:

Unaccustomed eccentric exercise leads to delayed onset muscle soreness as well as a loss in strength (30). Laboratory protocols to provoke this response typically use downhill running or eccentric isotonic muscle contractions using a dynamometer, machine weights or free weights. The protocol to use depends on the risks, benefits, and ease of use as well as similarity to the real-life activity being mimicked. A traditional method of eccentric exercise involves the testing of the knee extensors using a leg extension. Another widely used method involves contraction of the elbow flexors using an isokinetic dynamometer. Additionally, there are several other

protocols that engage the arms and legs, such as biceps preacher curls, isokinetic elbow extension, downhill running, and Smith squats.

When implementing various protocols, researchers typically use a resistance that is based on a percentage of the subject's one-repetition maximum (1-RM) or a percentage of their maximum isometric strength (ISO). In several studies, subjects were loaded or provided 100% of their maximum voluntary contraction (158, 95, 27, 150, 4) while other studies used a resistance that was greater than (64, 36) or less than 100% (120, 66, 27, 136). Furthermore, researchers can manipulate the difficulty of any given exercise by changing the time-under-tension for the contracting muscle, the rest period between sets and the total work volume (reps x sets). For example, Silva et al. (136) used an eccentric lowering phase that lasted for six to eight seconds while the protocol used by Trombold et al. (150) required each repetition to last approximately three seconds. Exaggerated lowering phases can be implemented because the most significant muscle force (i.e. greatest contractile strength) can be exerted during the eccentric lowering phase (45, 131, 25).

Knee Extensor Protocols:

Several studies isolate the quadriceps muscles by having subjects perform leg extensions on an isokinetic dynamometer (158, 132, 48). However, there is significant variation between protocols, which can be seen in the knee extensor summary table below. One aspect of particular interest is the degree in which these protocols vary in their total training volume (sets x reps). For instance, White et al. (158) employed a total training volume of 50 maximal eccentric contractions while the protocol implemented by Shafat et al. (132) had a much larger training volume totaling 300 maximal contractions. Subjects participating in the White et al. protocol experienced reductions of approximately 25-35% in peak torque as well as maximal voluntary

contraction (MVC) 24 hours after completing the eccentric bout. In contrast, subjects participating in the Shafat et al. protocol only had reductions of 15-25% in MVC 24- hours after exercise. This result is the opposite of what one might expect due to the larger volume employed in the Shafat et al. protocol. Although this difference may be explained because the Shafat et al. protocol used a smaller range of motion during the lifting phase, had a smaller sample size and provided a shorter rest period (30-s vs. 1-min between sets). A shorter range of motion puts subjects in a more favorable mechanical advantage. When the angle increases and the muscles lengthen, the mechanical advantage decreases along with the ability to produce maximum force which will limit the overall load used.

The subjects participating in the White et al. protocol also had a significant increase ($p<0.05$) in creatine kinase (CK) activity, with levels peaking in the control group at 1428 $\pm$ 2208 IU/L 72 hours following the exercise bout. A study by Mackey et al. showed similar reductions in MVC (~35%) and also demonstrated significant increases in CK activity (84). However, in this study, CK levels peaked at 96-hours post-eccentric exercise (as opposed to 72-hours) with levels reaching 18,129 $\pm$ 31,382 IU/L. It is difficult to determine exactly why there was a difference of 24 hours between peak elevations for the two studies. Aside from the large individual variability of CK activity, one explanation could be that the Mackey et al., experiment used 100 total contractions while the White et al. experiment only used 50 contractions. The larger volume employed by Mackey et al. may have prolonged the release of CK into the blood.

From these studies (Table 1), the average reduction in strength ranged from 15-40%. The CK response also varied widely, with peak activity ranging from 1000 IU/L to 31,000 IU/L. Typically, a greater reduction in strength appeared to coincide with a larger increase in CK

activity. Therefore, the increase in CK activity appears to be related to the amount of muscle damage sustained during an eccentric exercise bout.

Compound Quadriceps Protocols:

Another method to target the knee extensors is downhill running. Buford et al. (18), required subjects to complete an eccentric exercise bout where they were required to run on a treadmill positioned at a 10% grade downhill for 45 minutes at an intensity of ~60% of their VO_2Max. A slightly different downhill protocol was used by Nunan et al., in which subjects ran downhill at 7 mph on a 10% grade for 5 sets of 8 minute runs separated by 2 minutes of walking at a 0% grade between sets (106). Both of these protocols were effective in promoting DOMS with subjects reporting an increase from 0 mm to 50-70 mm on a 200 mm visual analog scale used in the Nunan experiment 48hrs after exercise (representing an increase in soreness of 25-35%). The drawbacks to downhill running protocols are that they require specialized equipment. In addition, subjects would have to be aerobically fit to complete these exercise bouts and aerobically induced fatigue could limit the number and magnitude of eccentric contractions. Furthermore, exercises which mimic more traditional resistance training would be more appropriate for our investigation.

Lastly, another way to target the quadriceps muscle is to employ a compound exercise. Variations of a smith squat protocol exercising the quadriceps muscles have been used by Udani et al., Davies et al., and Rawson et al. (Table 2) (151, 42, 120). As is indicated in the table below, there is large variability between exercise protocols. Subjects in both the Rawson et al. and Davies et al., protocol experienced significant increases in muscle soreness. Participants reported results of approximately 45 mm and 71 mm (out of 100 mm) on visual analog scales 48 hours after completing the eccentric bout. Furthermore, both studies showed peak CK activity at 24

hours post exercise with levels of 740 ± 666 IU/L for Davies et al. and log activity of approximately 2.7 for Rawson et al. Reductions in peak torque of approximately 21% were also noted at 24 hours in the Davies et al. study. These studies demonstrate that subtle variation in volume, load and rest parameters are all capable of eliciting appropriate DOMS responses.

An issue that may arise when implementing a protocol that involves multiple muscle groups like the Smith squat is that the targeted muscle (quadriceps) might not always fatigue first. The Smith apparatus is designed to relieve a lot of the burden of the supporting musculature in balancing and stabilizing the load because the barbell is fixed to two vertical rails. But it still leaves the possibility for muscle groups that assist and stabilize the completion of the squat to impede performance by fatiguing faster than the primary mover involved (quadriceps) in the activity. The source of the fatigue may make the corresponding data difficult to interpret. This is especially true in a study like that of Udani et al. (151), where the load used is relatively small and exercise volume is not uniform across subjects. In this case, it seems entirely possible for fatigue of the lower back, shoulders, or hamstrings to set in prior to fatigue of the quadriceps. The problem is the potential for different muscle groups to be the limiting factor for different individuals completing the same exercise.

Upper Limb Protocols:

Some studies indicate that greater muscle damage is created following eccentric exercise of the arm compared to the leg (66, 112). A study conducted by Jamurtas et al. (66), exercised both the elbow flexors and knee extensors on an isokinetic dynamometer using the same volume and eccentric load (percentage of limb peak torque strength). The results from this study showed that not only was recovery time longer for the elbow flexors but strength loss was greater compared to the knee extensors. In fact, the eccentric peak torque of the knee extensors elevated

about +10-15% above baseline at 72 ($p<0.05$) hours and 96 ($p<0.05$) hours post exercise while the elbow flexors were still weakened about -18-20% below baseline at those time points ($p<0.05$). Similarly, there were also significant differences ($p<0.05$) in isometric strength between the arms and the legs at 24, 48, 72, and 96 hours. With strength loss in the arms reaching about 22% at 24-hours before returning to about 18% below baseline at 96-hours compared with isometric strength loss in the legs of about 10% and 5% at those time points respectively ($p<0.05$).

A more dramatic reduction in isometric peak torque was seen in a similar study conducted by Paschalias et al. (112), where subjects arm strength declined ~60% compared to a 40% reduction in leg strength at 24 hours ($p<0.05$). There were also significant differences between the two limbs at 48 and 72 hours following exercise ($p<0.05$). Furthermore, both studies showed significant differences in mean CK levels between the limbs in the hours following exercise ($p<0.05$). With the subjects in the Jamurtas et al. (66) study peaking at 96 hours with CK levels of the elbow flexors reaching 3670 IU/L ($p<0.05$) compared to only 459 IU/L ($p<0.05$) for knee extensors. The subjects in the Paschalias et al. study peaked at 72 hours with levels reaching ~2500 IU/L ($p<0.05$) and ~1500 IU/L ($p<0.05$) for the arms and legs respectively. Significant differences ($p<0.05$) in CK levels were noted at 72 and 96 hours in both studies.

Other markers of muscle damage which were shown to be different between the limbs were DOMS upon palpitation of ~6 for the arm and 3 for the legs (1-10 scale) at 24 hours post exercise ($p<0.05$ for both) (112). With significant differences from baseline and between samples ($p<0.05$) noted at 48 and 72 hours as well (~7 vs. ~5.5 and ~6.5 vs. 5 for arm vs. leg). Additionally, there were significant differences in ROM at 24, 48, and 72 hours with the most

substantial being a reduction of ~25% for the arms compared to a loss of ~12% for the legs at 48 hours (112).

One study that did not find significant reductions in muscle strength or elevations in CK levels following an eccentric exercise bout of the elbow flexors was conducted by Miles et al. (95). In this protocol, subjects used an isokinetic dynamometer to perform 3 sets of 15 repetitions with 5 minutes rest between sets. Subjects experienced increased soreness and loss of strength immediately following the high force eccentric bout but their strength had returned to baseline by 24 hours. This recovery may be explained by the relatively small work volume implemented in the protocol, the elongated rest period between repetitions, the maximal effort of the subjects or the small sample size (n=8) affecting statistical power. A weakness of the isokinetic dynamometer is that it will move the isolated limb through the intended range of motion regardless of the amount of force being applied to its lever arm. Therefore, it is difficult to determine whether a subject is maximally resisting the lever arm or not resisting at all. The controlled lowering of free weights, while not completely infallible does remedy this problem to some degree by enforcing subjects to engage the load that is being placed on their exercised limb.

A method employed by Chen et al., and Lavendar et al. required subjects to perform eccentric contractions using free weights (a dumbbell) with their elbow resting on a preacher curl bench (27,76). In these studies, intensity parameters for eccentric exercise were based on the maximal voluntary contraction of the elbow flexors at an isometric joint angle of 90°. Subjects were verbally instructed to lower a dumbbell (whose weight corresponded to a percentage of their 1-repetition maximum (1-RM)) over a count of “5”. At the bottom position the investigators removed the load and the subject passively returned their arm to the starting position. Using

loads corresponding to 40% of MVC in both studies subjects had reductions in maximal isometric force of approximately 25-50% at 24 hours post eccentric exercise. Furthermore, range of motion declined between 15-22%, arm circumference increased 3-7 mm, ratings of muscle soreness increased about 20-30 mm (out of 100 mm) on elbow extension at 24 hours after the exercise bout for both studies. In the study conducted by Chen et al., more significant eccentric loading (i.e. a higher percentage of MVC) produced results that were indicative of greater muscle breakdown. This outcome is expected due to the progressive manner in which motor unit recruitment occurs during voluntary muscle activation, with more muscle fibers being activated to move a heavier load. That is contractile strength increases as more motor units are recruited.

Eccentric exercise bouts that isolate arm muscles will also have varied responses in strength reduction and peak CK activity like those seen with isolated muscles of the leg. Therefore, the amount of muscle damage is largely dependent on the volume, intensity, range of motion, and duration of eccentric exercise. It is apparent then, that there are several different protocols that are effective in eliciting DOMS, albeit to varying degrees. Therefore, as researchers it becomes prudent to develop a protocol that can be efficiently and consistently implemented during investigation to reduce variability and increase uniformity between trials. It has been seen that using exercises which target the arms will elicit a larger CK response, increase perceptions of muscle soreness, and invoke a longer recovery time compared to the legs when the volume and eccentric load are equal (66,112). Moreover, it was seen that the protocols implemented by Chen et al. (27) and Lavendar et al. (76) that used free weights (dumbbells) at varying intensities during elbow flexor contractions, had larger reductions in isometric force production compared to isokinetic dynamometers (Table 3). Additionally, it was observed that as the intensity of eccentric loading increased the indicators of muscle damage also increased (27).

Therefore, protocols which engage the arms with high intensity free weights in a controlled eccentric lowering phase produced the greatest degree of muscle damage.

A significant DOMS response should be observed by applying a similar eccentric method which targets the upper extremities. This method will benefit the investigators by providing a potentially larger data set to determine if DHA supplementation makes a significant difference in attenuating the inflammatory response along with the subsequent muscle breakdown associated with eccentric exercise. One final consideration when asking untrained participants to engage in extremely damaging physical exercise is the risk of overexertion and development of rhabdomyolysis, a condition that can result in renal strain due to the rapid breakdown of muscle tissue. This can be prevented by adjusting the volume and intensity that is employed during exercise. In the current investigation, rhabdomyolysis is unlikely to occur because the overall exercise volume was low, exercise was executed in a unilateral fashion with only one muscle group, and the involved muscle group was relatively small.

Supplements:

The discomfort associated with the DOMS response following exercise has led many investigators to seek ways to reduce it, whether through dietary supplements or pharmaceutical means. For example, Connolly et al. (33) investigated the effects of cherry juice supplementation to attenuate the symptoms of muscle damage following exercise. The researchers found that cherry juice supplementation reduced the strength loss and the perception of pain following a bout of acute eccentric exercise compared to placebo. The researchers believed that this was perhaps due to a preservation of muscle function considering that the cherry juice supplementation only resulted in a strength loss of 12% at 24 hours compared to 30% in placebo, rather than a reduction in muscle damage because subjects experienced no

difference in motion loss or muscle tenderness following the exercise bout. However, additional measurements of muscle damage such as serum CK were not provided. To speculate, it could be that supplementation provided a preservation of neural functioning which lessened the strength decline. Maximum voluntary contractions (MVC) can result in CNS fatigue and reductions in motor unit firing rates (53). As such, maximal isokinetic contractions are largely dependent on the ability to generate neural drive and an inability to produce a sufficient neural signal would result in reduced force output (53). The researchers suggested that the numerous antioxidants contained in the cherries contributed to the preservation of strength. Evidence in this regard was observed in people consuming approximately 8 oz of fresh cherries per day who experienced reductions in the concentration of circulating inflammatory markers found in their blood (69). However, known antioxidants, vitamin C and vitamin E have not conclusively shown protective effects on muscle degradation, which perhaps is due to their inability to target the specific reactive oxygen species (ROS) released in response to exercise (89). ROS contribute to the dysfunction of mitochondria, thereby leading to neurodegeneration in neurons, like motor neurons, which control muscle contraction, as well as other impairments depending on where the dysfunction occurs (38). Another possibility is that the effects of eccentric exercise on muscle breakdown are independent of ROS as a mediator. Since this study did not include a full array of muscle damage indicators and does not provide any clear mechanistic explanation for the benefit of cherry juice, it should be repeated by other investigators to verify that there is an effect.

Other well researched supplementation strategies involve the consumption of essential amino acids, carbohydrates, and derivatives of creatine (often monohydrate) prior to, during, after, exercise or in some combination thereof. Amino acids, and in particular branched chain amino acids, (BCAA, L-leucine, L-valine and L-isoleucine), have been shown to significantly

lower creatine kinase, muscle soreness, and other indices of muscle damage when ingested during and for several days after exercise (104, 87, 97, 135). These effects are believed to be caused by the reduction of BCAA catabolism in muscle tissue due to the endogenous BCAA intake and subsequent release into the bloodstream. Therefore, the ingested BCAA are likely being oxidized in place of endogenous BCAA. Additionally, the large concentration of L-leucine contained within BCAA supplements can stimulate protein synthesis to further preserve muscle tissue. However, other studies involving BCAA have failed to find any significant muscle preserving or performance enhancing effects (55). While carbohydrate consumption is known to quickly replenish glycogen stores, induce an insulin response and thus stimulate protein synthesis, differences in carbohydrate intakes alone seem to have no effect on either soreness or muscle damage following exercise (95, 31). This same negligible effect also holds true for the performance enhancing substance creatine monohydrate (70).

The role for supplements as a means to increase performance, health, body composition and recovery from aerobic and anaerobic exercise is an extremely profitable marketplace. In 2009, retail supplement sales reached $9.4 billion in the United States (109). This market potential has driven great interest for development of the next lucrative and successful product for the public. In turn, researchers are continually investigating different compounds or cellular intermediates that could affect the muscle building process. For example, b-hydroxy-b-methylbutyrate (HMB), a metabolite of the amino acid leucine, has been shown to contribute to muscle hypertrophy and decrease protein degradation in untrained subjects participating in resistance exercises (52, 111) but not in trained subjects (110). It is believed, that HMB reduces protein degradation by fortifying the cell membrane through cholesterol synthesis making it more resistant to proteolysis (101). One study showed HMB combined with a-ketoisocaproic

acid (KIC), a keto-analogue of leucine, were ineffective in attenuating indices of muscle damage following a bout of downhill running in recreational exercisers that had no prior experience with lower limb eccentric training (106). A prior study conducted in the same laboratory implementing an eccentric exercise protocol utilizing the elbow flexors found that HMB and KIC supplementation diminished the CK response (peak of ~155 IU/L for HMB-KIC and ~315 IU/L for placebo), decreased strength loss (-2% for HMB-KIC vs. -12% for placebo at 24 hours $p<0.05$) along with DOMS (visual analog score of 2.6 vs. 3.3 out of 10 for HMB-KIC and placebo respectively, $p<0.05$) (153). These contrasting outcomes suggest that the nature of exercise (aerobic component to downhill running), the involved limb, or perhaps differences between subjects contribute to the effectiveness of any supplemental intervention. At the same time, the small sample size involved in both studies (n=14 and n=8 for Nunan (106) and van Someren (153) respectively) could likely contribute to the inconsistent results. For the van Someren study, a post-hoc power analysis indicates that a sample size of 32 subjects would have been necessary for statistical power.

There is some research and anecdotal evidence to support the notion that aspects of physical performance can be improved through the use of a few current nutritional supplements. Currently, there is no clinically conclusive nutritional intervention that has consistently demonstrated specific reductions in muscle damage and enhanced recovery for studied populations.

Omega-3 Fats and Inflammation:

One of the positive benefits of omega-3 fatty acids is their apparent ability to reduce inflammation (154). This effect can be achieved at the level of gene expression, the production of inflammatory mediators and through differential synthesis of eicosanoids. Eicosanoids

produced by the oxidation of 20-carbon PUFA are responsible for regulating the length of action as well as the magnitude of the inflammatory response (70). The omega-3 fatty acid EPA plays a role in generating anti-inflammatory eicosanoids by acting as substrate for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (154). DHA cannot be converted to eicosanoids but it can act as a substrate for LOX and COX enzymes and generate local acting anti-inflammatory mediators termed resolvins along with EPA (154). These n-3 fatty acids compete with n-6 PUFA's (chiefly arachidonic acid) for COX and LOX enzymes, with the latter signaling the creation of pro-inflammatory eicosanoids (154). Consequently, it is likely that greater intakes of n-3 fatty acids will increase the concentration of anti-inflammatory eicosanoids, while proportionally reducing the production of pro-inflammatory eicosanoids since there will be larger concentrations of available n-3 substrate to compete for the COX and LOX enzymes (162, 60, 121). To illustrate, when human alveolar cells undergo a lipopolysaccharide (LPS) challenge, (injection of LPS act as endotoxins and results in the release of pro-inflammatory cytokines) DHA added in a 1:1 or 1:2 ratio with arachidonic acid (AA) can overcome the resulting inflammation by improving the PUFA ratio of the cell membranes (39). The net result is that 1:1 and 1:2 ratios of DHA to AA produce an overall anti-inflammatory response (measured as the balance of anti-inflammatory cytokines against pro-inflammatory cytokines) while ratios of 1:4 and 1:7 produce a pro-inflammatory response (39).

Furthermore, n-3 fatty acids can reduce anti-inflammatory gene expression by interacting with nuclear factor kappa B (NF-kB) and peroxisome proliferator-activated receptors (PPARs), transcription factors which are responsible for inflammatory cytokine signaling and cellular differentiation respectively (154).

When looking at the efficacy of treating patients with n-3 PUFA the results have been inconclusive. One study found that 3.4 g/d of pre-formed EPA (~1900 mg) + DHA (~1700 mg) consumed over an eight week period was enough to reduce fasting triglycerides by 27% in moderately healthy subjects compared to placebo; however, this treatment failed to depress inflammatory cytokine gene expression (140). In contrast, hemodialysis patients receiving 2.4 g/day of EPA (1600 mg) + DHA (800 mg) for 8 weeks had reduced serum insulin, TNF-α, IL-6 and high sensitivity CRP (119). Moreover, Trebble et al. demonstrated that supplementation with as little as 208 mg EPA + 101 mg DHA for a four week period was sufficient to reduce cytokine production (TNF-α and IL-6) in stimulated immune cells (149). Lastly, a study conducted by Bouwens et al. demonstrated that long term supplementation (26 weeks) of 1.8 g/d EPA (~1100 mg) + DHA (~850 mg) led to the alteration of the expression of 1040 genes in peripheral blood mononuclear cells by qualitative real time polymerase chain reaction (16). In this experiment, EPA + DHA supplementation downregulated genes in eicosanoid synthesis, prostaglandin synthesis, interleukin pathways (including IL-6 and IL-1), adipogenesis, scavenger receptor activity, PPAR signaling, and NF-kB signaling, among others (16). Taking all these studies into account, it appears that either the dose of DHA needs to be large enough to induce a change in cell composition or, if dosing is more conservative, the duration of supplementation needs to be long enough to achieve such a change, despite the observation in an in vitro study with lower dosing (149).

The use of n-3 fatty acids and DHA in particular as dietary supplements has steadily increased over the past 10 years due to their apparent cardio-protective properties, an area that has received considerable media attention. In fact, the popularity of fish oil supplementation trailed only multivitamins in a 2009 Consumer Labs survey (35). This has been secondary to

positive statements in the media, some of which are based on the research showing positive health outcomes for conditions such as arthritis and cognitive decline, as well as the fact that the American Heart Association recommends ingestion of n-3 fats to reduce risk of heart disease. For instance, one study found that the relative risk for coronary death and sudden cardiac death was 68% ($p<0.01$) and 55% ($p<0.001$) respectively for placebo compared to groups supplemented with 850 mg of EPA and DHA daily (85). Further, Harris and Von Schacky found that red blood cell levels of EPA and DHA (termed the Omega-3 index) of > 8% provided cardio-protective properties (58). This cardio-protection could possibly be attributed to the anti-arrhythmic effect of n-3 PUFAs (77). This was further evidenced by Aarsetøy et al., who determined that the Omega-3 index was positively associated with reducing ventricular fibrillation during ischemic events by incorporating n-3 PUFA in cardiac cell membranes preventing abnormal cardiac contractions (1).

Docosahexaenoic Acid:

DHA is a polyunsaturated omega-3 fatty acid commonly found in nature (Table 4). Some of the more abundant food sources include wild fish, livestock that are raised on pasture, and eggs from chickens receiving a diet high in omega-3.

A recent study published in the British Journal of Nutrition found that increasing blood concentration of n-3 PUFA may be possible without implementing changes in dietary habits by replacing grain-fed red meat with grass-fed (88). The study conducted by McAfee et al., substituted habitual grain-fed red meat intake with three servings of grass-fed red meat each week that equated to approximately 67 g of meat per day over a four week period. Subjects receiving grass-fed meat increased incorporation of n-3 fatty acids in their platelets of roughly 3.5% (23.13% → 26.86%, $p=.002$) while those consuming grain-fed red meat had a slight

reduction in n-3 fatty acid levels (22.81% → 22.62%). The researchers estimated that grass-fed meat contributed 94% of the long chain omega-3 fatty acid intake of the subjects over the course of the intervention compared to 87% for the grain-fed group. These data appear to suggest that substituting grass-fed meat for grain-fed would be a viable option for increasing omega-3 fatty acid intakes even though the difference between the meats amounted to 18 mg of n-3 PUFA per day with the grass-fed group totaling ~65 mg/day total of n-3 PUFA. The investigators believe that limitations in food composition tables and dietary analysis could be partly responsible for the observed disparity. Furthermore, this amount was sufficient enough to raise plasma concentration of n-3 PUFA from 3.93% to 5.39% over the span of four weeks ($p=.019$). In contrast, subjects receiving a fish oil supplement containing 2160 mg of n-3 PUFA per day for 8 weeks raised plasma concentration of n-3 PUFA from 5.26% to 10.93% ($p<0.0001$) and flaxseed supplementation containing 3510 mg of alpha-linolenic acid (ALA) raised plasma n-3 PUFA from 5.08% to 6.07% ($p<0.05$) over the same period (22). This demonstrates that pre-formed EPA and DHA have superior incorporation rates into plasma as well as the relative inefficiency in the synthesis of these long chain n-3 PUFA by ALA.

Microalgae grown and harvested from fermentors are also used as a source to derive DHA. Within algael cell cultures, DHA can be isolated and extracted for use in a variety of products. For instance, this algae-derived DHA can be added to certain foods like yogurt and peanut butter to boost their n-3 content. However, fish oil supplements, a $739 million dollar industry as of 2008, are more than likely the most concentrated source for the majority of people (61). Additionally, DHA can be synthesized endogenously from its omega-3 precursor α-linolenic acid. ALA is highly concentrated in flaxseeds, walnuts and to a much lesser extent, in green vegetables. However, the amount that can be converted into long chain polyunsaturates

like DHA and EPA is minimal due to poor conversion efficiency (6). As was previously mentioned, the average American consumes only 1.1-1.6 g of n-3 PUFA per day, with preformed EPA and DHA only contributing 0.1-0.2 g/day to this total (73). Specifically, results from the National Health and Nutrition Examination Surveys (NHANES) estimated DHA intake at 80-100 mg/d for adults in the United States (157). Therefore, more emphasis on consuming food sources rich in preformed DHA and EPA or supplementing with these PUFA's to obtain optimal blood levels that encourage a state of anti-inflammation should be advised.

In the body, DHA plays a predominant role in various biochemical processes. For one, it is the most abundant structural fatty acid of the brain and retina (56). Furthermore, it is theorized that the consumption of large quantities of DHA led to the brain development that is seen in modern day Homo sapiens (40). There is also some evidence suggesting that DHA plays a more anti-inflammatory role than EPA (156, 96, 164). In vitro experiments demonstrate that DHA downregulates lipopolysaccharide (LPS) expression of IL-6 and IL-1B mRNA to a greater extent than EPA in human derived macrophages (156, 96). This is believed to be due to the decline in the ability of NF-kB to bind with DNA after translocating into the nucleus of macrophages and an increased concentration of IkBa (an inhibitory subunit of NF-kB) in the cytoplasm. Furthermore, in one study, NF-kB signaling was altered more significantly by DHA than EPA through potent inhibition of nuclear NFkB-p65 binding, greater reductions in total NFkB-p65 levels in the nucleus and a longer maintenance of NFkB-p50 in cytoplasm (96). By modulating NF-kB subunits, n-3 PUFA can produce anti-inflammatory effects at the level of gene transcription. In human studies, subjects supplemented with 1.8 g/day of EPA and 0.3 g/d of DHA did not have significant alterations in inflammatory markers (164). This could be due to the fact that the supplemental dose of DHA was low, which was evidenced in the study by the

absence of change in cell composition of DHA (164). Moreover, there was an inverse relationship between soluble intercellular adhesion molecule-1 (inflammatory marker) with DHA, suggesting that DHA is a more potent anti-inflammatory than EPA (164). Growing evidence in this area has lead researchers to further investigate potential causal links between dietary DHA consumption and positive health outcomes.

Omega-3 Fats and Exercise:

Exercise can be primarily aerobic or anaerobic depending on the mode, intensity and duration of muscle contractions. One of the most abundant fuel sources for the generation of ATP in aerobic exercise is beta oxidation of fatty acids, while anaerobic exercise mostly relies on phosphocreatine and anaerobic glycolysis for ATP. As such, maximal performance is dependent on the sport-specific development of these energy systems. Some evidence indicates that EPA and DHA are ineffective at reducing inflammation under predominantly aerobic conditions (99, 147). Perhaps this effect is caused by reductions in stored glycogen in addition to the depletion of omega-3 fatty acids as a result of prolonged beta-oxidation.

The realm of exploring the effects of n-3 PUFA supplementation as a possible beneficial aide in exercise performance/recovery is still relatively new. A recent study conducted by Nieman et al., (Table 5) examined the effects of n-3 fatty acid supplementation on parameters of exercise performance and inflammation following endurance exercise (99). In this study, subjects were given 2000 mg EPA and 400 mg DHA daily for 6 weeks prior to and during a 3-day endurance cycling test where they cycled for 3 hours/d with a 10 km time trial during the final fifteen minutes of every hour. The researchers found that neither inflammation as measured by plasma IL-1ra, IL-6, and IL-8 nor exercise performance were significantly affected by n-3 supplementation. Serum concentration of EPA and DHA increased 311% and 40% respectively

in the subjects undergoing supplementation. A similar lack of effects on inflammatory markers were observed after six weeks in an endurance study conducted by Toft et al. where subjects received 3.6 g/day of n-3 PUFA containing approximately 1908 mg of EPA and 1116 mg of DHA (147). In this instance, subjects received supplementation prior to participating in a marathon. Due to the nature of the marathon training during this study, it is possible that the inflammatory effect was caused by a reduction in stored glycogen during the marathon race rather than muscle breakdown due to training adaptation. Therefore, it is reasonable to suggest that the direct application of these findings to inflammation brought on by eccentric exercise is not entirely applicable due to the training history of the subjects and the mode of activity.

Burke et al., conducted a study examining the effects of omega-3 supplementation on eccentric exercise induced muscle damage following bicep curls (19). Subjects in this study completed two bouts of eccentric elbow flexor contractions, the first after 14 days of omega-3 diet restriction and the second after 7 days of 3 g/d n-3 PUFA supplementation. Following the n-3 PUFA supplementation, the subjects' rise in DOMS as assessed by VAS was 15% lower compared to the first bout. While this finding is encouraging, the sample size was small and the primary measure was obtained through a subjective and somewhat limited diagnostic indicator (visual analog scale) when there are no other dependent measures, like blood indicators of damage to substantiate the finding. Furthermore, the data were likely influenced by the repeated bout effect which could have induced protective effects that resulted in the smaller degree of soreness (26, 90). Similarly, a study conducted by Lenn et al. also produced inconclusive results (78). In this investigation, subjects received 1.8 g/d of n-3 PUFA containing approximately 270 mg/d of DHA + 400 mg of EPA for 30 days resulting in a serum increase of 450% and 475% respectively. After completing 50 maximal eccentric contractions on an isokinetic dynamometer

the omega-3 group had smaller increases in DOMS four days after exercise $p<0.008$ (1.8 centimeters vs. 3.8 centimeters for omega-3 vs. placebo on a 10 point scale). However, peak CK, IL-6 and TNF-α were all higher following exercise for omega-3 treatment compared to placebo but these measures did not reach statistical significance. This study's results may have been confounded by inclusion of women, due to the possibility of gender differences in soreness/muscle damage (30). Furthermore, the study may have been underpowered because there were only 16 subjects and three study groups.

Lastly, Philips et al, conducted an eccentric exercise study measuring the inflammatory response after subjects consumed a multi-ingredient supplement that included DHA (117). The results indicated that daily consumption of mixed dietary supplements containing 800 mg DHA, 300 mg flavonoids, and 300 mg mixed tocopherols had significantly reduced the rise in IL-6 (peak concentration ~30 pg/mL vs. 55 pg/mL) and CRP (peak concentration ~0.6 mg/L vs. ~1.0 mg/L). However supplementation also produced slightly elevated albeit statistically insignificant levels of peak CK, higher VAS scores, and a greater reduction in range of motion which are all indicators of muscle damage. These observed effects cannot be solely attributed to DHA due to the presence of other anti-inflammatory compounds in the supplement. One must also consider that in this trial, subjects only began supplementation 7 days prior to the eccentric exercise bout. This suggests the possibility that the supplementation period was too short in duration to significantly fortify DHA stores in order to produce an observable effect on indicators of muscle soreness.

The optimal DHA dosage and duration are not presently known. However, one study denotes that the ingestion of 1.6 g/d over a 42 day period of a DHA supplement led to a threefold increase in plasma DHA levels (34). Additionally, studies suggest that a daily dose of 700-1000

mg DHA is necessary to bring red blood cell concentration to 7%, a reference point associated with lower risk for CVD (91). Furthermore, baseline DHA concentration plays a role in the efficiency of DHA incorporation into the RBCs (22). Cao et al., observed that those individuals with higher baseline concentrations of DHA experienced smaller rises from supplemental DHA on a gram of intake per percentage increase in concentration basis. Thus, those with the lowest plasma DHA will likely have the greatest response from DHA supplementation. Taking this information into account as well as the supplemental doses used in the previously reviewed studies (Table 6) it is seen that DHA supplementation needs to be administered either over a longer period of time (i.e., 8 weeks) or sufficient in concentration (at least 1500 mg/d) if administered over a shorter time frame (i.e., 4 weeks) to produce meaningful changes in concentrations. Considering the scheduling challenges of our study (study duration, gym availability, phlebotomist availability, semester breaks, and football home games), we believe that for the purposes of our investigation that a dosage of 2000 mg/day of DHA for four weeks would be an appropriate quantity and duration to ascertain beneficial outcomes of DHA on indices of muscle damage.

Selecting Arm over Leg:

The biceps eccentric movement was selected after careful consideration of previously reviewed eccentric exercise methods as well as pilot testing. While the leg extension is perhaps the most commonly used modality for eccentric contractions, we didn't believe that it was the most efficient or safest method available. For one, it is much more difficult for subjects to complete activities of daily living and function normally with extreme discomfort in one leg. It is believed that this would deter subjects from continued participation. Second, muscle breakdown and the resulting creatine kinase response are much greater following eccentric exercise bouts of

the arm compared to the leg (66, 112). Third, the leg extension causes increased lateral patellar displacement due to unnatural rotation of the patellar tendon about the femur, especially in those who already suffered from patellar subluxation (118). None the less, the most compelling reason to exclude the leg extension was because it places constant tension on the anterior cruciate ligament (ACL) throughout its range of motion (28). Severe ACL injuries are common to many athletic sports and typically require a 6-9 month recovery timeframe. Furthermore, Lutz et al., demonstrated that open kinetic chain exercises, where the hand or foot is free to move, (like the leg extension) produced significantly greater posterior tibiofemoral shear force at 0, 30, 60, and 90 degrees of maximal isometric force compared to closed kinetic chain exercises, where the hand or foot is fixed to a surface (83). This would make the application of a significant eccentric load extremely risky for knee injury. An alternative that was briefly considered was a smith squat protocol similar to the one used by Davies et al., and Rawson et al. (42, 120). Due to the available equipment in our laboratory, this method wouldn't be feasible or appropriate. We only have access to angled smith machines which follow a fixed bar path, forcing the body into an unnatural position. Proper squatting technique requires a straight bar path.

Several compound free weight exercises are excellent ways to induce DOMS, however they were ruled out due to difficulties in standardizing weight lifting technique amongst subjects. Additionally, the time commitment required and practicality of trying to teach inexperienced lifters how to properly and safely perform these exercises would be too great.

Another conventional eccentric exercise testing method involves using an isokinetic dynamometer to test the elbow flexors. This technique has been used by Trombold et al., Miles et al. and Ahmadi et al., among others (95, 150, 4). However, in our lab a Biodex Dynamometer was used in a pilot study to test subjects but we were unable to create a significant DOMS

response or loss in strength. This was regardless of the joint angle, eccentric lowering speed, rest parameters between sets and total exercise volume that was prescribed.

Summary:

Acute resistance training generates an inflammatory response and reduces muscle strength for several days. Currently, there is evidence suggesting, but not proving, that omega-3 fatty acid supplementation is beneficial in reducing inflammation, as well as treating several health maladies (e.g. hypertriglyceridemia, anxiety, arthritis, etc.). Along these lines there is limited evidence suggesting that omega-3 fatty acids maybe beneficial in diminishing muscle soreness, damage and inflammation following bouts of acute resistance training. However, some studies do not support effects of omega-3 fats to limit muscle soreness and muscle breakdown from eccentric exercise (147, 78, 19, 117). Therefore, additional investigation of the efficacy of omega-3 supplementation to attenuate the muscle damage and inflammatory response of individuals participating in strenuous resistance training with an eccentric component is required. The proposed study is unique because it evaluates the effects of DHA ingestion preceding an acute eccentric exercise bout in untrained participants. The time period following eccentric exercise in untrained individuals is when inflammation and muscle damage are likely to be most extensive due to a lack of muscle adaptation.

FIGURES AND TABLES FOR LITERATURE REVIEW

Figure 1:

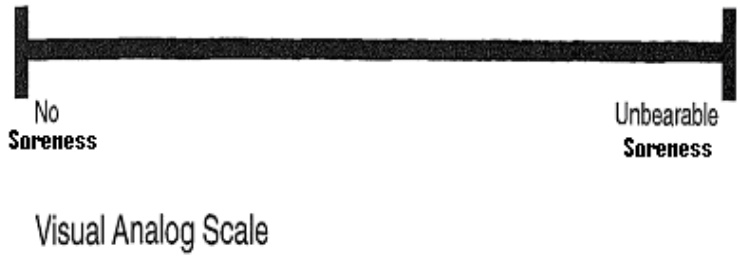

Figure 1 – Scale is 10 cm (100 mm) in length. Subject's made vertical marks through the above scale in pen and this mark was later measured with a ruler by the investigator.

Table 1. Knee Extension Using an Isokinetic Dynamometer

Study	**Subjects**	**Exercise**	**Sets**	**Reps**	**Rest**	**Mean ISO Decline**	**Peak CK**
(84) Mackey et al.	9 healthy men and women aged 19-27 years.	Maximal eccentric knee extensor contractions on an isokinetic dynamometer. ROM: 2.27 rad at an angular velocity of 1.57 rad/s.	17	6 (100 reps total)	30s per set	~40%	~18,000 ± 31,000 IU/L
(132) Shafat et al.	12 male (No RT)	Eccentric knee extensions, at a velocity of 0.52 rad/s. ROM: 60–120degrees.	30	10	30s per set	~15-25%	
(158) White et al.	27 untrained men (18-25 years)	Maximal isokinetic eccentric quadriceps contractions ROM of 1.75 rad (100°). Performed at an angular velocity of 1.05 rad/s (60°/s).	5	10	1min per set	~20-38%	~1000 ± 2200 IU/L

ISO – Isometric strength; RT – Resistance Training; ROM – Range of Motion

Table 2. Quadriceps Activation Using Smith Squat Protocols

Reference	Subjects	Exercise	Sets	Reps	Rest
(42) Davies et al.	9 healthy men (21-25 y). UT lower body.	Smith squats, load = 70% of body mass. lower than 90° during eccentric phase.. Active concentric	10	10	
(120) Rawson et al.	22 resistance trained men (19-25 y)	Smith Machine squat exercise at 50% 1-RM	5	15-20	2 minutes between sets
(151) Udani et al.	10 healthy UT male and female aged 18-45 years	Max # of smith squats in a five minute period. This effort was regarded as submaximal.		2x the amount performed during the 5 minute screening period	

1-RM – One repetition maximum; UT – Untrained

Table 3. Eccentric Exercise Using the Elbow Flexors

Author:	Mode/Intensity	Reduction in Maximal isometric Strength at 24h
(27) Chen et al.	Dumbbell at 100% MVC Dumbbell at 80% MVC	Approximately 50% Approximately 45%
(66) Jamurtas et al.	Isokinetic Dynamometer at 75% of Eccentric Peak Torque	Approximately 22%
(76) Lavendar et al.	Dumbbell at 40% MVC	Approximately 60-70%
(112) Paschalias et al.	Isokinetic Dynamometer at 100%1-RM	Approximately 60%

1-RM – One repetition maximum; MVC – Maximum voluntary contraction

Table 4. Food Sources Abundant in n-3 Fatty Acids per 100g serving

Food (100g)	DHA - 22:6 n-3 (mg)	EPA- 20:5 n-3 (mg)
Fish Oil, Salmon	18,230	13,024
Fish Oil, Menhaden	8,561	13,167
Fish Oil, Sardine	10,655	10,137
Fish Oil, Cod Liver	10,967	6,899
Fish Oil, Herring	4,207	6,273
Fish, Caviar, black and red granular	3,801	2,741
Fish, Mackerel Salted	2,965	1,619
Fish, roe, mixed species, cooked, dry heat	1747	1260
Fish, Shad, American, raw	1321	1086
Fish, roe, mixed species, raw	1363	983
Fish, Mackerel, Atlantic, raw	1401	898
Chicken, canned, no broth	211	11
Chicken, stewing, dark meat, meat only, cooked, stewed	110	30
Veal, variety meats and by-products, kidneys, cooked, braised	30	90
Lamb, variety meats and by-products, heart, cooked, braised	40	60
Lamb, variety meats and by-products, kidneys, cooked, braised	40	60
Egg, whole, dried	176	278
Egg, yolk, dried	239	203
Egg, whole, raw, fresh	46	88

*107. Nutrition Data. Internet: http://nutritiondata.self.com/foods-00007006700000000000-w.html (accessed February 13, 2011)

Table 5. Prior Investigations of n-3 and Various Forms of Exercise

Author	Subjects	Intervention	Dose	Outcome
(19) Burke et al.	11 healthy adult men and women aged 18 – 60 y.	Two bouts of 2 sets of eccentric biceps curls with a 4 s eccentric phase separated by 7 days.	2000 mg/d EPA and 1000 mg/d DHA for 7 days prior to 2nd eccentric bout.	Supplementation reduced DOMS.
(78) Lenn et al.	13 men and 9 women aged 18-30 y who did not participate in strength training in the past 60 days.	50 maximal isokinetic contractions of the elbow flexors.	1800 mg/d n-3 for 30 days prior to eccentric bout and for 7 days following the bout.	No difference between treatments on reducing DOMS, inflammation or muscle damage
(99) Nieman et al.	23 trained cyclists.	Cycled for 3 hours at an intensity of approximately 57% Watt max on three consecutive days with 10-km time trials in the final 15 minutes of each bout.	2000 mg EPA and 400 mg DHA for 6 weeks prior to intervention and for 3 days during the intervention.	Supplementation had no effect on exercise performance or markers of inflammation.
(117) Phillips et al.	40 untrained males 18-35 y.	3 sets of 10 repetitions using the elbow flexors, corresponding to 80% 1-RM in non-dominant arm o n a Magnum Arm Curl machine.	800 mg DHA, 300 mg mixed tocopherols and 300 mg of flavonoids for 7 days prior to exercise and for 7 days following exercise.	Supplementation significantly reduced IL-6 and CRP.
(147) Toft et al.	20 healthy endurance trained men.	Participation in a marathon race.	1908 mg EPA and 1116 mg DHA for 6 weeks prior to a Marathon.	Supplementation had no influence on muscle damage or inflammation.

Table 6. DHA Dosage Summary**:**

Author:	**Dosage:**	**Duration:**	**Study Outcome:**
(22) Cao et al.	1296 mg EPA + 864 mg DHA per day.	8 weeks	Changes in erythrocyte membrane composition for EPA and DHA were 300% and 42% respectively. Additionally, the increases were inversely related to baseline levels.
(34) Conquer et al.	1620mg/day DHA	6 weeks	Serum phospholipid levels of DHA increased approximately 5%
(91) McNamara et al.	700mg-1000mg/day DHA for Adults		Approximated amount required to bring erythrocyte composition to 7%

EPA - Eicosapentaenoic acid; DHA - docosahexaenoic acid

Chapter 3: JOURNAL MANUSCRIPT

THE EFFECTS OF DHA SUPPLEMENTATION ON MARKERS OF INFLAMMATION AND MUSCLE DAMAGE FOLLOWING AN ACUTE ECCENTRIC EXERCISE BOUT

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ABSTRACT

Aim: The purpose of this study was to investigate the influence of docosahexaenoic acid (DHA) on muscle damage and inflammation following an acute eccentric exercise bout. **Methods**: A double-blind placebo-controlled, study was performed using 41 healthy, untrained males aged 18-28 y who consumed either 2 g/d DHA or placebo (PL, corn oil) for 32 days. Supplements were consumed for 28 days prior to exercise. Participants completed an eccentric exercise procedure of the elbow flexors at 140% of 1-RM (6 sets x 10 repetitions). The time under tension (TUT) for each set of eccentric contractions was recorded manually from the investigators voice commands. Fasted blood samples for prostaglandin E2 (PGE2), interleukin-6 (IL-6), tumor necrosis factor-alpha, interleukin-1 receptor antagonist, C-reactive protein and creatine kinase (CK) were assessed on days 1, 2 and 4. Fasted serum DHA was measured at baseline (day -28) and on day 1. Peak isometric strength of the elbow flexors, delayed-onset muscle soreness, and range of motion were measured on day 1 prior to exercise and days 2, 3, and 4 following exercise. **Results**: DHA significantly reduced natural log of CK ($p<0.05$) response over 4 d. Additionally, IL-6 area under the curve (AUC) was reduced for DHA compared to PL (3.6 ± 2.5 pg/mL vs. 5.3 ± 2.7 pg/mL) ($p<0.05$). TUT/set was higher in the DHA group compared to placebo ($p<0.05$). There were no other significant differences between treatments. **Conclusion**: DHA supplementation produced lower indicators of muscle damage (CK) and inflammation (IL-6 AUC). DHA supplementation resulted in greater TUT/set.

Keywords: DHA, Creatine Kinase, IL-6

INTRODUCTION

Increased consumption of omega-3 (n-3) fatty acids has been associated with reductions in inflammation and cardiovascular disease risk factors (16). The anti-inflammatory effects of n-3 fatty acids are mediated through cyclooxygenase (COX) and lipooxygenase (LOX) enzymes (38). Pharmaceutical agents (i.e. celecoxib, rofecoxib) which target COX enzymes have been used in clinical investigations on resistance training to attenuate delayed onset muscles soreness (DOMS) (26, 36). However, these attempts are sometimes accompanied by unwelcomed side effects, such as gastrointestinal distress and decreased rates of protein synthesis (20, 23, 46). N-3 fatty acids may dampen muscle damage and inflammation following resistance training similar to pharmacological interventions without unintended side effects since they do not act through the same mechanism.

The n-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid, are largely thought to be responsible for the majority of the anti-inflammatory effects of n-3 fatty acids (38). As such, n-3 consumption should be centered on the ingestion of these particular fatty-acids as opposed to the essential fatty acid α-linolenic acid (ALA) because of poor conversion efficiency from ALA to EPA and DHA. Moreover, there is some evidence indicating that DHA may provide superior anti-inflammatory benefits compared to EPA (40, 22, 44).

DOMS is primarily caused by unaccustomed or intense resistance or eccentric exercise and could be a factor preventing the continuation of resistance training programs. Furthermore, reducing DOMS could provide performance advantages in competitive settings by enabling athletes to train longer and with greater frequency.

Acute eccentric exercise is a primary way to induce muscle damage in research settings. Eccentric contractions produce greater myofibrillar disturbances and allow for heavier loading

(9). Eccentric exercise is associated with increases in blood biochemical makers such as creatine kinase (CK) and Interleukin-6 (IL-6) as well as functional changes such as reductions in isometric strength (ISO), range of motion (ROM) and increased delayed-onset muscle soreness (DOMS), which are measured to monitor muscle damage (7, 15, 17, 25, 34).

Another result of damaging eccentric exercise is the development of inflammation, the body's natural response to injury and an essential component of the healing process. After bouts of strenuous exercise there will be an aggregation of inflammatory markers as a result of an accumulation of immune cells and fluids inside muscle tissue. Specifically, after exercise induced muscle damage, adhesion molecules expressed by the endothelial cells attract an influx of neutrophils and monocytes which migrate to the damaged tissue, with concentrations peaking at approximately 24 hours after initial injury (4, 8). These cells serve to degrade and clear the damaged tissue and stimulate recovery.

Cytokines can be released by these immune cells. Their synthesis can be regulated by stress hormones, reactive oxygen species and mechanical stress due to exertion related muscle damage (8). Consequently, these cytokines can be indirectly measured to gauge the muscle inflammatory response. In particular, circulating concentrations of interleukin-6 (IL-6) are considerably elevated during prolonged periods of muscle contractions (31, 28). Moreover, IL-6 can negatively influence muscle growth (3) and can signal the transcription of other inflammatory cytokines, in addition to C-reactive protein (CRP), an acute response phase protein (45). This chronic elevation in pro-inflammatory cytokines has been associated with several negative health outcomes (6, 11, 13, 41). Furthermore, increased CRP levels have been positively correlated with cardiovascular disease and obesity is associated with chronic inflammation (6, 13).

To date, there have been very few studies investigating the effects of n-3 fatty acids on muscle damage and inflammation and their results have been contradictory (18, 30, 35). It is difficult to determine the reasons for differences in results among studies performed thus far because of differences in duration of n-3 supplementation or dosage (18, 30, 35). The present investigation was designed to observe the effects of 2g/day DHA supplementation for 32 days on blood markers of muscle damage (CK, ISO, ROM, DOMS) and inflammation (IL-6, IL1-ra, TNF-α, PGE2, CRP) following a novel eccentric exercise bout of the elbow flexors.

MATERIALS AND METHODS

Subject Characteristics:

Fifty healthy, non-smoking adult males who were students at Virginia Tech, between the ages of 18-28 were recruited to participate. Subjects who had participated in strenuous resistance training within the last six months were excluded. In this study, strenuous resistance training is defined as 2 or more training bouts per week using weights or vigorous bodyweight resistance training on 2 or more occasions per week. Moreover, subjects who presented physical limitation, had a history of chronic inflammatory disease or acute injuries were excluded from participation. All study parameters were submitted and approved by the Virginia Tech Institutional Review Board prior to subject recruitment.

Design:

All subjects were assigned in random fashion to consume a dietary supplement (DHA or PL) for 28 days (d -28 to 1) prior to performing a set of baseline measures for muscle damage and inflammation on the morning of day 1. Immediately after the baseline measures, the acute eccentric exercise protocol was performed. The measures of muscle damage (serum CK, muscle strength, DOMS, joint angle) and inflammation were repeated approximately 1, 2, and 4 d after

the eccentric exercise bout. On each day of testing, the order was as follows: blood withdrawal, ROM, DOMS, ISO (**Figure 1**).

Generally, all testing took place in the morning hours between 6:30AM-Noon. Subjects participated in follow-up measurements at approximately the same time as their previous ROM, DOMS, and ISO measurements, although there was some deviation in time due to class schedules. The average blood collection time was 8:31 am ± 43 minutes.

Anthropometric measurement:

Baseline body composition was assessed on day -28. This assessment was accomplished by performing a three site (chest, abdomen and thigh) skinfold analysis following the American College of Sports Medicine (ACSM) procedure (1). The sums of the skinfold measurements were used to estimate body composition from the relevant Jackson-Pollock equations (14).

Range of Motion (ROM):

Upon arrival to our laboratory on days 1, 2, 3 and 4, subjects had their relaxed elbow joint angle measured on their non-dominant arm using a technique similar to that of Chen et al. and Lavendar et al. (7, 17). The subject was instructed to let their non-dominant arm hang by their side. The base of a goniometer was then placed over the lateral epicondyle. One arm of the goniometer was aligned in the center of the humerous of the upper arm and the other arm in the middle of the radius and ulna of the lower arm. To measure the flexed joint angle the subject was instructed to touch the palm of their hand to their shoulder joint without lifting their elbow. The same goniometer procedure was used to determine the joint angle. The averages of two measurements were taken for both positions and the flexed joint angle was subtracted from the relaxed joint angle to yield the corresponding range of motion. This procedure was completed after every blood collection except for the one obtained on day -28.

DOMS:

DOMS was assessed prior to the isometric test on days 2-4. Subjects were asked to complete 10 repetitions using a 5 lb weight with their exercised arm, which also served as their warm-up prior to isometric testing. During the bicep curl, subjects were asked to think about the level of soreness they were experiencing in their exercised limb. After completing this set, subjects indicated the level of arm soreness they experienced on a visual analog scale (VAS) measuring 100 mm in length by making a vertical mark. The VAS was anchored by the designations of "No Soreness" at 0 mm and "Unbearable Soreness" at 100 mm.

Peak Isometric Strength (ISO):

On day one, subjects had their baseline isometric strength (ISO) tested at 90° of elbow flexion in their non-dominant arm using a Biodex dynamometer (Biodex System 3 Pro Isokinetic Dynamometer). To prepare for this test, subjects were asked to perform a warm up using a standing biceps curl with a 5 lb weight for 8-10 repetitions. At the completion of this set, subjects rested for approximately 1-2 minutes before performing another standing biceps curl with a dumbbell weighing 15 lbs for 4-6 repetitions. After a 2-3 minute rest, the subjects completed an isometric strength test. For this test, the subjects were positioned so their non-dominant arm was at 90° of elbow flexion and approximately 45° of shoulder flexion. Subjects were instructed to apply maximal effort to the attached lever arm of the dynamometer for three seconds. Following this three-second contraction, subjects rested for 30 seconds before performing their next contraction. In total, the subjects performed three contractions and measures of peak isometric strength and average peak strength were recorded. With maximum peak isometric strength defined as the greatest torque produced during any of their three contractions, and the average peak isometric strength being the mean torque applied over the

course of the three contractions. For strength tests completed after day one, only the 5 lb weight was used to "warm-up" the subjects to prevent heavier loading from reducing the maximal effort produced by the subjects.

1-RM Testing:

On day 1 only, subjects were provided with a 20 oz sports beverage (Gatorade® G2 Thirst Quencher) containing approximately 35 g carbohydrates following the blood collection. This way subjects would not have to participate in strenuous exercise while in a fasted state. To determine a consistent intensity for eccentric loading based on individual strength, each subject performed a one-repetition maximum test (1-RM) with their non-dominant arm on a preacher curl bench. The subject was positioned so that their non-dominant arm was resting on a pad at approximately 45° of shoulder abduction and approximately 50° of elbow flexion. The subject was instructed to lift a sub-maximal load (dumbbell) for 5-10 repetitions, followed by a 1-minute rest interval. The warm-up was continued with two more sets of increased sub-maximal loading interspersed with 2- minute rest periods for 3-5 and 2-3 repetitions respectively. Following the rest period after the final warm-up set, the subject attempted a 1-RM. If the subject was successful, the load was increased and the subject attempted another 1-RM. If the subject was unsuccessful, the load was decreased and the subject attempted another 1-RM. Between 1-RM attempts the subject was given a 2-minute rest period. Ideally, the subject would complete their 1-RM testing within four attempts. Magnetic weights (PlateMate®) in 1.25 lb and 2.5 lb increments were added to dumbbells to provide more precise testing since the dumbbells increased in 5 lb increments. While performing the test, subjects lowered the dumbbell to approximately 5-10° before full elbow extension. The investigator provided a verbal command instructing the subject that the dumbbell reached the proper angle and that they were to curl the

weight upward. After completing 1-RM testing subjects were provided a 2-minute rest period prior to beginning the eccentric exercise bout.

Eccentric Exercise Protocol:

Following 1-RM testing on day 1, subjects engaged in an eccentric exercise bout of 6 sets of 10 eccentric only contractions with approximately 140% of their previously tested 1-RM. The subjects used their non-dominant arm to complete the test. The investigator placed the dumbbell in the subject's hand and the subject was required to lower the dumbbell in rhythm with the investigators cadence to mimic the eccentric lowering of a dumbbell preacher curl. On the count of "0" the investigator removed his hands from the load and proceeded to count "1, 2, 3, 4, 5" as the subject lowered the dumbbell (7, 17). When the load reached the bottom position of the eccentric movement the investigator removed the load from the subject's hand which indicated the completion of a repetition. The duration of each repetition was measured from the instructor's audible count by another investigator and recorded to correspond with each subject's time-under-tension (TUT). The subject then flexed his elbow to return to the starting position and the investigator placed the load back in the subject's hand to begin the next repetition. At the completion of each set, the subject was provided a 2-minute rest interval.

Dietary Guidelines and Treatment:

Participants were instructed to cease all other vitamin/mineral and performance enhancing supplements (all forms of creatine, D-aspartic acid, beta-alanine, pro-hormones and other nutritional supplements) for the duration of the study. Furthermore, subjects were instructed to avoid excess consumption of fatty fish and to avoid making alterations to their diet. During the first 28 days of the study the subjects were instructed to only consume their prescribed supplement at two pills per serving, twice daily with meals (4 pills total per day).

Supplements contained either DHA (500 mg/pill) or corn oil (500 mg/pill) totaling a daily dose of 2000 mg of the prescribed treatment. Weekly reminders to consume their supplements were distributed to the subjects via e-mail over the course of their 28- day supplementation period as well as prior to testing to encourage subjects to maintain a fasted condition. The number of supplements consumed was measured by a pill count and compliance was verified through serum fatty acid analysis (10) and through an exit survey.

Blood Collection:

A certified phlebotomist withdrew blood samples using a serum separator tube ~~(SST)~~ from the antecubital vein on days -28, 1, 2, and 4 of the study following a 10-hour overnight fast. Blood was centrifuged at a speed of 3000g for 15 minutes at 4°C. Serum was separated from whole blood and was stored at -80°C until analyzed in the lab.

Serum Analysis:

Serum was collected and analyzed for IL-6, IL-1ra, TNF-α, CK, CRP and PGE2 on days 1, 2, and 4. Additionally, serum DHA was measured on day -28 and day 1.

Serum DHA concentration was determined by a lipid extraction and methylation procedure developed by Corl et al. (10). Serum fatty acid methyl esters were analyzed by gas chromatography (Agilent 6890N GC) using a CP-Sil 88 capillary column (100 m × 0.25 mm i.d. with 0.2 µm thickness; Varian, Inc., Palo Alto, CA) and run in duplicate. Run conditions were as follows: the oven temperature was initially set at 70°C, then increased at 8°C/min to 110°C, then increased at 5°C/min to 170°C and held for 10 min, then increased 4°C/min to 225°C and held for 15 min. The inlet and detector temperatures were 250°C, the split ratio was 100:1, and a 1 µL injection volume was used. The hydrogen carrier gas flow rate was 1 mL/min. Hydrogen flow to the detector was 25 mL/min, airflow was 400 mL/min, and the flow of nitrogen makeup gas was

40 mL/min. Fatty acid peaks were identified by using pure methyl ester standards (Nu-Check Prep Inc., Elysian, MN). Serum CK was assessed using an enzymatic, spectrophotometric procedure from Pointe Scientific (Canton, MI). Analysis of TNFα, IL-1ra, CRP and IL-6 were conducted via enzyme linked immunosorbant assay kits from R&D Systems (Minneapolis, MN) while PGE2 was analyzed via competitive immunoassay (Ann Arbor, MI). All measurements were completed in duplicates and samples were re-assessed if coefficients of variation (CV) were >20%. Intra- and inter-assay coefficients of variation were 7.8% and 26.8% for CK, respectively. For inflammatory markers, intra-assay CV's were 7.1%, 9.1%, 8.2%, 7.2%, and 27.1% for CRP, TNF-α, IL-1ra, IL-6, and PGE2, respectively. Inter-assay CV's for inflammatory markers were 22.0%, 12.8%, 12.5%, 11.5%, and 30.5% for CRP, TNF-α, IL-1ra, IL-6, and PGE2, respectively.

Statistics:

All baseline data on subject characteristics, blood values, ROM, and ISO were compared using a two-sided t-test to determine whether there was uniformity between groups. A 2-way analysis of variance (ANOVA) with repeated measures was used to detect differences by treatment and time for TNF-α, CK, CRP, IL-6, IL-1ra, DHA, ISO, DOMS and ROM with significance set to $p<0.05$. Natural log transformation of CK activity was used because these data were not normally distributed. Area under the curve (AUC) was approximated by multiplying the number of days by the mean value of each time point using the equation (T1*(M1+M2)/2) + (T2*(M2+M3)/2)…+ (Tx*(Mx + My)/2), where M is the measurement and T is time. AUC was calculated for CK, IL-6, IL-1ra, PGE2, and CRP. Correlation analysis of change scores (value on Day 4 – value on Day 1; except for DOMS, which was Day 4 – Day 2) for dependent measures was performed using Pearson's correlation coefficients. All change score measurements were based on absolute values and not percentages. Post-hoc analysis when there

was a significant group by time interaction was performed using a Student t-test to determine differences in treatment at individual time points.

Eccentric exercise data (weight used and TUT per set), like baseline data, were analyzed using a 2-sided pooled t-test in conjunction with Bartlett, Levene, O'Brien, and Brown-Forsythe tests to verify equal variance between treatments ($p<0.05$) to determine if differences in testing variables occurred between groups. All statistical analysis was performed using JMP® 9.0 (SAS Institute Inc. 2011).

RESULTS

Subjects:

In total, 50 male subjects were recruited to participate in this investigation as well as the follow up study that continued for 13 additional days (Drager, in progress) with 41 subjects completing the entire study. Three subjects from the DHA group and six subjects from the placebo group did not complete the study and are not included in the analysis for reasons unrelated to supplementation. Six subjects had scheduling conflict, two no longer wanted to participate, and one subject had to leave the study due to beginning a new medication which could potentially interfere with the inflammatory processes being measured. In total, there were 21 participants in the DHA group and 20 participants in the PL group. Baseline characteristics did not differ between groups (**Table 1**).

The average body weight gain from baseline to day 4 by group was 0.7 ± (1.7) kg for DHA and 0.2 ± 1.0 kg for PL (p-value = 0.2787).

Compliance:

The exit survey was completed by 40 of the 41 subjects. When answering the survey, the majority of all subjects (65%) believed they were in the DHA treatment group. In the DHA

group 14 of 20 subjects correctly guessed their treatment (70%) and one subject did not answer. In the PL group, 6 of 20 subjects correctly guessed their treatment (30%). Perception of the supplement's ability to improve recovery time following exercise was answered "yes" by 50% of all participants. When asked how frequently they consumed the assigned supplements, four participants responded 75% of the time (2 per group) while the remaining subjects admitted that they had 90-100% supplement compliance. Over the course of this period, 176 total supplements should have been consumed by each subject. Subjects in the DHA group consumed 164 ± 4 supplements while the placebo group consumed 164 ± 5 supplements. Within the DHA group, 8 of 21 subjects believed that they were receiving the DHA treatment due to experiencing a fishy after-taste or in the case of one subject, "gas like fish oil." No other adverse effects were reported from the treatments. Analysis of serum DHA demonstrated a significant effect of time ($p<0.0001$), treatment ($p<0.0001$) and interaction between treatment over time ($p<0.0001$) as a proportion of total serum fatty acids from Day -28 to Day 1 (**Figure 6**). Plasma DHA increased by approximately 3.5% over this duration for the supplement group (**Table 6**) while not changing for the PL group.

Maximum Isometric Strength:

Overall, the acute eccentric exercise bout resulted in a reduction in maximal isometric muscle strength (ISO) on the day following eccentric exercise of 43 ± 17% and 41±18% for the DHA and PL groups, respectively **(Figure 2)**. This reduction peaked on day 2 and returned to approximately 66 ±21% of baseline by the 4th day of the study for the DHA group and 68 ± 21% for PL. Statistical analysis displayed a significant time effect but no effect due to treatment or interaction between treatment and time. Changes in ISO (Peak Torque Day 4 – Peak Torque Day 1) correlated negatively with changes in CK ($r = -0.5243$ and $p = 0.180$) and IL-6 ($r = -0.4227$

and p = 0.0047) and positively with changes in ROM (r = 0.5678 and p < 0.0001) (**Table 5**). Differences in ISO AUC were not significant.

Eccentric Exercise and Time-Under-Tension:

The difficulty of the eccentric exercise procedure on a 1-10 scale (10 being the most difficult) was determined through exit surveys. The average perceived difficulty rating from the DHA group was 7.9 ± 1.8 while the placebo group rated the difficulty as 8.5 ± 1.1 (p=.2416).

There was no difference in the amount of weight used during the eccentric exercise procedure between groups (**Table 2**) (p=.8487). However, there was a difference in time under tension (TUT) between treatments, with the DHA group having a higher total TUT (p=.0013). The baseline TUT for set one between groups did not differ (p = 0.3068). The DHA treatment resulted in mean TUT of 44 ± 6 s, 41 ± 7 s, 41 ± 7 s, 39 ± 7 s, 38 ± 7 s, and 36 ± 7 s, for sets 1-6, respectively. Placebo treatment resulted in mean TUT for sets 1-6 of 42 ± 7 s, 39 ± 7 s, 37 ± 7 s, 35 ± 8 s, 35 ± 7 s, and 33 ± 7 s, respectively. Treatments were not different by set.

Range of Motion (ROM):

The largest reduction in ROM was experienced on the day after the eccentric exercise bout (Day 2). Subjects had an average decline of ~21 ± 19% in their total range of motion on this day (Figure 3) for the DHA group and an average decline of ~25 ± 24% for PL. By the 4^{th} day, DHA participants ROM was reduced 14 ± 18% of baseline compared to 19 ± 22% for PL. As demonstrated in **Table 3**, reductions in ROM were similar for both treatments and statistical differences by treatments were not observed. Changes in ROM correlated positively with changes in ISO and negatively with CK (r = -0.4379 and p = 0.0033) and IL-6 (r = -0.3872 and p = 0.0103). Differences in ROM AUC were not significant.

Delayed Onset Muscle Soreness (DOMS):

Participant's perception of peak muscle soreness occurred on day 3 of the intervention (2 days after eccentric exercise), which corresponded to scores of 44 ± 21 mm and 51 ± 17mm for DHA and PL groups respectively (scale of 100 mm) (**Figure 4**). The following day, subject soreness perception was still elevated but had to 29 ± 22mm for DHA treatment and 36 ± 22mm for PL. Analysis of variance did not demonstrate a statistical effect of treatment. Changes in DOMS correlated positively with changes in CK ($r = 0.3591$ and $p = 0.0180$) and IL-1ra ($r = 0.4798$ and $p = 0.0011$). Differences in DOMS AUC were not significant ($p = 0.1937$).

Creatine Kinase:

There was a significant time effect for natural log transformed CK ($p<0.0001$) with activity peaking on day 4 (**Table 3**). Post hoc student t-test found that CK was lower for DHA compared to PL on day 4 ($p<0.05$). Values at baseline were 4.83 ± 0.53 for DHA and 5.11 ± 0.77 for PL compared to values at day 4 which were 6.27 ± 1.83 and 7.22 ± 1.78 for DHA and PL, respectively. Furthermore, there was a statistically significant treatment effect that demonstrated serum CK to be lower for the DHA group ($p=.0476$) over the period following the eccentric exercise (**Figure 5**). Change in CK correlated positively with changes in IL-6 ($r = 0.5460$ and $p = 0.0002$) and negatively with changes in ISO ($r = -0.5243$ and $p = 0.0003$), DOMS ($r = -0.3591$ and $p = 0.0180$) and ROM ($r = -0.4379$ and $p = 0.0033$). Differences in CK AUC were not statistically significant.

Indicators of Inflammation:

Analysis of serum TNF-α was discontinued after initial analysis results revealed concentrations below detection for all samples. For serum IL-6, the highest concentration was seen on day 4 (Table 4) and overall the DHA treatment trended to have lower values ($p=.0586$).

Area under the curve (AUC) for serum IL-6 was significantly lower for the DHA group (p= 0.0456).

For serum IL-1ra, there was no effect by time (p=.0540) or by treatment. A two-sided pooled t-test demonstrates that there was a group difference on day 1 for serum PGE2, prior to exercise testing (p=.0152) with participants in the DHA group having a starting serum PGE2 level of 965.8 ± 286.7 pg/mL compared to 750.8 ± 255.1 pg/mL (**Table 4**). However, there was no treatment or time effect following the exercise bout.

Analysis of serum CRP demonstrated no significant treatment or time effects. Changes in IL-6 correlated positively with changes in CK (r = .5460 and p = 0.0002) and IL-1ra (r = 0.5370 and p = 0.0002) and negatively with changes in ISO (r = -0.4227 and p = 0.0047) and ROM (r = -0.3872 and p = 0.0103). Changes in IL-1ra correlated positively with changes in IL-6 and DOMS (r = 0.4798 and p = 0.0011). Differences in IL-1ra, CRP, and PGE2 AUC were not significant.

DISCUSSION

The primary focus of this investigation was to examine the effects of supplemental DHA ingestion for 32 days on muscle damage and inflammation following a damaging eccentric exercise bout. It was hypothesized that DHA may act in a similar capacity as pharmaceutical interventions (COX-2 inhibitors) on the inflammatory process that result from exercise. Additionally, this investigation would shed light on the use of DHA as a compound to aid recovery following damaging exercise.

The eccentric exercise procedure was effective in eliciting muscle damage as assessed functionally using decline in strength, soreness, and reduced ROM and biochemically with a dramatic increase in serum CK. Peak isometric strength loss occurred at 24 hours following

eccentric exercise, peak DOMS at 48 hours and peak serum CK activity at 72 hours. Reductions in ISO and perception of DOMS were similar to other protocols using eccentric contractions of the elbow flexors (7, 15, 17, 25). Lavendar et al. (17) saw reductions in isometric muscle strength of the elbow flexors of ~50% in both young (12-25yrs) and old (41-57yrs) untrained men the day after dumbbell eccentric contractions. Similarly, Chen et al. (7) found reductions of ~45% in isometric strength in the day following eccentric exercise when using dumbbells corresponding to ~100% maximum voluntary contraction (MVC) in young untrained males. As with the investigations of Chen et al. (7) and Lavendar et al. (17), the present investigation also used an eccentric dumbbell procedure and found an average reduction in ISO of 41-43% between groups.

There were several differences in the eccentric exercise protocol used in the two aforementioned studies and the present study; however, all had similar effects on muscle strength in young, untrained males. Lavendar et al. and Chen et al. used an eccentric load corresponding to 40% MVC and 100% MVC, respectively, while the present study used ~140% of subject's 1-RM (7, 17). Additionally, Lavendar et al. performed 30 contractions (6 sets x 5 repetitions) and Chen et al. performed 30 contractions (1 contraction approximately every 45s) and while the present study had participants perform a total of 60 contractions (6 sets x 10 repetitions).

Changes in serum CK also followed similar patterns to those observed in prior studies (7, 15, 17, 25). Chen et al. (7) observed CK activity of approximately 5000 IU/L in the 100% MVC group on the 3rd day following eccentric exercise, while the present study found average serum activity at that same time point of 3151 IU/L and 4301 IU/L for DHA and PL, respectively. Lavendar et al. (17) found mean absolute CK activity of approximately 5000 IU/L in young participants and approximately 10,000 IU/L in older subjects.

Thus, eccentric exercise protocols that varied widely in intensity (40-140% of 1-RM) appeared to have similar effects on muscle damage as assessed by reduction in muscle strength and increase in serum CK. For Lavendar et al. and Chen et al., intensities were calculated from ISO at an angle of 90°. In the present investigation, intensity was derived from a 1-RM protocol that required a full range of motion. Therefore, the differences in intensity is likely less than the range of 40-140% because of mechanical advantage and the ability to generate force is reduced as the angle changes over the course of a 1-RM rep. Additionally, Chen et al. (7) allowed for 45s between each rep which would minimize the effect of fatigue during the bout and contribute to the participant's ability to maintain time-under-tension.

Subject range of motion (ROM) varied widely on an individual basis following exercise. Some variability may be due to subconscious manipulation of arm arrangement by the subjects to be consistent with their perceived outcome of the exercise bout. A better indicator for muscle trauma may have been upper arm circumference, since this change would likely be more uniform. However, ROM decreased following eccentric exercise testing in the present study as well as Chen et al. and Lavendar et al. (7, 17). The results were similar across the studies with the present study having reductions of approximately 21% and 25% for DHA and PL respectively on day 2, while mean reductions of approximately 25% were seen for Chen et al. and mean reductions of approximately 15-22% were seen for Lavendar et al. at that same time point (7, 17). All studies demonstrated a gradual increase in ROM in the days following the exercise bout. Considering that all the studies elicited similar effects on ISO it is unsurprising that ROM recovered in the same manner. Furthermore, this helps substantiate that muscle damage was similar between the studies since there were similar alterations in dependent measures.

DOMS increased, as expected, following the eccentric exercise bout. Mean DOMS scores were similar for the present study and the studies of Chen et al. and Lavendar et al. (7, 17). For the present study, mean DOMS was 44 mm for DHA and 51 mm for PL while measures of approximately 47 mm for Chen et al. and measures of 25 mm and 42 mm were observed for Lavendar et al. (7, 17). Peak DOMS occurred at the same time point in every study (day 3). When the same age groups are taken into account, all studies demonstrated similar mean DOMS responses to eccentric exercise. In conjunction with reductions in ISO and ROM, it can be argued that similar muscle damage occurred during these studies.

In the present study, DHA treatment dampened the overall magnitude of the CK response which suggests that there were less myofibrillar disturbances (9) than the placebo group. However, there is some evidence to suggest that indirect markers of muscle damage have poor associations with direct markers of damage, such as Z-line streaming (39). Therefore, the reduction in CK among the DHA group must be carefully considered. On the surface, the reduction in CK suggests that DHA supplementation prevents muscle damage. Nevertheless, the other indices of muscle damage (ISO, DOMS, ROM) did not demonstrate differences between treatments. Conversely, CK is a more precise measurement compared to the other indices of damage (ISO, DOMS, ROM) and therefore has a greater ability to detect differences between groups. Thusly, it could be argued that DHA treatment resulted in less muscle damage based on the outcome of the present study. This outcome is supported because the average levels of DOMS tended to be lower for the DHA group, albeit insignificant. Furthermore, changes in CK were negatively correlated with changes in ISO and ROM. As such, it is possible that less muscle damage results in a lower CK response following exercise (9). There also exists the possibility that random assignment resulted in genetically different groups and it has been observed that a

difference in CK could possibly be attributed to genetic variations in the use of calcium during exercise leading to subject variability, due to variation in IGF-II alleles (2).

The DHA group experienced a reduction in CK despite no differences in other markers of muscle damage such as ISO and ROM, which was also seen in a study conducted by Bloomer (3). Bloomer tested the effects of combined antioxidant therapy (vitamin E, vitamin C, and selenium) on eccentric exercise in young, healthy, untrained, females (18-31yrs) (3). He found significant differences between treatment and placebo on plasma CK between 24-96 hours post exercise. Furthermore, there was significantly lower muscle soreness in the supplement group 48 and 72 hours post-exercise. However, no differences were noted between treatments for ROM or ISO. Bloomer speculated that vitamin E may have prevented CK leakage by stabilizing skeletal muscle cell membranes without reducing muscle damage (3). Perhaps, the DHA treatment in the present study offered a similar protection against CK release without protecting the muscle from damage. One study observed that fish oil reduced peak CK activity after an acute myocardial infarction (37). It is possible that fish oil offers similar protection on skeletal muscle as it does on cardiac muscle by inducing changes in the lipid bilayer. On the basis of the present data, it is not clear that muscle damage occurred due to the reduction in CK. Other measurements of damage, like magnetic resonance imaging (MRI), or z-line streaming from muscle biopsy would provide more direct assessments of muscle damage and support more conclusive results.

Phillips et al. (30) studied the effects of a multi-ingredient dietary supplement which contained 800mg of DHA on serum IL-6 after participation in an eccentric exercise protocol using 80% 1-RM of the non-dominant elbow flexors. Subjects consumed the treatment for 7 days prior to the eccentric exercise bout and for 7 days following the bout. In this study, muscle damage was assessed biochemically by CK and lactate dehydrogenase (LDH). No differences

between treatments on markers of muscle damage were reported. In a similar study, Lenn et al. (18) investigated the effects of 1.8 g/day of omega-3 fatty acids from fish oil compared to 120 mg of soy isolate or placebo. Subjects were supplemented for 30 days prior to completing an eccentric exercise bout of 50 maximal contractions of the elbow flexors using a dynamometer. There was a reduction in isometric strength of roughly 25%, in the 48 hours following eccentric exercise, but there was no difference between treatments in isometric strength or CK. In a study by Tartibian et al. (33), untrained male subjects consumed 324 mg EPA/day and 216 mg/day DHA placebo for 30 days prior to an eccentric exercise bout involving bench stepping for a period of 40 minutes. Tartibian et al. (33) found significantly lower CK, LDH, and myoglobin in the n-3 group compared to placebo (soybean/corn oil) and control. In the present study, muscle damage, assessed by CK, was lower for DHA treatment as well.

In the Phillips et al. (30) study, there was elevation in IL-6 in the 3 days following exercise in the placebo group, with levels reaching approximately 52pg/mL from a baseline of approximately 20pg/mL. The treatment group's levels went from approximately 15 pg/mL to approximately 25 pg/mL over that same time period. Additionally, there was a significant difference in the change of CRP from baseline to the third day following exercise, with the placebo treatment resulting in increased levels of CRP while the supplement group had reductions in CRP. In the Lenn et al. (18) study, mean IL-6 increased from 235 ± 436 pg/mL at baseline to 294 ± 524 pg/mL 24 hours following exercise in the fish oil group. The soy and placebo treatments had IL-6 of 1 ± 3 pg/mL and 11 ± 11 pg/mL at baseline, respectively and levels of 3 ± 4 pg/mL and 9 ± 9 pg/mL at 24 hours following exercise, respectively. However there were no differences between IL-6 by treatment or over time. The Taritibian et al. (33), study had baseline values of IL-6 of 0.9 ± 0.2 pg/mL in the omega-3 group and 1.1 ± 0.6 pg/mL

in the placebo group. Forty-eight hours after the eccentric exercise bout, IL-6 level were 1 ± 0.2 pg/mL and 2.8 ± 0.7 pg/mL in the omega-3 and placebo groups respectively. There were significantly lower IL-6 levels in the omega-3 group at 24 and 48 hours following exercise.

In the present study, IL-6 increased marginally by day 4 to mean levels of 1.3 ± 1.3 pg/mL for DHA and 2 ± 1.1 pg/mL for PL, from baseline levels of 1.1 ± 0.8 pg/mL and 1.4 ± 1.1 pg/mL for DHA and PL, respectively. The results of the present study were considerably lower than those seen in the studies by Phillips et al. (30) and Lenn et al. (18), and were more in line with the results of the study conducted by Tartibian et al. (33). In the study by Tartibian et al., (33) peak IL-6 levels were observed immediately following the exercise bout, while in the study of Lenn et al. (18), peak IL-6 concentration was observed 3 hours following exercise. In the case of the present study and the study conducted by Phillips et al., (30), peak IL-6 elevations were observed on the third day after the exercise bout. Overall, IL-6 AUC was lower in the present study which is similar to the results found in the Tartibian et al. study. This could possibly coincide with the fact that the Tartibian et al. study found treatment differences in CK, like the present study, which was not seen in the studies of Phillips et al. and Lenn et al.

In the reviewed studies, increased levels of IL-6 at baseline appear to be associated with more pronounced increases in IL-6 following an eccentric stimulus. Furthermore, there is considerable variation in the baseline levels and the increase in IL-6 in all of these studies, even though all studies evaluated IL-6 using ELISA. This result is hard to explain because all subjects came from similar populations (young, healthy, untrained males) with the exception of the study by Lenn et al. (18) which also included young, healthy females. Additionally, the only study which did not use eccentric contractions of the elbow flexors was Tartibian et al. (33). Furthermore, the reviewed studies and the present investigation supplemented with omega-3

fatty acids for approximately the same duration prior to exercise, except for the investigation of Phillips et al. (~30 days vs. 7 days). There were differences in the intensity of exercise, with the present investigation using approximately 140% 1-RM for 60 repetitions, the study of Phillips et al. using 80% 1-RM for 50 repetitions, and the study of Lenn et al. using maximal resistance of a dynamometer for 50 repetitions. The higher intensity in the present study did not lead to larger increases in IL-6 compared to the other investigations which is counter-intuitive.

In the present study, area under the curve (AUC) for IL-6 was different between treatments ($p<0.05$). Concentrations of IL-6 become elevated during prolonged periods of muscle contraction, more so than any other cytokine (31, 28). Additionally, IL-6 is responsible for signaling the transcription of other inflammatory cytokines as well as C-reactive protein (CRP) (45). Acute elevations of IL-6 have been linked with enhanced levels of fat oxidation, and it may even play a role in restricting the inflammatory process by stimulating the production of IL-1 receptor antagonist (IL-1ra) and halting the production of TNF-α (27, 43).

The exact role of IL-6 in the inflammatory process is still not fully understood; to this point it is believed that it mostly acts in an anti-inflammatory capacity when elevated in response to an acute event (like novel anaerobic and aerobic exercise) because it is produced locally in exercising skeletal muscle, can be elevated by exercise even in the absence of muscle damage, and exhibits growth factor capabilities (28). Therefore, it is difficult to interpret this outcome as IL-6 is known to exert differential effects on inflammation (43, 19). Elevations in IL-6 result in reductions in muscle protein turnover, although, with slight increases in protein degradation (24). That is, higher levels of IL-6 are associated with small increases in skeletal muscle breakdown, likely due to reduced plasma amino acid availability (24). As such, minimizing increases in IL-6 could potentially spare muscle protein breakdown.

Further, cytokines which are influenced by IL-6, such as IL-1ra, CRP, and TNF-α were measured in this investigation and they did not demonstrate statistical significance between treatments. There is some evidence suggesting that acute elevations in serum IL-6 coincide with muscle damage (34). In the present investigation, changes in serum IL-6 were positively correlated with changes in CK and IL-1ra and negatively correlated with changes in ISO and ROM. These associations seem to suggest that increases in IL-6 were related to the degree of muscle damage and inflammation. The association between IL-1ra and IL-6 would indicate that higher IL-6 levels result in an increased production of the anti-inflammatory IL-1ra which likely accompanies an increase in inflammatory IL-1. However, IL-6 in serum can be influenced by several factors (27). For instance, muscle induced generation of IL-6 is influenced by vitamins C and E, which are capable of inhibiting the release of IL-6 in exercised muscle into the circulation, which would cause differing effects on local and systemic IL-6 concentrations (12). Based on the above, there is a possibility that dietary differences could contribute to the measured amount of IL-6 in serum, although unlikely.

The statistically significant changes that were observed in CK and IL-6 AUC seem to indicate a reduction in muscle damage and inflammation, respectively, for the DHA treatment group. However, this interpretation should be cautiously applied because there were no differences in any other markers of inflammation, muscle damage or area under the curve for TNF-α, PGE2, IL-1ra, CRP, DOMS, ROM, ROM, and ISO. Therefore, more investigations should be completed to determine if DHA is beneficial in attenuating muscle damage or inflammation in an acute setting due to the differential findings of this and of other investigations (18, 30, 33, 35).

Although not the original intention of study, analysis of the eccentric exercise protocol revealed the DHA treatment group to have a greater ability to maintain a consistent time course during each eccentric set resulting in a higher TUT/set. This suggests lower fatigue over the eccentric exercise protocol. However, the method of measuring TUT in this experiment lacked precision; therefore interpretation from this result is cautiously approached. A difference in TUT was similarly observed by Peoples and McLennan in vivo (29) in the hindlimbs of male Wistar rats who consumed fish oil compared to saturated or n-6 fats for 8 weeks. The rats who consumed n-3 had increased n-3 fats in their skeletal muscle membranes, superior twitch tension throughout the contraction bouts, and were able to produce greater tension during the final set of contractions. The investigators believe that Ca^{2+} cycling processes of skeletal muscle may be influenced by fish oil, further they speculate that endogenous protective mechanisms are increased in skeletal muscle due to the rise in n-3. Additionally, oxygen consumption among the n-3 group was significantly lower during periods of contraction. This suggests a superior ability to utilize glycolytic metabolism during these contractions which is supported by the fact that fish oil improves glycogen synthesis (which may lead to a larger pool of available glucose) and glucose oxidation in obese rats compared to obese controls (42). It is interesting to note that while the DHA group had increased levels of TUT, they also had lower mean ratings for perceived difficulty of eccentric exercise and mean ratings of DOMS compared to placebo, albeit not statistically significant. Perhaps these measures were not different because the longer TUT experienced by the DHA treatment group.

Differences in TUT likely could not be explained by differences in strength between the groups due to similar starting weights used for eccentric exercise and similar baseline ISO results. It is possible that there could be potential differences in muscle fiber type distribution

amongst subjects. This is likely not the case considering that all participants came from the same population but there is still variability involved. It is not known how or if n-3 fatty acids influence fiber type distribution. However, the more insulin sensitive and oxidative muscle fibers (type 1 and type 2a) have a greater proportion of n-3 fatty acids in their membrane phospholipid (32). For the investigation conducted in this laboratory, it could be that DHA supplementation led to an improved functional capacity of these muscle fiber types by increasing the proportion of n-3 fatty acids in their membranes which resulted in an improved ability to maintain muscle tension during the eccentric exercise protocol. Whether this could be due to calcium cycling or increases in endogenous protection due to the unsaturation of n-3, another reason or just random variability is not clear. However, it is possible that the DHA group was better able to maintain proper repetition tempo due to some other difference between the two groups that was unrelated to fatigue.

DHA supplementation in the present study compared favorably to interventions using COX-2 inhibitors. In a study conducted by Paulsen et al. (26) there were no differences in muscle recovery or markers of inflammation after an eccentric exercise bout when 400mg of celecoxib was administered in the 9 days following exercise. In the present investigation, DHA resulted in significantly smaller levels of CK and IL-6 AUC, possibly resulting in less muscle damage and inflammation. In addition, a study by Trappe et al. (36) found no differences in CK response or muscle soreness when participants were provided the maximum over-the-counter dosage of ibuprofen or acetaminophen immediately following an eccentric exercise bout. The use of ibuprofen and acetaminophen also resulted in significantly lower skeletal muscle fractional protein synthesis rates compared to placebo. DHA seems like it could be an alternative to these other treatments.

The magnitude in the increase of serum DHA was expected and similar to other studies (5, 21). Overall, changes in membrane composition of n-3 fatty acids are directly proportional to quantity of n-3 intake and inversely proportional to baseline levels (i.e. higher baseline levels are less sensitive to the same quantity of intake (5, 21). Therefore, DHA levels would be expected to increase if the duration of supplementation were longer but the extent of change would begin to dampen.

CONCLUSION

DHA treatment produced a lower CK and AUC IL-6 responses compared to placebo following eccentric exercise ($p<0.05$). However, all other indirect markers of muscle damage and inflammation did not differ between groups. Therefore, although a difference in these markers is intriguing, a reduction in muscle damage and inflammation from supplementation cannot be definitively determined. It is possible, for example, that DHA stabilized muscle cell membranes to reduce efflux of CK. Additional, more invasive research is required to verify an effect on muscle damage. DHA supplementation resulted in a higher time-under-tension per set compared to placebo which could explain the lack of statistical difference observed in markers of muscle soreness and damage (ISO, DOMS, ROM). However, this was crudely measured and interpretation of this finding should be considered cautiously. The trend for DHA treatment to have lower mean DOMS in conjunction with smaller elevations in CK should be evaluated further.

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FIGURES AND TABLES FOR MANUSCRIPT

Figure 1. Study Progression
Figure 1 represents the progression of the study beginning at the supplementation only period (Day -28), which lasted for 4 weeks through the end of the study (Day 4). Boxes marked by "X" indicate when measurements were taken while boxes marked by "-" indicate that a measurement was not taken.

	Day -28	Day 1	Day 2	Day 3	Day 4
1. Blood Collection	X	X	X	-	X
2. ROM	X	X	X	X	X
3. DOMS	X	X	X	X	X
4. ISO	X	X	X	X	X
5. Eccentric Exercise	-	X	-	-	-

Figure 2: Maximum Mean Isometric Strength by Day

Figure 2 indicates that there were no treatment differences in maximum mean ISO at any time point.

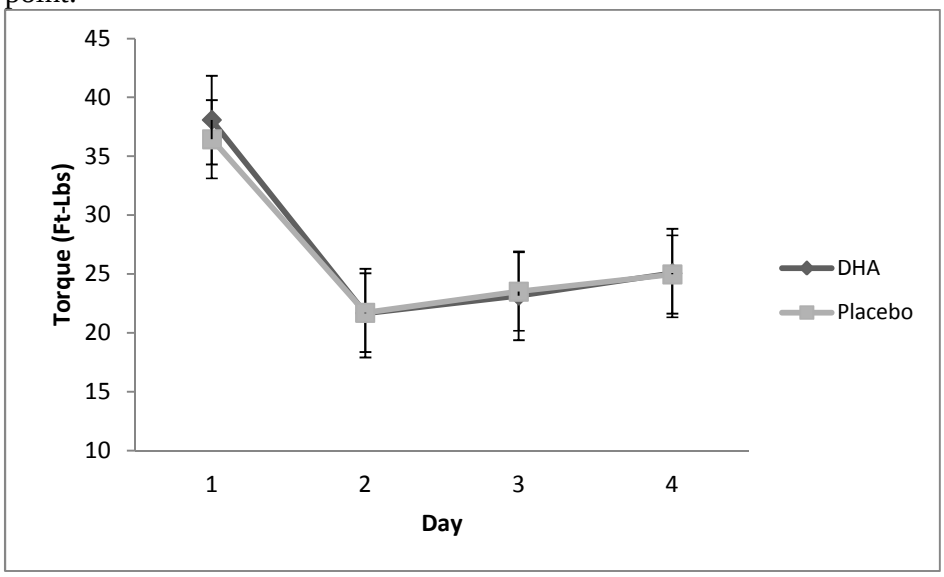

Figure 3: Range of Motion by Day

Figure 3 indicates that there were no treatment differences in mean range of motion at any time point.

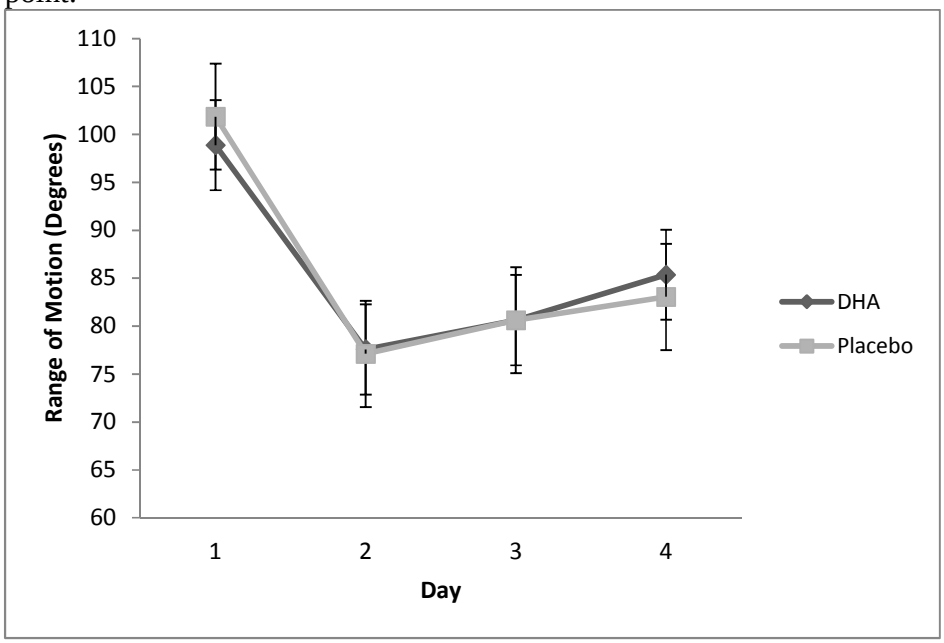

Figure 4: Delayed-Oneset Muscle Soreness by Day
Figure 4 indicates that there were no treatment differences in DOMS at any time point. DOMS, Delayed-Onset Muscle Soreness.

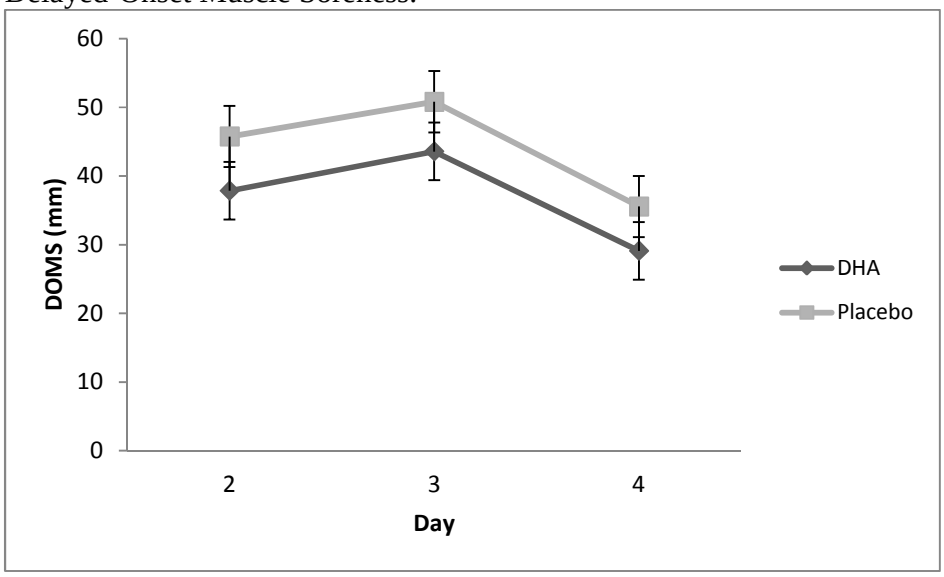

Figure 5: Natural Log of CK by Day

In Figure 5, * indicates $p<0.05$ and statistically different from placebo for treatment and post hoc analysis identifying day 4; Ln(CK), Natural Log of CK

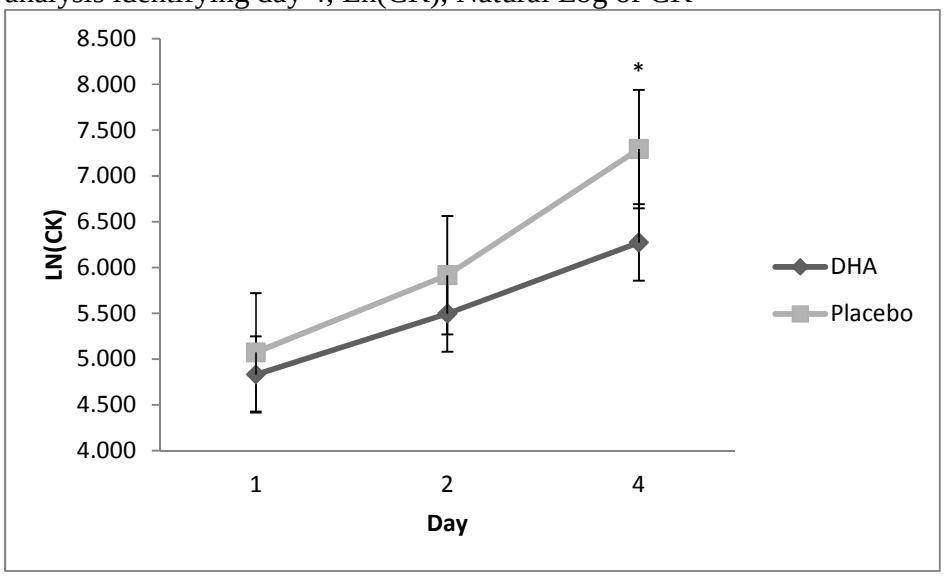

Figure 6: Serum DHA at Baseline and After Supplementation
In Figure 6**,** *ab indicates difference (p<0.0001) between treatments on day 1 (a) and a difference over time for DHA between day -28 and day 1 (b). Day -28 represents the 28 days that proceeded day 1. DHA, Docosaheaenoic Acid

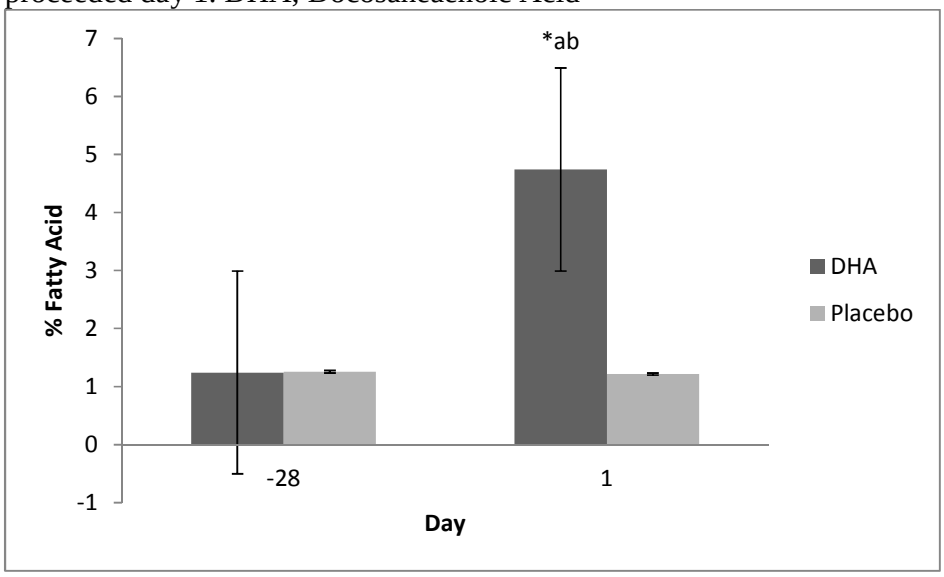

Table 1: Subject Baseline Characteristics

	Age (years)	Body Mass (kg)	Body fat (%)	BMI
DHA n=21	22.2 (2.8)	76.3 (12.6)	13.8 (5.6)	24.4 (4.6)
Placebo n=20	21.3 (2.5)	80.6 (18.6)	15.9 (5.0)	25.4 (5.40)

Values are means with standard deviation in parenthesis. There were no statistical differences in baseline subject characteristics between groups ($p<0.05$)

Table 2: Eccentric Exercise Comparison Data

	Weight Used (kg)	Set 1 (s)	Set 2 (s)	Set 3 (s)	Set 4 (s)	Set 5 (s)	Set 6 (s)	Mean TUT/Set in seconds
DHA	21 (4)	44 (6)	41 (7)	41 (7)	39 (7)	38 (7)	36 (7)	40 (7)*
Placebo	20 (4)	42 (7)	39 (7)	37 (7)	35 (8)	35 (7)	33 (7)	37 (8)

Values are means with standard deviation in parentheses. * Indicates statistical significance ($p<0.05$) between treatments. TUT, time-under-tension.

Table 3: Creatine Kinase (IU/L)

IU/L	Day 1	Day 2	Day 4
Absolute Concentration			
DHA	145.95 (96.21)	345.67 (353.21)	3151.81 (7620.85)
Placebo	237.55 (296.43)	562.05 (555.38)	4301.35 (5421.33)
Natural Log transformation			
DHA	4.83 (0.53)	5.50 (0.78)	6.27 (1.83)*
Placebo	5.11 (0.77)	5.94 (0.90)	7.22 (1.78)

Values are means with standard deviation in parentheses. * Indicates statistically significant difference ($p<0.05$) between treatments.

Table 4: Markers of Inflammation

	Day 1	Day 2	Day 4	AUC
IL-6 (pg/mL)				
DHA	1.1 (0.8)	1.2 (0.9)	1.3 (1.3)	3.62 (2.47)*
Placebo	1.4 (1.1)	1.7 (1)	2 (1.1)	5.28 (2.68)
IL-1ra (pg/mL)				
DHA	335.5 (152.5)	298.6 (100.1)	309.1 (95.9)	924.8 (310.3)
Placebo	480.4 (427.3)	454.0 (407.2)	465.7 (422.6)	1386.9 (1237.5)
PGE2 (pg/mL)				
DHA	965.8 (286.7)	862.9 (374.8)	877.9 (360)	2655.1 (981.7)
Placebo	750.8(255.1)	757 (371.5)	744.4 (366.5)	2255.3 (968.6)
CRP (mg/L)				
DHA	0.90 (1.01)	1.26 (1.70)	1.36 (2.19)	3.70 (5.16)
Placebo	1.69 (1.81)	2.29 (2.17)	1.87 (1.46)	6.14 (5.00)

Values are means with standard deviation in parentheses. * Indicates statistical significance ($p<0.05$) between treatments. Interleukin-6 (IL-6); Interleukin-1 receptor antagonist (IL-1ra) Prostaglandin-E2 (PGE2); C-reactive Protein (CRP).

Table 5: Associations between changes in selected dependent measures

Measure	**P-Value**	ΔLN(CK)	ΔIL-6	ΔIL-1ra	ΔPGE2	ΔCRP	ΔISO	ΔDOMS	ΔROM
ΔLN(CK)	Correlation	1	0.5460*	0.2018	-0.2062	0.1257	-0.5243*	0.3591*	-0.4379*
	p-value	0	.0002	.1944	.1847	.4220	.0003	.0180	.0033
ΔIL-6	Correlation	0.5460*	1	0.5370*	-0.2266	0.2409	-0.4227*	0.2496	-0.3872*
	p-value	.0002	0	.0002	.1440	.1196	.0047	.1064	.0103
ΔIL-1ra	Correlation	0.2018	0.5370*	1	-0.0265	0.1694	-0.1372	0.4798*	-0.1359
	p-value	.1944	.0002	0	.8660	.2776	.3804	.0011	.3849
ΔPGE2	Correlation	-0.2062	-0.2266	-0.0265	1	-0.0274	0.2228	-0.1848	0.0834
	p-value	.1847	.1440	.8660	0	.8616	.1509	.2355	.5947
ΔCRP	Correlation	0.1257	0.2409	0.1694	-0.0274	1	-0.2159	-0.0117	-0.0274
	p-value	.4220	.1196	.2776	.8616	0	.1643	.9404	.8614
ΔISO	Correlation	-0.5243*	-0.4227*	-0.1372	0.2228	-0.2159	1	-0.2828	0.5678*
	p-value	.0180	.0047	.3804	.1509	.1643	0	.0662	<.0001
ΔDOMS	Correlation	0.3591*	0.2496	0.4798*	-0.1848	-0.0117	-0.2828	1	-0.0267
	p-value	.0180	.1064	.0011	.2355	.9404	.0662	0	.8652

*Indicates statistical significance ($p<0.05$). LN(CK) Natural Log of CK, IL-6 Interleukin-6, IL-1ra Interleukin-1 receptor antagonist, PGE2 Prostaglandin E2, CRP C-reactive Protein, ISO Peak Isometric Strength, DOMS Delayed-onset Muscle Soreness, ROM Range of Motion.

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Chapter 4: SUMMARY AND RECOMMENDATIONS

SUMMARY

The purpose of this study was to examine the effects of docosahexaenoic acid (DHA) on markers of inflammation (serum IL-6, IL-1ra, TNF-α, PGE2, CRP) and muscle damage (ISO, DOMS, ROM). DHA has demonstrated anti-inflammatory effects through the cyclooxygenase enzyme (COX) (154), which is often a target of pharmaceutical intervention in the reduction of muscle soreness following exercise (81, 113). Similar studies have attempted to alleviate muscle damage using n-3 fatty acid supplementation. However these studies tested either aerobic conditions or lacked a significant loading period or dosage of n-3 fatty acids prior to testing (19, 78, 117, 147).

It was hypothesized that the ingestion of DHA at 2000mg/day for 4 weeks would reduce the inflammatory (serum TNFα, IL-6, IL-1ra, PGE2, CRP) and muscle damage (serum CK, muscle strength reduction, muscle soreness) that occur within four days after an acute, novel eccentric resistance exercise bout compared to placebo ingestion. The dosage was selected in part because overall changes in membrane composition of n-3 fatty acids are directly proportional to quantity of n-3 intake and inversely proportional to baseline levels (i.e. higher baseline levels are less sensitive to the same quantity of intake) (22, 91).

Fifty healthy, untrained male subjects between the ages of 18-28 years were recruited for participation in this study. Forty-one subjects completed the entire loading and testing period (32 days total). Subjects underwent baseline assessment for body composition as well as serum DHA concentration (% of total fatty acids) which occurred on day (-28) of the investigation. Following, baseline measurements subjects ingested either 2g/day of DHA or 2/g placebo (PL, corn oil) for a period of 32 days without other alterations to their diet or activity. Following, the 28 day loading period, subjects submitted fasted blood samples on days 1, 2, and 4 of the study

timeline, which were measured for serum CRP, IL-6, IL-1ra, TNF-α, and PGE2. Subjects completed measurements of ISO, DOMS, and ROM on days 1, 2, 3, and 4 of the study timeline. Additionally, subjects completed an eccentric only exercise bout with their non-dominant elbow flexors using free moving dumbbells which corresponded to ~140% of their 1-RM. Subjects were instructed to lower the dumbbell in cadence with the investigators audible commands with "0" designating the initiation of the repetition. The amount of time corresponding to each repetition was recorded from the investigators audible signals. In total, participants completed 6 sets of 10 repetitions interspersed with 2-minute rest periods.

All baseline data on subject characteristics, blood values, ROM, and muscle torque were compared using a two-sided t-test to determine whether there was uniformity between groups. A 2-way analysis of variance (ANOVA) with repeated measures was used to detect differences by treatment and time for TNF-α, CK, CRP, IL-6, IL-1ra, DHA, ISO, DOMS and ROM with significance set to $p<0.05$. Natural log transformation of CK was used because this data were not normally distributed. Area under the curve (AUC) was approximated by multiplying the number of days by the mean value of each time point using the equation (T1*(M1+M2)/2) + (T2*(M2+M3)/2)…+ (Tx*(Mx + My)/2), where M is the measurement and T is time. AUC was calculated for CK, IL-6, IL-1ra, PGE2, and CRP. Correlation analysis of change scores (results from Day 4 – results from Day 1; except for DOMS, which was Day 4 – Day 2) for dependent measures was performed using Pearson's correlation coefficients. All change score measurements were based on total values and not percentages. Post-hoc analysis of natural log of CK was performed using a Student t-test to determine differences in treatment at individual time points.

Eccentric exercise data (weight used and TUT per set), like baseline data were analyzed using a 2-sided pooled t-test in conjunction with Bartlett, Levene, O'Brien, and Brown-Forsythe tests to verify equal variance between treatments ($p<0.05$) to determine if differences in testing occurred between groups. All statistical analysis was performed using JMP® 9.0 (SAS Institute Inc. 2011).

Performance:

There were differences in CK and IL-6 AUC with the DHA group having lower response ($p<0.05$).There was also a trend for IL-6 to be lower in the DHA group. Furthermore, there was a statistically significant difference between TUT/set between treatments ($p<0.05$) with the DHA group maintaining a higher level of TUT per set. There were no differences in PGE2, IL-1ra, CRP, TNF-α, ISO, DOMS, or ROM.

Interpretation

It is difficult to ascertain the benefits of DHA considering the varying results. That is, CK (an indirect marker of muscle damage) was significantly lower for the DHA treatment group, however no other markers of muscle damage were affected by DHA. Therefore, it is difficult to make any conclusions regarding the ability of CK to decrease muscle damage. Bivariate correlation confirms that the change in CK is associated with negative changes in ISO, ROM and positively associated with changes in DOMS. This result suggests that the reduction in CK would result in less muscle damage which is consistent with prior literature that found increases in myofibrillar disturbances to match rises in CK (30).

Inflammation

It was also seen that changes in IL-6 were positively correlated with IL-1ra and negatively associated with ISO and ROM. It has been suggested that IL-6 may exert anti-

inflammatory effects in acute settings by stimulating the production of IL-1ra (161). In this experiment, the DHA group had lower AUC IL-6 ($p<0.05$) and trended to have lower IL-6 in general. Furthermore, the increases in IL-1ra were also associated with increases in DOMS. It seems that the increased levels of IL-1ra could possibly be mirroring increases in IL-1. That is, perhaps increases in IL-1 would be causing a negative feedback which could upregulate the amount of IL-1ra to return to homeostasis.

Eccentric Exercise

It was shown that the DHA group were able to maintain a higher TUT/set compared to the placebo group ($p<0.05$) during the eccentric exercise procedure. While the precision of this measurement was less than ideal it does follow a similar finding that was produced in another study supplementing n-3 fatty acids in rats (116). Additionally, it would seem that increased ability to maintain TUT would be due to increased glucose availability and oxidation in the exercised muscle and n-3 intakes have been associated with this benefit (160). Furthermore, a lack of statistical difference between subjects in terms of DOMS, ISO and ROM could be partially explained by the fact that the DHA group was actually working slightly harder during the course of the intervention.

It is possible that the effects of DHA cannot be realized in an acute setting. No anti-inflammatory or performance benefit was observed in similar acute exercise studies performed by Nieman et al. (99) and Toft et al. (147). However, there is evidence suggesting that n-3 supplementation may be beneficial in strength training over the course of multiple weeks (124). In addition, n-3 fatty acid supplementation has been shown to play a role in regulating protein metabolism (43, 141). Therefore, the study of n-3 fatty acid as an ergogenic aid should be continued. Rodacki et al. (124) observed greater muscle activation under electromyography

when elderly women were supplemented with fish oil compared to those who were not. The researchers believed that these faster muscle contractions are produced as a result of increased acetylcholine sensitivity and changes in myocyte membrane fluidity (124). It is possible that any neural adaptations that manifest as a result of n-3 supplementation are unlikely to be observed in only one session and prolonged testing would be needed in order to capture this effect.

The influence of n-3 on protein metabolism is also intriguing for those seeking performance advantages. Smith et al. (141) found increased muscle protein synthesis rates in older adults who supplemented with n-3 fatty acids for 8 weeks under concomitant hyperaminoacidemic and hyperinsluemic conditions. In conjunction with these findings, muscle biopsy revealed increased mTOR and p70s6k phosphorylation, which are predominant signals for myocyte growth (7, 14, 108). Lastly, Smith et al. believe that the anti-inflammatory effects of n-3 fatty acids likely do not play a role in increased muscle anabolism due to the lack of change in inflammation observed in their study.

As an attempt to explain the mechanism by which n-3 lead to increased muscle protein synthesis, one could hypothesize that the result could be caused by increased IGF-1 levels (21). Shen et al. (134) observed significant IGF-1 increases in rats supplemented with high intakes of n-3 fatty acids for 20 weeks. IGF-1 is partly responsible for signaling the mTOR pathway and mediating skeletal muscle hypertrophy (11, 12, 32). Another possible explanation for increased protein synthesis could be due to the correlation between n-3 fatty acids and cortisol levels (67, 94, 102). Increased cortisol levels are associated with protein degradation and the inhibition of protein synthesis (59). Therefore, a reduction in plasma cortisol as a result of n-3 fatty acid ingestion could lead to increases in skeletal muscle accretion over time.

Implications

Based on the evidence of this investigation it is inconclusive if DHA supplementation attenuated muscle damage or inflammation in an acute setting. As such, it is possible that the entire benefit of n-3 fatty acids cannot be realized in an acute setting. This is supported by the notion that interventions of n-3 fatty acids in resistance training programs that lasted multiple weeks saw an ergogenic benefit (124). Moreover, n-3 fatty acids have been shown to exert positive effects on regulating protein metabolism (43,141) which could lead to greater accretion of skeletal muscle protein over time. The present study demonstrates that DHA supplementation suggested reduction in muscle damage and inflammation after eccentric exercise with two key measures (serum CK and IL-6) but since some of the other measures were not significantly different by treatment, additional research should be conducted to confirm this finding.

RECOMMENDATIONS

Although DHA did not offer any protective or anti-inflammatory benefit in the present study, the investigation of this compound in addition to eicosapentaenoic acid (EPA) should be continued. Future investigations should examine the benefits of n-3 supplementation in conjunction with a resistance training program over the course of many weeks. Ideally, more direct markers of muscle damage would be used (MRI imaging, muscle biopsy, etc.) to confirm any statistical difference in indirect markers to allow for a more accurate conclusion. In addition, TUT should be more closely scrutinized and precisely monitored with n-3 supplementation. If n-3 supplementation/status does influence the rate of muscle fatigue this information would be extremely valuable for athletes or other exercise enthusiasts who are seeking to maximize performance capacity.

Conclusion

DHA treatment produced a lower serum CK and IL-6 response as well as a higher time under tension per set compared to placebo which could explain the lack of statistical difference observed in muscle soreness and damage. Evidence suggests that the effects of n-3 fatty acid supplementation on exercise performance and recovery may not be fully realized in an acute setting. Further investigation of n-3 fatty acid supplementation as a recovery aid over the course of many bouts is warranted.

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Appendix A: Subject Recruitment and Screening

Individuals are invited to participate in a research study out of the **Department of Human Nutrition, Foods, and Exercise**

Purpose of study?

- Determine whether daily consumption of a supplement (corn oil or omega-3 fat) will influence the muscle soreness, damage, and inflammation that occurs after weight training

What does it involve?

- Consumption of a supplement for 45 days
- Perform a strenuous weight training bout
- Go through several weeks of a weight training program we develop for you
- Seven blood withdrawals over 45 days

When?

- Summer 2010, Fall 2010 or Spring 2011
- We will recruit groups of subjects to begin the study over the year

Who is eligible to be considered?

- Healthy males (no orthopedic limitations to exercise or chronic health conditions such as diabetes, cardiovascular disease, bleeding, or inflammatory diseases, nonsmokers)
- Ages 18-28
- Schedule compatible with testing and training

Benefits?

- Development of personal weight training program by experienced exercise professionals
- Financial compensation up to $175

If you are interested, please contact VTHNFE@gmail.com — for more information.

Example e-mail correspondence:

Hello,

I am glad that you're interested in our research study. Let me briefly describe the study requirements. Subjects will take either a placebo fatty acid or an omega-3 fatty acid supplement for a period of 45 days. After 28 days the subjects will complete a series of exercise tests. Firstly, the subjects will have their elbow flexor isometric strength tested (Bicep curl at a stationary joint angle). Then, they will participate in an acute eccentric exercise protocol that is designed to elicit delayed onset muscle soreness (negative portion of a dumbbell bicep preacher curl). In addition, the subjects will be required to complete 6 resistance training sessions as well as provide blood samples at various times throughout the study. The resistance training sessions will be composed of the following exercises, the leg press, vertical machine bench press, a low cable back row, a vertical machine shoulder press, a lat pulldown, and a low cable bicep curl. The first day of resistance training will be used to identify the subject's 4-6 repetition maximum for each of the exercises listed. The total compensation for completing the study would be $175. In total there would be 7 "training days" but there would be a couple more days where brief strength tests and subsequent measures of muscles soreness are required. As far as the schedule is concerned, the tests and blood draws will be conduced in the morning hours generally between 7:30AM-11:00AM. If you're still interested in participating I need you fill out the attached screening form.

Thank you,
Frank DiLorenzo

--
Frank DiLorenzo B.S., CSCS
MS Candidate
Department of Human Nutrition, Foods and Exercise
Virginia Tech

Nutrition and Exercise Initial Screening Form

Name:

Please state your age:

Height: Weight:

Where are you living this summer? Campus / Blacksburg / Christiansburg / Other _____

Will you be out of town for any period September 20 through November 4th? Y / N
If yes, please describe dates.

Will you be in town during the fall semester of 2010 or spring semester in 2011? If so, please list any out-of-town trips you may be aware of.

Do you exercise regularly now? Y / N
If yes, please describe your current exercise program (i.e. running, lifting, swimming, etc. and how often):

What other physical activities have you participated in, and when was the last time you participated?

Do you have any concerns or previous problems with blood withdrawals? Y / N
If yes, please describe.

Do you have any injuries, physical limitations, or medical conditions which would prevent you from participating in strenuous exercise? If yes, please describe.

Do you smoke? Y / N

MEDICAL AND HEALTH HISTORY

Name: _____ Age: _____

Address: _____ e-mail: _____

Phone Numbers: Home: _____ Work :_____ Cell: _____

Local Address: _____
_____

Person to Contact in Case of an Emergency: _____

Relationship: _____Phone: _____

Primary Care Physician: _____Phone: _____

Medical Insurance Carrier: _____

Are you currently employed by Virginia Tech? _____

Are you an international visitor/student (e.g. J1 or F1 visa) _____

Current Body Weight:_____ Height_____

MEDICAL HISTORY

Please indicate any current or previous conditions or problems you have been told by a physician you have or had in the past:

	Yes	**No**
Heart disease or any heart problems:	_____	_____
Aneurysm	_____	_____
Rheumatic Fever:	_____	_____
Respiratory disease or breathing problems (e.g. asthma):	_____	_____
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Musculoskeletal problems (i.e. muscle or bone diseases, osteoporosis) :	_____	_____
Fainting and Dizziness:	_____	_____
High Cholesterol:	_____	_____
Diabetes:	_____	_____
Thyroid problems:	_____	_____
Mental illness:	_____	_____
Hypoglycemia:(i.e. low blood sugar)	_____	_____
Epilepsy or seizures:	_____	_____
Blood clotting problems (e.g. hemophilia**):**	_____	_____
Liver disorders (e.g. hepatitis B)	_____	_____
Cancer	_____	_____

Irritable bowel disease _____ _____
Crohn's disease _____ _____
Lupus _____ _____

If you answered "yes" to any of the previous questions, please indicate the date and describe:
_____
_____
_____
_____

Please list any hospitalizations/operations/recent illnesses (type/date):
_____
_____
_____
_____
_____

	Yes	**No**
Have you ever been diagnosed as having high blood pressure?	_____	_____
Are you currently being treated for high blood pressure?	_____	_____

If "yes", please explain:
_____
_____
_____

Please list all **medications** (prescription and over-the-counter) you are currently taking, have taken in the past week, or expect to take in the next two months:
_____
_____
_____

For what reason(s) are you taking these medications?
_____
_____
_____

Do you take any dietary supplements?_____ If so, what kind and how often?

Has your weight been stable over the past year?____ past 3 months? If not, describe how it has changed?

Do you have a fear of needles or have difficulty having blood withdrawn?_____

Health Habits

	Yes	**No**
Do you smoke cigarettes?	_____	_____

Packs per day: _____

	Yes	No
Do you engage in regular exercise?	_____	_____

If “yes”, please list:

Activity	Frequency (times per week)	Duration (minutes)
_____	_____	_____
_____	_____	_____
_____	_____	_____

Do you ever faint, experience shortness of breath or chest discomfort with exertion?
_____

If “yes”, please explain: _____
_____

Are there any orthopedic limitations you have that may restrict your ability to perform exercise and if “yes”, please explain:
_____
_____
_____

Family History

Has anyone in your family been diagnosed or treated for any of the following?

	Yes	No	Relationship	Age
Heart attack	____	____	_____	____
Aneurysm	____	____	_____	____
Heart disease	____	____	_____	____
High blood pressure	____	____	_____	____
Stroke	____	____	_____	____
Kidney disease	____	____	_____	____
Diabetes	____	____	_____	____

Schedule --indicate with an X those times you have classes, work etc that you CANNOT be involved in study activities over the next two months:

	Mon	*Tue*	*Wed*	*Thursday*	*Fri*
6:00-7:00am					
7:00-8:00					
8:00-9:00					
9:00-10:00					
10:00-11:00					
11:00-12:00					
12:00-1:00					
1:00-2:00					
2:00-3:00					
3:00-4:00					
4:00-5:00					
5:00-6:00					
6:00-7:00					
7:00-8:00					

Any explanation required for above

_____

_____

_____

Please sign to indicate that the information provided on this form is correct:

_____ _____ _____

Print name Signature Date

Virginia Polytechnic Institute and State University

Informed Consent for Participation in Research Projects Involving Human Subjects

Project Title: Effect of Oil Ingestion on Muscle Damage and Inflammation Following Weight Training Exercise

Investigators: Janet W. Rankin, Ph.D. (PI), Chris Drager (M.S. Candidate), Frank DiLorenzo (M.S. Candidate)

I. Purpose: The purpose of this study is to determine whether ingestion of a supplement (corn oil or omega- 3 fat) affects the muscle damage and inflammation that occurs after strenuous weight training exercise. Some muscle damage and inflammation occur when someone does unaccustomed strenuous weight training or begins a new weight training program. This typically causes muscle soreness in the several days following the exercise. Inflammation is the reaction of the body to various stresses including cellular damage or infection. This study will help to determine whether the ingestion of a supplement reduces the effects on muscle soreness, damage, or inflammation. We can determine this through measurements of muscle strength, soreness, and your blood we measure before compared to after the exercise.

We will recruit forty, healthy, nonsmoking young male individuals between the ages of 18 and 25 who have not been participating in resistance training for at least 6 months for involvement in this study. Individuals should be of normal body weight and be free from disease or orthopedic injury that would prevent them from lifting weights. Those with diabetes, inflammatory (e.g. Crohn's disease, inflammatory bowel disease), chronic diseases of the liver, heart or kidney or bleeding disorders should not participate. You should not consume any dietary supplements or medications without getting these approved by the experimental team over the course of the study.

II. Procedures: Prior to being included in this research study, you will complete a brief screening questionnaire that will help to determine if you meet our initial selection criteria and the scheduling requirements. If selected, you will be invited to attend an informational session held on the Virginia Tech campus.

If you are selected and volunteer to participate, you will be asked to cease any dietary supplementation and agree to not change your general diet, physical activity, or medication, over the study period (approximately six weeks) except in ways requested by the experimenters.

To start your involvement in the study, you will come to our laboratory to have your body composition (estimated by measuring fat fold thickness on three sites of the body with calipers), body weight and height measured. You'll also provide a blood sample to the investigators. Following this visit, you will be asked to consume four daily supplements (two in morning and two in evening) that will contain either corn oil or an omega-3 fat (similar to that in fish oil and used to supplement some foods that claim to be high in omega-3 fats) for four weeks. At the end of this period, you will come to the laboratory in the morning prior to eating breakfast to have your arm strength measured and then perform a strenuous weight lifting bout. Your arm strength will be measured on a Biodex machine that allows the experimenters to test specific

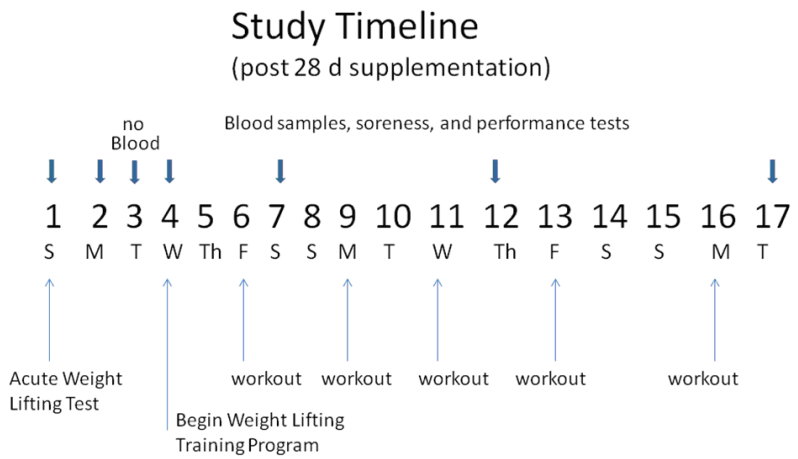
joint angles. Your elbow will be positioned at 90 degrees and you will be required to apply force against the machine's lever arm. You will be required to contract your arm 5 times for 3 seconds per contraction. The machine will record the amount of force being applied during each contraction. You will then proceed to the gym to have your one repetition maximum tested using a dumbbell on a preacher curl bench. After a brief resting period, you will complete the strenuous weightlifting bout. This involves doing 6 sets of 10 repetitions of elbow flexion of your non-dominant arm on a preacher curl bench using a dumbbell at an intensity of about 140% of your maximal strength. During this bout the investigator will place the dumbbell in your hand. You will be responsible for lowering the weight in rhythm with the investigators cadence. Once you have lowered the weight to the bottom position the repetition is complete and the investigator will remove the dumbbell from your hand. You will then move your arm back to the starting position to begin the next repetition.

The next day you will come into the laboratory in the morning to allow us to take another blood sample, measure your muscle strength again using the Biodex machine, and ask you to estimate the magnitude of muscle soreness using a visual scale (you will lift a dumbbell through a full range of motion and then mark on a scale the amount of soreness you experienced during the movement). The following day (day three), you will return to the lab again to have your muscle strength and soreness measured (See schematic of schedule in figure below). On day four, you will begin a full resistance weight training program (three days after the acute eccentric training bout) after you provide a third blood sample. The training program will involve seven different exercises using weight machines in a weight room. Volume and intensity for each exercise will be prescribed as three sets of 8 repetitions at a weight of 60-75% of the individual's estimated 1 repetition maximum (which will be determined from a 4-6 repetition maximum test during the first training session). You will be asked to perform this workout three times per week (e.g. Monday, Wednesday and Friday) for two weeks (total of 6 training sessions). Personal trainers will be available to help you or answer your questions. We will measure your arm muscle strength and ask about muscle soreness plus take three additional blood samples in the mornings on the 7^{th}, 12^{th}, and 17^{th} day after the initial weight training test. You will continue taking your supplements each of these days of the study (total of 45 days).

To summarize, if you are selected for this study and agree to participate, you will be involved for approximately six weeks, four of which simply involve taking the supplement correctly and completely each day. At the end of this supplementation-only period, you will have your muscle strength tested and do a strenuous arm weight training bout. On the third day after the exercise bout, you will begin a 17-day whole-body weight training program designed to increase your muscle strength. Over the entire study, we will collect seven blood samples of approximately 2 teaspoons each (9 ml). The initial, baseline visit will take about 30 minutes, the second visit (weight lifting test) will take approximately 45-60 minutes, each subsequent muscle strength/soreness/blood draw day will take approximately 30-45 minutes, and each weight lifting training session will take approximately 60 minutes. These times are estimates and it could take shorter or longer to complete each procedure.

You will be expected to discontinue the use of any vitamin/mineral supplement for the entire duration of this study and to check with the investigators before any over- the-counter (such as aspirin or ibuprofen) or prescription medications are taken. You will arrive at the laboratory fasted for each performance test and complete the entire protocol each time. If at anytime during the study there are any changes to your personal health or medical status, or you experience any unusual symptoms, you understand that you need to inform the investigators immediately. For example, it is very important that we know if you become or have recently

been ill (e.g. flu) because you should not exercise hard and this would also influence our results. We will ask you questions prior to each test to insure that you are not ill. We will also ask you about how you feel at the end of the performance test (e.g. if your stomach is upset, etc).



III. Risks:

Muscle fatigue will occur during the acute eccentric weight lifting exercise bout and muscle soreness is highly likely to occur in the days following the exercise bout. Similarly, fatigue willl be experienced during the weight lifting training with some soreness the next several days. It is possible that you could injure yourself while lifting the weights but we will reduce this chance by training you in correct technique (e.g. avoid breath holding during lift) and insisting that you use weight machines rather than free weights that require a spotter. You need to let us know immediately if you have severe pain or soreness. In the event of an injury, you will be instructed to terminate the testing/training procedure immediately and appropriate medical care will be provided; a first aid kit will be on site at all times. In the case of an emergency, a cell phone will be on hand at all testing, and appropriate medical personnel will be contacted. Any costs involved in transportation and/or care for medical help will be borne by you and not by Virginia Tech.

Some short-term pain will be experienced during the blood withdrawal that happens prior to supplementatin (baseline) and on six additional days. There is also a small risk of fainting before, during, or after blood draws. If this occurs, we will have you lay down with your feet slightly elevated. If you continue to experience problems we will call for medical help. Bruising and infection at the site of blood withdrawal has a low risk of occurance. You should let us know if the site becomes very red or painful.

The primary dietary sources are fish, oils, and foods containing added omega-3 fats. The average adult in the US ingests about 1.6 g/d total omega-3 fats with about 10% of this as EPA and DHA, the type used in this study. The American Heart Association recommends that most individuals increase their omega-3 fat ingestion to 0.5 g/d and that those with high blood triglyceride ingest 2 g/d. Various human clinical trials have been performed using up to 6 g/d for up to 2 years without serious side effects. The FDA considers up to 3 g/d of omega-3 fat as GRAS (generally recognized as safe). Potential side effects of omega-3 fat ingestion include reduced blood clotting rate and possible increase in blood sugar in diabetics. Thus, it is

important that you do not participate if you have a blood clotting disorder (e.g. hemophilia) or have diabetes.

You understand that all personnel involved in drawing and handling blood have undergone training for Blood Borne Pathogen Exposure Control administered by the Environmental Health and Safety Services of the Occupational Health Lab Safety Division at Virginia Tech or other medical facility. You understand that precautions will be taken by research personnel during handling of your blood samples. You further understand that the standard operating procedures set by Virginia Tech's governing body will be executed in the event that blood exposure occurs (blood spilled onto open skin of researcher) in that your blood would then be tested for HIV and hepatitis to determine exposure to the experimenter. There are two HIV/AIDS test sites in the area that offer HIV testing. If you are a Virginia Tech student, you have access to the Schiffert Health Center, otherwise, you must use the Montgomery County Health Department. You will have the option of an anonymous test or a confidential test. The confidential test requires that you give your name and social security number to the testing facility, if you are positive, your name will be sent to the State Health Department (state law requires this). Your name will remain confidential, but this will be on your medical record. Both sites require pre-test and post-test counseling, and you will have to return in person two weeks later to get your results. You will not be allowed to call in for your results. Again, this would occur only if someone is exposed to your blood; we will do all that we can to insure this does not occur.

IV. Benefits: You will be provided with the results of your measurements, if you desire, including performance test results and markers of inflammation.

V. Extent of Anonymity and Confidentiality: Due to the inability to assure anonymity, you understand that confidentiality of your results will be preserved. You understand that this means that all of your answers to questions, measurements and laboratory values will be kept confidential. A code number will be assigned to you. All questionnaires, data collection sheets, data analysis sheets, blood and storage containers will be identified by code number only and not by your name. You understand that a master list of participants' code numbers will be kept in a secure filing cabinet separate from completed data, which will also be maintained in a locked filing cabinet. You further understand that only the investigators of this study will be allowed access to any data.

VI. Compensation: You will be compensated for participation in this research project. You will be given $25 for completion of the baseline blood withdrawal and 28-day supplementation period, an additional $50 for completion of the arm weight lifting bout with accompanying blood withdrawals, and an additional $100 for completion of the two weeks resistance training program with accompanying blood withdrawal and muscle strength/soreness measurements. Thus, a total compensation of $175 will be received upon full participation in the study.

VII. Freedom to Withdraw: You can withdraw from this study at any time. You are free to not answer any questions or to not participate in any procedure included in this study. You understand that there may be circumstances under which the investigator may determine that you should not continue to participate in this project. This could include evidence of health risk, injury, or non-compliance to procedures. If a minor emergency arises during your participation in this study, you will discontinue your participation and seek care from your personal

physician. If a major emergency arises during your participation in this study, emergency personnel will be called (911), and they will care for you. Any costs associated with medical care received or transportation to a medical facility will be at the expense of the individual, and not Virginia Tech.

This research project has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Human Nutrition, Foods and Exercise.

X. Subject's Responsibilities: You voluntarily agree to participate in this study. You have the following responsibilities:

1) Consume four supplements per day throughout the entire experiment (45 days) with two in the morning and two in evening (or inform us of any noncompliance)
2) Arrive for exercise testing and blood draws in the fasted condition (not eat or drink anything since 10 pm night before)- on baseline before supplementation, and days 1, 2, 4. 7, 12 and 17 shown on above figure. Arrive for strength and soreness testing on day 3 (there will be *no blood drawn on this day* and a fasted condition *does not* have to be maintained on this day).
3) Perform all performance tests to completion with maximal effort
4) Perform weight training program as prescribed
5) Provide honest estimates of your muscle soreness
6) Maintain your weight within 2 pounds through the study
7) Maintain your activity level and general diet the same throughout the study except for those changes introduced by the study (i.e. weight training program)
8) Consume no dietary supplements beyond those we provide during the study
9) Do not consume any over-the-counter medications without advance notification from the research team
10) Inform the research team of any change in prescription medication
11) Allow for blood to be drawn at seven time points (as described in #2)
12) Notify the investigators of any changes in health (i.e. illness, injury, pain, etc.) that occur during the study

VI. Subject's Permission: You have read and understand the Informed Consent and conditions of this project. You have had all of your questions answered. You hereby acknowledge the above and give your voluntary consent for participation in this project. If you participate, you may withdraw at any time without penalty. You agree to abide by the rules of this project.

_____

Participant's Signature Date

_____

Investigator's Signature Date

Should you have any questions about this research or its conduct, you may contact:

Janet W. Rankin, Ph.D. Professor
Department of Human Nutrition, Foods, and Exercise
Virginia Tech
(540) 231-6355

Dr. David M. Moore,
Institutional Review Board for Research Involving Human Subjects Chair
(540) 231-4991

Appendix B: Subject Data Collection Documents and Procedures

Anthropometric Data

Subject: _____ Age:_____ Height: _____in _____cm

Weight: _____kg _____lb

BMI

Weight (kg) / Height (m^2) = _____/_____ = _____

Skinfold Data

Date: _____

				Average
Chest	_____	_____	_____	_____
Abdomen	_____	_____	_____	_____
Thigh	_____	_____	_____	_____

Sum of Skinfolds: _____

Percent Body Fat: _____

Body Weight (no shoes, indoor clothing)

Day	Weight (kg)	Time of blood draw
Pre-test		
1		
2		
4		
7		
12		
17		

Body Composition Skinfold Testing Procedure

1. Measurements are taken on the right side of the body
2. Place the caliper 1-2 cm away from the thumb and finger
    2.1.It should be perpendicular to the skin fold and halfway between the crest and the base of the fold.
3. Release the caliper lever so its spring tension is exerted on the skinfold
4. Maintain pinch while reading caliper
5. Read dial on caliper to the nearest mm (ACSM)
6. Cycle through each of the skinfold sites
7. Take duplicate measures at each site
    7.1 If within 1 or 2 mm take average
    7.2 If not within 1 or 2 mm take a 3rd measurement.
    7.3 If still no match, then take average of 2 closest measurements

Biodex Isometric Strength Data Sheet

Subject's ID #: _____

Non-Dominant Arm: _____ Chair Distance from Dynamometer: _____

Dynamometer Position: _____

Arm Rest Height: _____ Arm Rest tilt/Position: _____

Lever Arm Height: _____ Dynamometer Tilt: _____

Dynamometer Height: _____ Seat Back Position: _____

ROM Flexion: _____ ROM Extension:_____

***Have the subject warm-up performing a standing bicep curl for 1 set of 10 repetitions using a 5 lb weight. Next have the subject perform 1 set of 4 repetitions using a 15 lb weight.**

Test	Date	Time	Researcher	Maximal Peak TQ (ftlbs)	Average Peak TQ (ftlbs)
Baseline (Day 1)					
24 hr Post Eccentric (Day 2)					
48 hr Post Eccentric (Day 3)					
96 hr Post Eccentric (Day 4)					
24 hr Post 1st Session (Day 7)					
24 hr Post 3rd Session (Day 12)					
24 hr Post Final Session (Day 17)					

Notes:

1 RM Testing Protocol

1. Instruct the athlete to warm up with a light resistance that easily allows 5 to 10 repetitions.

2. Provide a 1-minute rest period.

3. Estimate a warm-up load that will allow the athlete to complete three to five repetitions by adding 5 to 10 pounds (~2-4 kg) or 5% to 10% for upper body exercise

4. Provide a 2-minute rest period.

5. Estimate a conservative, near-maximal load that will allow the athlete to complete two to three repetitions by adding 5 to 10 pounds (~2-4 kg) or 5% to 10% for upper body exercise

6. Provide a 2- to 4-minute rest period.

7. Make a load increase: 5 to 10 pounds (~2-4 kg) or 5% to 10% for upper body exercise

8. Instruct the athlete to attempt a 1-RM.

9. If the athlete was successful, provide a 2- to 4-minute rest period and go back to step 7. If the athlete failed, provide a 2- to 4-minute rest period, then decrease the load by subtracting 2.5 to 5 pounds (~1-2 kg) or 2.5% to 5% for upper body exercise AND then go back to step 8. Continue increasing or decreasing the load until the athlete can complete one repetition with proper exercise technique. Ideally, the athlete's 1-RM will be measured within three to five testing sets.

Eccentric Exercise Testing

Subject's ID#: _____ Date: ____/_____/_____

Non-Dominant Arm: _____

Exercise	**Warm-Up (1x5-10)~60s (1x3-5)~120s (1x2-3)~120s**	**Max Attempt #1**	**Max Attempt #2**	**Max Attempt #3**	**Max Attempt #4**
Seated DB Preacher Curl					

Dumbbell Weight Used for Eccentric__ _____ _____

Time (sec) it took to lower dumbbell

	Set 1	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Set 6**
Rep 1						
Rep 2						
Rep 3						
Rep 4						
Rep 5						
Rep 6						
Rep 7						
Rep 8						
Rep 9						
Rep 10						

Notes:

Subject Supplementation Compliance Record

Subject:	Starting Pills 1st Bottle	Endings Pills 1st Bottle	Starting Pills 2nd Bottle	Ending Pills 2nd Bottle

Muscle ROM and Soreness Scales

Subject's ID #: _____

Non-Dominant Arm: _____ Age: _____

Visual Analog Scale

Have the subject perform a standing bicep curl with a 5lb weight for 10 repetitions. Afterward, ask the subject to indicate their perception of muscles soreness on the above line.

Test Soreness	Date	Time	Researcher	VAS
D2-Post Eccentric				
D3-24hr Post Ecc				
D4-Pre-Training				
D7-24 hr Post Training				
D12- 24hr Post Training				
D17- 24hr Post Training				

For the flexed elbow measurement have the subject attempt to touch their palm to their shoulder without lifting their elbow. Relaxed measurement taken during standing with the arm resting along the side of the body.

Test ROM	Flexed Elbow (2 measures)	Relaxed Elbow (2 measures)	ROM (Avg. Relaxed – Avg. Flexed)
D1-Pre-Eccentric			
D2-Post Eccentric			
D3-24hr Post Ecc			
D4-Pre-Training			
D7-24hr Post Training			
D12- 24hr Post Training			
D17- 24hr Post Training			

Appendix C: Blood Collection Procedures

Steps for blood collection on day -28 (Blood Fatty Acids, Serum SOD, Serum Catalase)

Need: Disposable Pipet, gloves, freezer bag, plastic tubes

Apply gloves and label tubes with subject ID#, Blood draw day (-28, 1, 2, etc), and sample type prior to beginning.

Blood will be drawn into 1 tube:
1. 2- 10mL serum separator tube (SST)

Immediately invert SST tube 5 times

1. Take the tube to Janet Rinehart's lab in Wallace Hall
2. Centrifuge tube @ 3000 x g for 15 minutes at 4˚C in Janet's centrifuge.

1) Serum aliquoting
   a) Once tubes have finished spinning, remove from centrifuge & carefully place in rack
   b) Remove the top with a kim wipe & discard in a biohazard waste container
   c) With a disposable transfer pipet, aliquot the serum from the tube in the following order:
      i) DHA: ~3.0mL (two 1.5 mL tubes).
      ii) Catalase: ~250µL (.25mL)
      iii) SOD: ~125µL (.125mL)
      iv) Any Remaining Serum (labeled Extra)
   d) After all samples have been aliquoted, put in the appropriate bags for each subject and place in freezer (-80ºC)
   e) Discard tubes and transfer pipet in biohazard waste container

Will Need:
1. 1- 10mL SST
2. Labeled subject tubes
3. 4 plastic tubes
4. Kim wipes
5. Disposable pipets
6. Tube racks
7. Subject bag

MEASURE	SAMPLE
Serum Fatty Acid (DHA)	3.0mL
Serum Catalase	100µL
Serum SOD	50µL

Steps for blood collection on Day 1 (Blood Fatty Acids, Serum IL-6, IL-1 ra, TNF, PGE2, CRP, CK, Serum Catalase, SOD,)

Need: Disposable pipet, gloves, freezer bag, plastic tubes.

Apply gloves and label tubes with subject ID#, Blood draw day (-28, 1, 2, etc), and sample type prior to beginning.

Blood will be drawn into 2 tubes:
2. 2- 10mL serum separator tube (SST)

Immediately invert SST tubes 5 times

3. Take the tube to Janet Rinehart's lab in Wallace Hall
4. Centrifuge tube @ 3000 x g for 15 minutes at 4˚C in Janet's centrifuge.

2) Serum aliquoting
   a) Once tubes have finished spinning, remove from centrifuge & carefully place in rack
   b) Remove the top with a kim wipe & discard in a biohazard waste container
   c) With a disposable transfer pipet, aliquot the serum from the tube in the following order:
      i) DHA: ~3.0mL (two 1.5 mL tubes).
      ii) TNF: ~600µL (.60mL)
      iii) IL-1 ra: ~600µL (.60mL)
      iv) IL-6: ~400µL (.40mL)
      v) PGE2: : ~400µL (.40mL)
      vi) Catalase: ~100µL (.1mL)
      vii) SOD: ~50µL (.05mL)
      viii) CRP: ~50µL (.05mL)
      ix) CK: ~50µL (.05mL)
      x) Any Remaining Serum (labeled Extra)
   d) After all samples have been aliquoted, put in the appropriate bags for each subject and place in freezer (-80ºC)
   e) Discard tubes and transfer pipet in biohazard waste container

Will Need:
2- 10mL SST
Labeled subject tubes
10 plastic tubes
Kim wipes
Disposable pipet
Tube racks
Subject bag

MEASURE	SAMPLE
Serum Fatty Acid (DHA)	3.0mL
Serum TNF	600µL

Serum IL-1 ra	600μL
Serum IL-6	400μL
Serum PGE2	400μL
Serum Catalase	100μL
Serum SOD	50μL
Serum CRP	50μL
Serum CK	50μL

Steps for blood collection on Day 2 and 4 (Serum IL-6, IL-1 ra, TNF, PGE2, CRP, CK)

Need: Disposable pipet, gloves, freezer bag, plastic tubes.

Apply gloves and label tubes with subject ID#, Blood draw day (-28, 1, 2, 4, etc), and sample type prior to beginning.

Blood will be drawn into 1 tube:
3. 1- 10mL serum separator tube (SST)

Immediately invert SST tube 5 times

5. Take the tube to Janet Rinehart's lab in Wallace Hall
6. Centrifuge tube @ 3000 x g for 15 minutes at 4˚C in Janet's centrifuge.

3) Serum aliquoting
   a) Once tubes have finished spinning, remove from centrifuge & carefully place in rack
   b) Remove the top with a kim wipe & discard in a biohazard waste container
   c) With a disposable transfer pipet, aliquot the serum from the tube in the following order:
      i) TNF: ~600µL (.60mL)
      ii) IL-1 ra: ~600µL (.60mL)
      iii) IL-6: ~400µL (.40mL)
      iv) PGE2: : ~400µL (.40mL)
      v) CRP: ~50µL (.05mL)
      vi) CK: ~50µL (.05mL)
      vii) Any Remaining Serum (labeled Extra)
   d) After all samples have been aliquoted, put in the appropriate bags for each subject and place in freezer (-80ºC)
   e) Discard tubes and transfer pipet in biohazard waste container

Will Need:
10mL SST
Labeled subject tubes
7 plastic tubes
Kim wipes
Disposable pipet
Tube racks
Subject bag

MEASURE	SAMPLE
Serum TNF	600µL
Serum IL-1 ra	600µL
Serum IL-6	400µL
Serum PGE2	400µL
Serum CRP	50µL
Serum CK	50µL

Appendix D: Raw Data

Table 1a. Baseline Measurements:

Subject DHA Treatment	BW PRE-Test (KG)	Pre-Test (LBS)	DAY 1 (LBS)	DAY 2 (LBS)	DAY 4 (LBS)	Weight Change (DAY 4 – Pre-test)
201	67.27	147.99	145.75	145.75	148	0.01
202	52.95	116.49	119.75	121	119.25	2.76
204	78.73	173.21	170.75	170.25	172.25	-0.96
205	73.73	162.21	166.25	164.75	166.75	4.54
207	89	195.8	193	192.75	191.5	-4.3
208	68.9	151.58	154.75	155.5	155.25	3.67
209	84.9	186.78	185	185.5	187	0.22
213	66.14	145.51	140.25	142	140	-5.51
217	109.8	241.56	239.5	243.5	240	-1.56
219	65.57	144.25	146.25	145.25	146	1.75
224	85.9	188.98	197.25	196.5	197.75	8.77
226	68.75	151.25	149.75	152	149.5	-1.75
227	66.5	146.3	145	142	146.75	0.45
228	79.9	175.78	172.75	180.75	184.25	8.47
229	74.8	164.56	164.5	167.75	166.25	1.69
234	94.89	208.76	212.5	213	212.75	3.99
239	71.93	158.25	158.25	157.25	157.5	-0.75
240	82.5	181.5	188	191.5	189.5	8
242	76.82	169	170	170	168.75	-0.25
247	81.14	178.51	181.25	182	180	1.49
249	61.8	135.96	137.5	138.25	136.5	0.54
MEAN	**76.28**	**167.82**	**168.48**	**169.39**	**169.31**	**1.49**
SD	**12.28**	**27.02**	**27.27**	**27.89**	**27.56**	**3.71**
n=21						

Table 1b. Baseline Measurements (continued):

Subject Placebo Treatment	BW PRE-Test (KG)	Pre-Test (LBS)	DAY 1 (LBS)	DAY 2 (LBS)	DAY 4 (LBS)	Weight Change (DAY 4 – Pre-test)
203	79.73	175.41	174.5	174	174	-1.41
206	90.91	200	204.25	205.25	204	4
210	127.61	280.74	286.5	287	286	5.26
211	90.45	198.99	196.25	195.5	195.25	-3.74
212	77.84	171.25	175	174.75	172.5	1.25
214	78.3	172.26	173	172.25	170.5	-1.76
215	76.1	167.42	170	168.75	167	-0.42
216	66.6	146.52	148.5	149.75	148	1.48
218	81.02	178.24	177.5	177.75	179	0.76
220	62.61	137.74	139.75	138	138	0.26
222	66.93	147.25	144.75	147	150.25	3
225	78.5	172.7	173	171.5	173.5	0.8
230	124.9	274.78	276.25	275	275.5	0.72
232	56.7	124.74	124.5	124	124.25	-0.49
233	95.45	209.99	210.5	209	212	2.01
241	67.61	148.74	147.25	147.25	147.75	-0.99
243	63.64	140.01	141.75	143.75	140.75	0.74
244	85.23	187.51	188.25	188.25	186.5	-1.01
246	71.36	156.99	154.75	155.75	154.75	-2.24
250	70.11	154.24	155.5	155.75	154.75	0.51
MEAN	**80.58**	**177.28**	**178.09**	**178.01**	**177.71**	**0.44**
SD	**18.15**	**39.92**	**40.84**	**40.61**	**40.72**	**2.07**
n=20						

Table 2a. Baseline Subject Characteristics:

Subject DHA Treatment	BF%	Body Mass (KG)	BMI	AGE
201	10.74	67.27	21.8	23
202	8.3	52.95	18.6	26
204	12.31	78.73	22.4	25
205	15.9	73.73	21.4	22
207	18.16	89	26.3	21
208	12.24	68.9	24.6	23
209	21.97	84.9	28.2	25
213	10.3	66.14	22.83	19
217	24.78	109.8	33.6	24
219	5.51	65.57	20.6	22
224	18.91	85.9	26	18
226	8.42	68.75	21.3	20
227	8.95	66.5	21.3	22
228	10.3	79.9	23.2	19
229	11.64	74.8	25.3	19
234	22.04	94.89	37.65	20
239	5.73	71.93	20.09	27
240	16.61	82.5	25.01	22
242	18.99	76.82	24.83	23
247	17.99	81.14	27.6	28
249	10.3	61.8	20.6	19
MEAN	**13.81**	**76.28**	**24.44**	**22.24**
SD	**5.47**	**12.28**	**4.45**	**2.78**
n=21				

Table 2b. Baseline Subject characteristics:

Subject Placebo Treatment	BF%	Body Mass (KG)	BMI	AGE
203	16.7	79.73	25.6	24
206	20.48	90.91	27.7	27
210	19.5	127.61	36.12	22
211	15.45	90.45	24.92	23
212	18.07	77.84	24.79	19
214	15.62	78.3	23.4	22
215	17.31	76.1	24.2	22
216	13.26	66.6	20.5	19
218	16.58	81.02	24.22	18
220	9.65	62.61	19.94	20
222	9.65	66.93	23.63	20
225	22.04	78.5	27.1	20
230	23.94	124.9	40.96	20
232	7.8	56.7	18.46	20
233	23.93	95.45	29.14	22
241	16.55	67.61	21.54	19
243	9.34	63.64	21.33	27
244	20.31	85.23	28.72	22
246	10.45	71.36	21.3	19
250	11.61	70.11	23.5	20
MEAN	**15.91**	**80.58**	**25.35**	**21.25**
SD	**4.85**	**18.15**	**5.27**	**2.45**
n=20				

Table 3a.Supplement compliance:

Pill Count							
Subject DHA Treatment	**Starting (Bottle 1)**	**Ending (Bottle 1)**	**Removed 1st Bottle**	**Starting (Bottle 2)**	**Ending (Bottle 2)**	**Removed 2nd Bottle (DAY 17)**	**Total Pills Removed**
201	140	38	102	140	64	76	178
202	140	40	100	140	80	60	160
204	140	44	96	140	86	54	150
205	140	64	76	140	110	30	106
207	140	26	114	140	74	66	180
208	140	30	110	140	77	63	173
209	140	26	114	140	72	68	182
213	140	39	101	140	92	48	149
217	140	34	106	140	83	57	163
219	140	42	98	140	92	48	146
224	140	62	78	140	74	66	144
226	140	0	140	140	108	32	172
227	140	16	124	140	90	50	174
228	140	0	140	140	102	38	178
229	140	32	108	140	83	57	165
234	140	0	140	140	104	36	176
239	140	36	104	140	DNR	N/A	N/A
240	140	28	112	140	93	47	159
242	140	16	124	140	88	52	176
247	140	24	116	140	88	52	168
249	140	0	140	140	96	44	184
MEAN	**140.00**	**28.43**	**111.57**	**140.00**	**87.80**	**52.20**	**164.15**
SD	**0.00**	**18.04**	**18.04**	**0.00**	**12.09**	**12.09**	**17.99**

n=21

DNR - Did not return

Table 3b. Supplement compliance**:**

Pill Count							
Subject Placebo Treatment	**Starting (Bottle 1)**	**Ending (Bottle 1)**	**Removed 1st Bottle**	**Starting (Bottle 2)**	**Ending (Bottle 2)**	**Removed 2nd Bottle (DAY 17)**	**Total Pills Removed**
203	130	34	96	130	62	68	164
206	130	40	90	130	74	56	146
210	130	8	122	130	86	44	166
211	130	20	110	130	74	56	166
212	130	30	100	130	90	40	140
214	130	11	119	130	DNR	N/A	N/A
215	130	28	102	130	87	43	145
216	130	28	102	130	77	53	155
218	130	16	114	130	80	50	164
220	130	20	110	130	77	53	163
222	130	40	90	130	77	53	143
225	130	22	108	130	74	56	164
230	130	0	130	130	DNR	N/A	N/A
232	130	0	130	130	DNR	N/A	N/A
233	130	0	130	130	74	56	186
241	130	6	124	130	82	48	172
243	130	2	128	130	80	50	178
244	130	22	108	130	100	30	138
246	130	10	120	130	76	54	174
MEAN	**130.00**	**17.74**	**112.26**	**130.00**	**79.38**	**50.63**	**160.25**
SD	**0.00**	**12.97**	**12.97**	**0.00**	**8.27**	**8.27**	**13.89**

n=20

DNR - Did not return

Table 4a. Serum DHA data:

Fatty Acids (% of total in serum)						
Subject DHA Treatment	**Day**	**Linoleic Acid**	**Arachidonic acid**	**EPA**	**DHA**	**Treatment**
201	-28	33.9263	5.77794	0.26346	0.81393	DHA
202	-28	39.1606	7.46695	0.353	0.80277	DHA
204	-28	33.5394	7.02633	0.2857	0.83553	DHA
205	-28	27.499	4.2123	0.45096	0.72305	DHA
207	-28	28.7555	5.13668	1.05946	2.00961	DHA
208	-28	37.5895	5.71174	0.26154	1.23634	DHA
209	-28	36.548	8.60859	0.58329	1.95729	DHA
213	-28	39.0856	7.2431	0.37441	0.95915	DHA
217	-28	35.4427	8.14748	0.20243	1.61711	DHA
219	-28	31.0759	5.28403	0.3864	1.00673	DHA
224	-28	29.5525	6.19742	0.42108	0.9058	DHA
226	-28	38.6991	8.74037	0.44061	1.70625	DHA
227	-28	33.1459	7.0175	0.38755	1.35234	DHA
228	-28	36.2688	8.85559	0.27264	1.48246	DHA
229	-28	36.1399	9.7539	0.48961	1.68078	DHA
234	-28	33.4579	4.54378	0.28429	0.69634	DHA
239	-28	37.3617	6.16965	0.21693	1.52789	DHA
240	-28	30.2654	8.57825	0.54592	0.80694	DHA
242	-28	34.1062	7.66074	0.39813	1.19787	DHA
247	-28	34.1485	5.92785	0.34826	1.61518	DHA
249	-28	31.4174	6.00343	0.65076	1.15794	DHA
MEAN		34.15	6.86	0.41	1.24	
SD		3.36	1.51	0.19	0.41	
n=21						

Table 4b. Serum DHA data (continued):

Fatty Acids (% of total in serum)						
Subject DHA Treatment	**Day**	**Linoleic Acid**	**Arachidonic acid**	**EPA**	**DHA**	**Treatment**
201	1	35.3357	5.44825	0.59685	4.91072	DHA
202	1	38.8954	4.90453	1.1207	5.36274	DHA
204	1	35.2486	5.80401	0.48597	4.32144	DHA
205	1	34.2691	6.23991	0.62218	4.4283	DHA
207	1	29.8996	6.03219	0.89507	5.51039	DHA
208	1	35.8147	4.80903	0.65235	5.05207	DHA
209	1	34.4425	7.22366	0.82804	5.28365	DHA
213	1	35.6028	6.2125	0.76583	4.21882	DHA
217	1	32.8643	6.54776	0.54691	5.57279	DHA
219	1	33.9784	3.88868	0.74887	5.2128	DHA
224	1	31.8924	4.79885	0.34128	3.05254	DHA
226	1	39.8122	8.38992	0.62465	4.39946	DHA
227	1	35.1812	4.39349	0.64561	5.954	DHA
228	1	36.9759	6.15187	0.6842	4.59753	DHA
229	1	34.5189	7.24522	0.73962	5.33929	DHA
234	1	32.6904	7.63314	0.47205	5.19249	DHA
239	1	40.667	4.85787	0.604	4.33427	DHA
240	1	36.6655	6.63709	0.69348	2.58984	DHA
242	1	33.4829	7.52482	0.50442	5.07564	DHA
247	1	36.4187	5.99727	0.23789	4.86163	DHA
249	1	36.243	4.77549	0.83842	4.3179	DHA
MEAN		**35.28**	**5.98**	**0.65**	**4.74**	
SD		**2.49**	**1.17**	**0.19**	**0.79**	
n=21						

Table 4c. Serum DHA data (continued):

Fatty Acids (% of total in serum)						
Subject Placebo Treatment	**Day**	**Linoleic Acid**	**Arachidonic acid**	**EPA**	**DHA**	**Treatment**
203	-28	30.5455	6.35738	0.60964	1.97898	Placebo
206	-28	26.1264	7.05259	0.22796	0.86584	Placebo
210	-28	35.7134	6.16889	0.27331	0.86834	Placebo
211	-28	31.7393	8.85502	0.38548	0.90978	Placebo
212	-28	33.3667	5.33376	0.30502	0.98661	Placebo
214	-28	34.136	10.9922	0.60102	1.87594	Placebo
215	-28	30.7475	5.30768	0.36216	0.86233	Placebo
216	-28	31.4863	7.92758	0.48332	1.33506	Placebo
218	-28	35.996	9.61363	0.43224	1.50294	Placebo
220	-28	32.5558	8.26428	0.25516	1.13561	Placebo
222	-28	33.5631	7.53226	0.54355	1.40616	Placebo
225	-28	36.7638	4.69421	0.36444	1.33735	Placebo
230	-28	27.7719	6.07218	0.45459	1.2532	Placebo
232	-28	37.0204	7.98445	0.27506	0.66185	Placebo
233	-28	37.4138	5.4855	0.20799	1.19419	Placebo
241	-28	32.4156	7.97313	0.53005	2.1773	Placebo
243	-28	36.889	7.96178	0.29844	1.30765	Placebo
244	-28	35.3755	8.61979	0.57619	1.27776	Placebo
246	-28	25.5763	7.67322	0.62675	1.05775	Placebo
250	-28	33.9545	6.33491	0.47093	1.11725	Placebo
MEAN		**32.96**	**7.31**	**0.41**	**1.26**	
SD		**3.43**	**1.56**	**0.13**	**0.38**	
n=20						

Table 4d. Serum DHA data (continued):

Fatty Acids (% of total in serum)						
Subject Placebo Treatment	**Day**	**Linoleic Acid**	**Arachidonic acid**	**EPA**	**DHA**	**Treatment**
203	1	29.8923	6.90333	0.53518	1.85929	Placebo
206	1	31.1174	7.63538	0.31198	0.85562	Placebo
210	1	26.112	5.01906	0.27876	0.79021	Placebo
211	1	29.4873	9.79004	0.65015	1.03013	Placebo
212	1	33.9587	5.13676	0.28739	0.77575	Placebo
214	1	33.3855	9.58577	0.41341	1.57363	Placebo
215	1	37.0541	6.66781	0.31274	0.87509	Placebo
216	1	36.5347	8.39256	0.32601	1.20948	Placebo
218	1	36.7554	9.03354	0.56513	1.40538	Placebo
220	1	34.0481	7.9634	0.25764	1.14174	Placebo
222	1	33.1274	10.2301	0.45331	1.6981	Placebo
225	1	32.4833	5.16511	0.60782	1.62112	Placebo
230	1	32.2051	6.22961	0.29586	1.11163	Placebo
232	1	40.2775	5.06822	0.19664	0.72332	Placebo
233	1	33.2243	4.20919	0.32272	1.38911	Placebo
241	1	28.4727	5.08392	0.54263	2.22453	Placebo
243	1	37.4108	6.307	0.6546	0.95626	Placebo
244	1	35.3817	8.21717	0.45281	1.1545	Placebo
246	1	27.1509	8.99836	0.59389	1.09535	Placebo
250	1	40.6189	6.27673	0.62225	0.85374	Placebo
MEAN		**33.43**	**7.10**	**0.43**	**1.22**	
SD		**3.91**	**1.81**	**0.15**	**0.40**	
n=20						

Table 5a. Eccentric Exercise data:

Eccentric Time Under Tension (DHA Treatment)										
Subject DHA Treatment	**Weight Used (lbs)**	**Set 1 (s)**	**Set 2 (s)**	**Set 3 (s)**	**Set 4 (s)**	**Set 5 (s)**	**Set 6 (s)**	**Approximate TUT duration (total sec)**	**Avg. TUT (sec/set)**	**Avg. TUT (sec/rep)**
201	52.5	47	46	46	40	40	35	254	42.333	4.233
202	22.5	50	47	49	47	48	45	286	47.667	4.767
204	40	46	50	50	45	46	38	275	45.833	4.583
205	35	49	47	47	49	48	44	284	47.333	4.733
207	47.5	46	45.5	41	34	34	28.5	229	38.167	3.817
208	52.5	35	37	38	29	32	24	195	32.5	3.25
209	57.5	49	44.5	45	38	36.5	31	244	40.667	4.067
213	43.75	47	45	41	36	38	30	237	39.5	3.95
217	46.25	48	46	46	43	43	42	268	44.667	4.467
219	45	42	37	40	39	38	39	235	39.167	3.917
224	42.5	40	35	34	32	34	37	212	35.333	3.533
226	43.75	50	46	48	47	45	46	282	47	4.7
227	35	49	47	45.5	48	43	39	271.5	45.25	4.525
228	45	49	49	48	48	45.5	42	281.5	46.917	4.692
229	50	43.5	41	36.5	33	30	31	215	35.833	3.583
234	50	37.5	29.5	27.5	26.5	23	24	168	28	2.8
239	65	36	34	35	31	33	35	204	34	3.4
240	50	44	44	43	42	39	41	253	42.167	4.217
242	60	31	24	30.5	29	22.5	23	160	26.667	2.667
247	42.5	50	39	34	39	41	41	244	40.667	4.067
249	40	43	37	35	34	33	30	212	35.333	3.533
MEAN	**46.0**	**44.4**	**41.5**	**41.0**	**38.6**	**37.7**	**35.5**	**238.57**	**39.76**	**3.98**
SD	**9.17**	**5.56**	**6.77**	**6.46**	**7.11**	**7.33**	**7.15**	**37.35**	**6.22**	**0.62**

n=21

Table 5b. Eccentric Exercise Data (continued):

Eccentric Time Under Tension (Placebo Treatment)										
Subject Placebo Treatment	**Weight Used (lbs)**	**Set 1 (s)**	**Set 2 (s)**	**Set 3 (s)**	**Set 4 (s)**	**Set 5 (s)**	**Set 6 (s)**	**Approximate TUT duration (total sec)**	**Average TUT (sec/set)**	**Average TUT (sec/rep)**
203	45	50	48	49	46	47	48	288	48	4.8
206	31.25	49	48	47	47	46	42	279	47	4.7
210	55	41	39.5	39	39	32	32	223	37	3.7
211	47.5	42	37	45	41	43	41	249	42	4.2
212	40	42	37	33	27	34	30	203	34	3.4
214	50	45	39	38	34	34	32	222	37	3.7
215	36.25	47	48	46	44	40	41	266	44	4.4
216	43.75	40	34	28	30	27	25	184	31	3.1
218	36.25	44	43	41	43	41	40	252	42	4.2
220	45	40	29	31	25	27	27	179	30	3
222	55	47.5	43	41	40	44	42	258	43	4.3
225	60	47	44.5	43.5	42	40.5	33	251	42	4.2
230	40	39.5	38	36	31	33	26	204	34	3.4
232	35	45	37	33	27	28	24	194	32	3.2
233	55	46	48	40	36	26	28	224	37	3.7
241	42.5	39	32	38	38	38	37	222	37	3.7
243	45	41	30.5	31.5	28.5	28.5	27.5	188	31	3.1
244	50	19	26	18.5	13.5	22	22	121	20	2
246	50	43	41	35	37	35	34	225	38	3.8
250	46.25	32	28	29	29	29	32	179	30	3
MEAN	**45.4**	**42.0**	**38.5**	**37.1**	**34.9**	**34.8**	**33.2**	**220.4**	**36.7**	**3.7**
SD	**7.43**	**6.66**	**6.84**	**7.31**	**8.25**	**7.17**	**7.07**	**39.51**	**6.59**	**0.66**

n=20

Table 6a. Isometric Strength Data (ISO):

Maximum Isometric Strength (Ft-Lbs) DHA Treatment				
Subject DHA Treatment	1	2	3	4
201	33.1	17.8	21.1	23.8
202	31.9	24.8	27.3	26.7
204	43.2	33.6	37.7	42.9
205	39.4	35.5	38.6	40.2
207	31.8	17.6	22	28.7
208	31.4	14.9	15.8	13.2
209	47.7	27.8	29.2	31.8
213	36.6	19.1	19.5	20.3
217	44.1	23.8	25.8	31.7
219	47	18.1	24.2	25
224	36.9	18.8	15	16.5
226	32.3	22.1	20.1	22.1
227	37	16.2	17	21.2
228	41.7	16.7	20	20.4
229	37.1	14	18.9	22.4
234	36.6	11.9	11.9	11.8
239	39.8	28.3	29.2	35.3
240	41.7	27.4	30.5	24.8
242	39.1	17.8	21.2	25.6
247	37.6	24.8	25	19.4
249	33.8	24.3	16.2	22.9
MEAN	**38.1**	**21.7**	**23.2**	**25.1**
SD	**4.7**	**6.2**	**6.9**	**7.7**

n=21

Table 6b. Isometric Strength Data (continued):

Maximum Isometric Strength (Ft-Lbs) Placebo Treatment				
Subject Placebo Treatment	1	2	3	4
203	33.8	18.5	21.7	25.9
206	34.7	16.3	15.6	15.5
210	30	12.3	11.8	10.4
211	45.7	29	26.3	20.1
212	38.8	21.3	32.2	31.3
214	40.1	18.9	23.8	23.5
215	32.2	23.2	21.3	26
216	31.3	21.8	25.8	29.6
218	29.9	33	32.2	28.3
220	36	12.5	19.4	14.2
222	44.6	30.7	27.4	30
225	30.6	20.3	20.8	23.1
230	42.7	11.8	14.7	18
232	38.1	20.9	30.5	27.8
233	32.1	18.8	23.9	18.7
241	42	31.6	33.2	39.1
243	30.1	20	19.7	19.6
244	40.4	19.5	26.3	24.1
246	44.1	29.6	32.3	36.6
250	31.9	24.4	11.5	37.5
MEAN	**36.5**	**21.7**	**23.5**	**25.0**
SD	**5.3**	**6.2**	**6.6**	**7.6**

n=20

Table 7a. Range of Motion (ROM) data:

ROM (Degrees) DHA Treatment				
	Day			
Subject DHA Treatment	1	2	3	4
201	82.5	53	74.5	77
202	97	73	77	90.5
204	89	74.5	67.5	74
205	114.5	105	105	108.5
207	81.5	68	70	89
208	79.5	60.5	45	59
209	67.5	56	68.5	61.5
213	87	46.5	36.5	49.5
217	84	79	77	95.5
219	101.5	54	72	86.5
224	94.5	76.5	77.5	71
226	94	82	90	83
227	91.5	72	76	83
228	97.5	83.5	85.5	83
229	89	64.5	68.5	63
234	112	73	82	82.5
239	120.5	96.5	104	108.5
240	129.5	104.5	106.5	112.5
242	114.5	98	108	106
247	129.5	112.5	108.5	114
249	120	96.5	93.5	95
MEAN	**98.9**	**77.6**	**80.6**	**85.4**
SD	**17.0**	**18.4**	**19.0**	**18.0**
n=21				

Table 7b. Range of Motion data (continued):

ROM (Degrees) Placebo Treatment				
	Day			
Subject Placebo Treatment	1	2	3	4
203	81	52	51	78.5
206	102	86	77.5	84
210	86.5	52	63.5	48
211	91.5	75	74.5	70
212	73	49.5	56	59.5
214	93	47	45	61
215	99	85	86	91
216	100.5	67	88.5	95.5
218	95	84.5	96.5	103
220	86	32.5	33.5	33.5
222	103.5	81.5	83	84.5
225	80.5	48	44	59
230	126	93	103.5	97.5
232	106	88	101	96
233	113.5	81.5	85	83.5
241	117	103	102	101.5
243	122.5	105.5	108	109
244	106.5	82	76	76.5
246	125.5	111	117.5	112.5
250	128.5	118	120.5	117
MEAN	**101.9**	**77.1**	**80.6**	**83.1**
SD	**16.0**	**23.2**	**24.6**	**21.9**
n=20				

Table 8a. Delayed-Onset Muscle Soreness (DOMS) data:

DOMS (mm) DHA Treatment			
	Day		
Subject DHA Treatment	2	3	4
201	62	46	21
202	21	44	34
204	67	76	25
205	23	40	7
207	30	53	18
208	26	68	51
209	18	40	47
213	36	21	23
217	14	20	10
219	53	45	23
224	38	27	10
226	19	34	19
227	60	79	74
228	17	25	30
229	16	29	8
234	9	22	11
239	67	51	37
240	69	73	73
242	73	79	67
247	19	12	17
249	58	31	6
MEAN	**38**	**44**	**29**
SD	**22**	**20**	**21**
n=21			

Table 8b. Delayed-Onset Muscle Soreness data (continued):

DOMS (mm) Placebo Treatment			
	Day		
Subject Placebo Treatment	2	3	4
203	39	45	10
206	35	35	2
210	60	77	44
211	49	68	63
212	29	41	36
214	60	63	60
215	26	36	48
216	61	43	11
218	12	18	2
220	66	73	72
222	10	48	28
225	49	40	34
230	68	52	19
232	57	55	46
233	71	49	42
241	55	19	9
243	61	67	71
244	17	50	52
246	40	67	38
250	50	70	24
MEAN	**46**	**51**	**36**
SD	**18**	**16**	**22**
n=20			

Table 9a. Eccentric Exercise Difficulty Rating (10 being the most difficult):

Exercise Difficulty Rating (1-10)		
Subject DHA Treatment	**Treatment**	**Acute Eccentric**
201	DHA	9
202	DHA	5
204	DHA	9
205	DHA	8
207	DHA	8
208	DHA	8
209	DHA	8
213	DHA	9
217	DHA	8
219	DHA	9
224	DHA	7
226	DHA	7
227	DHA	10
228	DHA	7
229	DHA	9
234	DHA	2
239	DHA	8
240	DHA	n/a
242	DHA	10
247	DHA	7
249	DHA	9
MEAN		**7.9**
SD		**1.7**
n=20		

Table 9b. Eccentric Exercise Difficulty Rating (10 being the most difficult):

Exercise Difficulty Rating (1-10)		
Subject Placebo Treatment	**Treatment**	**Acute Eccentric**
203	Placebo	9
206	Placebo	9
210	Placebo	9
211	Placebo	9
212	Placebo	9
214	Placebo	8
215	Placebo	9
216	Placebo	9
218	Placebo	8
220	Placebo	10
222	Placebo	8
225	Placebo	8
230	Placebo	10
232	Placebo	8
233	Placebo	9
241	Placebo	5
243	Placebo	9
244	Placebo	8
246	Placebo	7
250	Placebo	8
MEAN		**8.5**
SD		**1.1**
n=20		

Table 10a. Serum Creatine Kinase (CK) data:

Creatine Kinase (IU/L) DHA Treatment			
	Day		
Subject DHA Treatment	**1**	**2**	**4**
201	110	106	237
202	153	111	86
204	165	169	122
205	314	337	159
207	100	149	157
208	135	1269	2189
209	48	125	2177
213	160	318	66
217	75	126	73
219	147	855	2423
224	456	329	297
226	274	200	256
227	87	126	187
228	71	242	635
229	185	1253	33900
234	97	455	11379
239	80	133	284
240	84	158	3247
242	115	142	132
247	103	508	8042
249	106	148	140
MEAN	**146**	**346**	**3152**
SD	**94**	**345**	**7437**
n=21			

Table 10b. Serum Creatine Kinase (continued):

Creatine Kinase (IU/L) Placebo Treatment			
	Day		
Subject Placebo Treatment	**1**	**2**	**4**
203	74	677	3708
206	140	250	124
210	259	734	4463
211	74	472	7663
212	211	779	10308
214	180	1550	16464
215	143	141	140
216	224	160	76
218	256	219	251
220	54	450	14956
222	261	770	540
225	156	192	3156
230	122	384	882
232	68	102	923
233	1424	2178	5760
241	97	237	111
243	290	273	246
244	457	1371	13604
246	66	119	2131
250	195	202	219
MEAN	**238**	**563**	**4286**
SD	**289**	**541**	**5295**

n=20

Table 11a. Serum C-Reactive Protein (CRP) data:

C-Reactive Protein Data (mg/L) DHA Treatment			
	Day		
Subject DHA Treatment	**1**	**2**	**4**
201	0.184	0.152	0.111
202	0.527	0.282	0.186
204	0.351	0.594	0.351
205	0.540	0.696	0.628
207	0.107	0.112	0.075
208	0.085	0.123	0.500
209	0.176	0.250	0.221
213	0.801	0.588	0.282
217	3.450	5.694	6.285
219	0.176	0.235	0.217
224	2.260	2.093	1.931
226	1.444	1.045	0.603
227	0.127	0.101	0.110
228	0.316	1.986	1.071
229	0.434	0.451	1.305
234	3.151	5.850	8.635
239	0.743	0.734	0.576
240	0.552	0.545	0.580
242	1.974	2.916	2.700
247	1.325	1.994	2.042
249	0.076	0.071	0.059
MEAN	**0.299**	**0.326**	**0.308**
SD	**0.987**	**1.656**	**2.133**

n=21

Table 11b. C-Reactive Protein data (continued):

C-Reactive Protein Data (mg/L) (Placebo)			
	Day		
Subject Placebo Treatment	**1**	**2**	**4**
203	2.693	4.249	4.071
206	1.573	3.262	3.406
210	1.484	1.603	1.733
211	2.466	2.644	2.480
212	0.357	0.384	0.285
214	1.235	2.275	1.799
215	7.908	5.244	2.197
216	0.283	0.473	0.567
218	1.373	1.671	3.672
220	0.661	0.422	0.285
222	0.262	1.029	2.904
225	0.912	1.592	0.946
230	4.322	5.541	4.554
231	0.398	0.684	0.993
232	0.635	0.648	0.501
233	3.104	2.602	1.985
238	0.863	2.333	2.460
241	1.629	1.079	0.439
243	0.671	0.868	0.537
244	1.025	1.200	0.801
246	0.920	8.658	3.906
250	0.335	0.305	0.260
MEAN	**1.60**	**2.22**	**1.85**
SD	**1.708**	**2.045**	**1.373**
n=20			

Table 12a. Serum Interleukin-1 Receptor Antagonist (IL-1ra) data:

Interleukin 1 Receptor Antagonist (pg/mL) DHA			
	Day		
Subject DHA Treatment	**1**	**2**	**4**
201	369.4	369.4	367.5
202	206.6	206.2	215.6
204	368.9	314.5	294.1
205	192.9	200.5	155.3
207	188.2	251.1	275.2
208	239.3	228.9	306.9
209	339.1	360.4	460.7
213	261.5	244.0	245.4
217	788.3	588.5	478.8
219	375.1	234.8	260.3
224	465.3	377.5	353.8
226	391.9	299.4	356.1
227	221.7	201.8	243.1
228	262.2	284.8	247.8
229	111.6	178.5	138.1
231	394.7	407.6	367.1
234	463.0	359.3	370.1
238	430.6	451.9	412.6
239	290.4	264.3	320.1
240	267.6	214.3	235.4
242	250.8	256.6	278.5
247	414.0	383.1	448.7
249	577.2	453.0	440.6
MEAN	**342.2**	**310.0**	**316.2**
SD	**144.0**	**100.6**	**92.5**
n=21			

Table 12b. Serum Interleukin-1 Receptor Antagonist data (continued):

Interleukin 1 Receptor Antagonist (pg/mL) Placebo			
	Day		
Subject Placebo Treatment	1	2	4
203	380.7	309.8	332.5
206	399.6	358.5	353.8
210	218.0	235.5	213.7
211	362.7	375.0	377.9
212	207.1	157.4	132.8
214	229.3	201.4	318.0
215	234.8	264.5	260.3
216	288.0	380.2	293.8
218	290.5	270.6	342.6
220	364.9	378.4	430.9
222	344.0	339.8	490.4
225	492.3	553.1	362.1
230	>2000.0	>2000.0	>2000.0
232	436.6	352.4	359.9
233	365.9	385.7	333.5
241	705.1	528.6	365.9
243	194.4	203.4	243.9
244	683.4	695.1	768.3
246	1189.2	907.8	1138.1
250	221.3	183.4	195.1
MEAN	**480.4**	**454.0**	**465.7**
SD	**416.5**	**396.9**	**411.8**
n=20			

Table 13a. Serum Interleukin-6 (IL-6) data:

Interleukin 6 (pg/mL) (DHA)			
	Day		
Subject DHA Treatment	**1**	**2**	**4**
201	0.967	0.823	0.568
202	0.572	0.381	0.420
204	1.287	0.878	0.911
205	1.653	1.239	0.521
207	1.418	1.407	0.785
208	0.515	0.400	1.839
209	1.124	1.699	2.443
213	1.144	0.964	0.887
217	3.825	4.282	1.120
219	0.417	1.027	0.824
224	0.576	0.632	0.864
226	0.450	0.829	0.666
227	0.639	1.245	0.684
228	0.437	0.708	0.471
229	0.267	0.421	1.005
234	1.953	2.550	x>5
239	0.845	0.584	1.176
240	1.221	0.899	1.104
242	0.970	0.753	0.613
247	1.374	1.630	x>5
249	1.599	0.914	1.136
MEAN	**1.107**	**1.155**	**0.949**
SD	**0.761**	**0.858**	**0.477**
n=21			

Table 13b. Serum Interleukin-6 data (continued):

Interleukin 6 (pg/mL) (Placebo)			
	Day		
Subject Placebo Treatment	**1**	**2**	**4**
203	0.960	2.888	2.556
206	0.788	1.637	1.473
210	1.923	2.872	4.147
211	1.137	0.989	1.193
212	0.867	0.444	1.611
214	0.485	1.522	2.230
215	1.655	1.111	1.621
216	1.965	4.329	2.738
218	1.297	1.198	1.621
220	0.421	1.242	1.392
222	0.889	1.360	2.604
225	1.423	1.855	1.649
230	1.261	2.601	1.516
232	1.215	1.453	1.550
233	0.692	0.844	1.234
241	3.466	1.875	0.961
243	0.372	0.925	1.068
244	2.042	1.472	2.259
246	x>5	3.248	x>5
250	1.008	0.802	0.820
Mean	**1.256**	**1.733**	**1.802**
SD	**0.714**	**0.955**	**0.776**
n=20			

Table 14a. Serum Prostaglandin-E2 (PGE2) data

PGE-2 (pg/mL) DHA Treatment			
	Day		
Subject DHA Treatment	-28	1	17
201	759.000	753.000	798.000
202	755.000	917.000	1041.000
204	1521.000	1286.000	1333.000
205	967.000	1201.000	1335.000
207	1088.000	724.000	1075.000
208	983.000	887.000	481.000
209	722.000	565.000	623.000
213	557.000	289.000	317.000
217	838.000	746.000	608.000
219	474.000	417.000	785.000
224	570.000	761.000	469.000
226	1207.000	270.000	499.000
227	1192.000	1343.000	1441.000
228	1234.000	657.000	618.000
229	1084.000	1264.000	1219.000
234	1293.000	1453.000	1074.000
239	1295.000	1556.000	1280.000
240	1203.000	908.000	1223.000
242	1070.000	1026.000	1183.000
247	792.000	536.000	512.000
249	677.000	561.000	521.000
MEAN	**966**	**863**	**878**
SD	**280**	**366**	**351**
n=21			

Table 14b. Serum Prostaglandin-E2 data (continued):

PGE-2 (pg/mL) Placebo Treatment			
	Day		
Subject Placebo Treatment	**-28**	**1**	**17**
203	757.000	669.000	453.000
206	844.000	657.000	647.000
210	568.000	650.000	554.000
211	940.000	416.000	475.000
212	232.000	519.000	252.000
214	739.000	698.000	394.000
215	769.000	916.000	876.000
216	1115.000	824.000	628.000
218	505.000	559.000	718.000
220	230.000	86.000	225.000
222	819.000	993.000	901.000
225	743.000	196.000	323.000
230	689.000	1223.000	1179.000
232	988.000	1555.000	1205.000
233	999.000	899.000	1326.000
241	1103.000	1255.000	1395.000
243	771.000	802.000	1166.000
244	728.000	656.000	457.000
246	1032.000	1219.000	982.000
250	444.000	687.000	732.000
MEAN	**751**	**774**	**744**
SD	**249**	**349**	**357**
n=20			

Appendix E: Statistical Summary

Table 15. Time-under-tension per set (TUT/set) analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	-3.0374	t Ratio	-3.15851
Std Err Dif	0.9617	DF	244
Upper CL Dif	-1.1432	Prob > |t|	0.0018*
Lower CL Dif	-4.9316	Prob > t	0.9991
Confidence	0.95	Prob < t	0.0009*

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	566.793	566.793	9.9762	0.0018*
Error	244	13862.752	56.815		
C. Total	245	14429.546			

Table 16. T-test for TUT/set; set 1 analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	-2.0311	t Ratio	-1.03562
Std Err Dif	1.9612	DF	39
Upper CL Dif	1.9359	Prob > |t|	0.3068
Lower CL Dif	-5.9981	Prob > t	0.8466
Confidence	0.95	Prob < t	0.1534

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	42.0586	42.0586	1.0725	0.3068
Error	39	1529.3804	39.2149		
C. Total	40	1571.4390			

Table 17. Load Used During Eccentric Exercise analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	-0.5744	t Ratio	-0.21578
Std Err Dif	2.6619	DF	39
Upper CL Dif	4.8099	Prob > |t|	0.8303
Lower CL Dif	-5.9587	Prob > t	0.5849
Confidence	0.95	Prob < t	0.4151

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	3.3799	3.3799	0.0466	0.8303
Error	39	2830.9189	72.5877		
C. Total	40	2834.2988			

Table 18. Baseline Fatty Acid Analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	0.01315	t Ratio	0.103655
Std Err Dif	0.12687	DF	39
Upper CL Dif	0.26978	Prob > |t|	0.9180
Lower CL Dif	-0.24348	Prob > t	0.4590
Confidence	0.95	Prob < t	0.5410

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	0.0017717	0.001772	0.0107	0.9180
Error	39	6.4309376	0.164896		
C. Total	40	6.4327093			

Table 19. Fatty Acid Comparison Day 1 analysis

Tests that the Variances are Equal

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	3.0594	1	39	0.0881
Brown-Forsythe	4.2689	1	39	0.0455*
Levene	5.3144	1	39	0.0266*
Bartlett	8.1369	1	.	0.0043*
F Test 2-sided	3.9104	20	19	0.0044*

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
316.9918	1	29.912	<.0001*

t Test
17.8043

Table 20. Peak Isometric Strength (ISO) analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Trt	1	1	39	0.0377	0.8471
day	3	3	117	111.4035	<.0001*
trt*day	3	3	117	0.4360	0.7277

t Test

Placebo-DHA

Assuming equal variances

Difference	-0.3350	t Ratio	-0.23804
Std Err Dif	1.4073	DF	162
Upper CL Dif	2.4441	Prob > |t|	0.8122
Lower CL Dif	-3.1141	Prob > t	0.5939
Confidence	0.95	Prob < t	0.4061

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
trt	1	4.598	4.5985	0.0567	0.8122
Error	162	13147.362	81.1566		
C. Total	163	13151.960			

Table 21. Delayed-Onset Muscle Soreness (DOMS) analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
trt*day	2	2	78	0.0289	0.9715
Trt	1	1	39	1.7717	0.1909
Day	2	2	78	12.6256	<.0001*

t Test

Placebo-DHA

Assuming equal variances

Difference	7.192	t Ratio	1.887756
Std Err Dif	3.810	DF	121
Upper CL Dif	14.735	Prob > |t|	0.0615
Lower CL Dif	-0.351	Prob > t	0.0307*
Confidence	0.95	Prob < t	0.9693

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Trt	1	1589.621	1589.62	3.5636	0.0615
Error	121	53974.346	446.07		
C. Total	122	55563.967			

Table 22. Range of Motion (ROM) analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
trt	1	1	39	0.0001	0.9936
day	3	3	117	74.5631	<.0001*
trt*day	3	3	117	0.8545	0.4669

t Test

Placebo-DHA

Assuming equal variances

Difference	2.969	t Ratio	0.561131
Std Err Dif	5.291	DF	39
Upper CL Dif	13.671	Prob > |t|	0.5779
Lower CL Dif	-7.733	Prob > t	0.2890
Confidence	0.95	Prob < t	0.7110

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
trt	1	90.302	90.302	0.3149	0.5779
Error	39	11185.002	286.795		
C. Total	40	11275.305			

Table 23. Natural Log Creatine Kinase (CK) analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
trt	1	1	39	4.1855	0.0476*
day	2	2	78	30.6652	<.0001*
trt*day	2	2	78	1.1776	0.3134

t Test

Placebo-DHA

Assuming equal variances

Difference	0.28283	t Ratio	1.368294
Std Err Dif	0.20670	DF	39
Upper CL Dif	0.70093	Prob > |t|	0.1791
Lower CL Dif	-0.13527	Prob > t	0.0895
Confidence	0.95	Prob < t	0.9105

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
trt	1	0.819449	0.819449	1.8722	0.1791
Error	39	17.069762	0.437686		
C. Total	40	17.889211			

Table 24. C-Reactive Protein analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	2.5142	0.1209
Day	2	2	78	2.4759	0.0907
Treatment*Day	2	2	78	0.6867	0.5063

t Test

Placebo-DHA

Assuming unequal variances

Difference	0.7972	t Ratio	1.731402
Std Err Dif	0.4604	DF	29.53238
Upper CL Dif	1.7382	Prob > |t|	0.0938
Lower CL Dif	-0.1438	Prob > t	0.0469*
Confidence	0.95	Prob < t	0.9531

Tests that the Variances are Equal

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	1.2020	1	39	0.2797
Brown-Forsythe	1.1439	1	39	0.2914
Levene	1.7255	1	39	0.1967
Bartlett	6.1246	1	.	0.0133*
F Test 2-sided	3.1918	19	20	0.0132*

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
2.9978	1	29.532	0.0938

t Test

1.7314

Table 25. Interleukin-1 Receptor Antagonist analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	2.6246	0.1133
Day	2	2	78	3.0302	0.0540
Treatment*Day	2	2	78	0.1209	0.8862

t Test

Placebo-DHA

Assuming unequal variances

Difference	144.91	t Ratio	1.432145
Std Err Dif	101.19	DF	23.56176
Upper CL Dif	353.96	Prob > |t|	0.1652
Lower CL Dif	-64.13	Prob > t	0.0826
Confidence	0.95	Prob < t	0.9174

Tests that the Variances are Equal

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	1.7129	1	39	0.1983
Brown-Forsythe	1.5638	1	39	0.2186
Levene	4.1655	1	39	0.0481*
Bartlett	17.6154	1	.	<.0001*
F Test 2-sided	7.8477	19	20	<.0001*

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
2.0510	1	23.562	0.1652

t Test

1.4321

Table 26. Interleukin-6 (IL-6) analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	3.7968	0.0586
Day	2	2	78	2.7594	0.0695
Treatment*Day	2	2	78	0.4818	0.6195

t Test

Placebo-DHA

Assuming equal variances

Difference	0.33601	t Ratio	1.132322
Std Err Dif	0.29675	DF	39
Upper CL Dif	0.93624	Prob > |t|	0.2644
Lower CL Dif	-0.26422	Prob > t	0.1322
Confidence	0.95	Prob < t	0.8678

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	1.156594	1.15659	1.2822	0.2644
Error	39	35.180804	0.90207		
C. Total	40	36.337398			

Table 27. Prostaglandin-E2 analysis

2-Way ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
group	1	1	39	2.6102	0.1142
day	2	2	78	0.8302	0.4398
group*day	2	2	78	0.8801	0.4188

t Test

Placebo-DHA

Assuming equal variances

Difference	-215.01	t Ratio	-2.53243
Std Err Dif	84.90	DF	39
Upper CL Dif	-43.28	Prob > |t|	0.0155*
Lower CL Dif	-386.75	Prob > t	0.9923
Confidence	0.95	Prob < t	0.0077*

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
group	1	473576.8	473577	6.4132	0.0155*
Error	39	2879929.6	73844		
C. Total	40	3353506.4			

Table 28. Acute Eccentric Exercise Rating analysis

Tests that the Variances are Equal

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	1.1473	1	38	0.2909
Brown-Forsythe	1.1197	1	38	0.2967
Levene	1.4409	1	38	0.2374
Bartlett	4.4700	1	.	0.0345*
F Test 2-sided	2.7255	19	19	0.0345*

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
1.6000	1	31.288	0.2152

t Test

1.2649

Table 29. Subject Age analysis
t Test
Placebo-DHA
Assuming equal variances

Difference	-0.9881	t Ratio	-1.17701
Std Err Dif	0.8395	DF	39
Upper CL Dif	0.7100	Prob > |t|	0.2463
Lower CL Dif	-2.6861	Prob > t	0.8768
Confidence	0.95	Prob < t	0.1232

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	10.00145	10.0015	1.3853	0.2463
Error	39	281.55952	7.2195		
C. Total	40	291.56098			

Table 30. Body Fat Percentage analysis
t Test
Placebo-DHA
Assuming equal variances

Difference	2.0982	t Ratio	1.264673
Std Err Dif	1.6591	DF	39
Upper CL Dif	5.4540	Prob > |t|	0.2135
Lower CL Dif	-1.2576	Prob > t	0.1067
Confidence	0.95	Prob < t	0.8933

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	45.0978	45.0978	1.5994	0.2135
Error	39	1099.6730	28.1967		
C. Total	40	1144.7708			

Table 31. Body Mass Index analysis
t Test
Placebo-DHA
Assuming equal variances

Difference	0.9149	t Ratio	0.586851
Std Err Dif	1.5590	DF	39
Upper CL Dif	4.0684	Prob > |t|	0.5607
Lower CL Dif	-2.2385	Prob > t	0.2803
Confidence	0.95	Prob < t	0.7197

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	8.57511	8.5751	0.3444	0.5607
Error	39	971.06551	24.8991		
C. Total	40	979.64062			

Table 32. Baseline Weight analysis
t Test
Placebo-DHA
Assuming equal variances

Difference	9.456	t Ratio	0.869875
Std Err Dif	10.870	DF	39
Upper CL Dif	31.442	Prob > |t|	0.3897
Lower CL Dif	-12.531	Prob > t	0.1948
Confidence	0.95	Prob < t	0.8052

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	915.876	915.88	0.7567	0.3897
Error	39	47204.908	1210.38		
C. Total	40	48120.784			

Table 33. Weight Change analysis
Tests that the Variances are Equal

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	4.3412	1	39	0.0438*
Brown-Forsythe	3.6957	1	39	0.0619
Levene	4.5385	1	39	0.0395*
Bartlett	6.0168	1	.	0.0142*
F Test 2-sided	3.1931	20	19	0.0143*

Welch's Test
Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
1.2146	1	31.707	0.2787

t Test
1.1021

Table 34. Area Under the Curve TUT analysis
t Test
Placebo-DHA
Assuming equal variances

Difference	-15.768	t Ratio	-1.51921
Std Err Dif	10.379	DF	39
Upper CL Dif	5.226	Prob > |t|	0.1368
Lower CL Dif	-36.763	Prob > t	0.9316
Confidence	0.95	Prob < t	0.0684

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	2547.086	2547.09	2.3080	0.1368
Error	39	43040.137	1103.59		
C. Total	40	45587.223			

Table 35. Area Under the Curve ISO analysis

ANOVA Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	0.0076	0.9310

t Test

Placebo-DHA

Assuming equal variances

Difference	-0.467	t Ratio	-0.08711
Std Err Dif	5.357	DF	39
Upper CL Dif	10.369	Prob > |t|	0.9310
Lower CL Dif	-11.302	Prob > t	0.5345
Confidence	0.95	Prob < t	0.4655

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	2.231	2.231	0.0076	0.9310
Error	39	11465.397	293.985		
C. Total	40	11467.628			

Table 36. Area Under the Curve DOMS analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	14.402	t Ratio	1.322513
Std Err Dif	10.890	DF	39
Upper CL Dif	36.430	Prob > |t|	0.1937
Lower CL Dif	-7.625	Prob > t	0.0969
Confidence	0.95	Prob < t	0.9031

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	2124.878	2124.88	1.7490	0.1937
Error	39	47380.402	1214.88		
C. Total	40	49505.280			

37. Area Under the Curve ROM analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	-0.135	t Ratio	-0.00716
Std Err Dif	18.790	DF	39
Upper CL Dif	37.872	Prob > |t|	0.9943
Lower CL Dif	-38.141	Prob > t	0.5028
Confidence	0.95	Prob < t	0.4972

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	0.19	0.19	0.0001	0.9943
Error	39	141057.00	3616.85		
C. Total	40	141057.19			

Table 38. Area Under the Curve CK analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	1506.2	t Ratio	0.680473
Std Err Dif	2213.5	DF	39
Upper CL Dif	5983.5	Prob > |t|	0.5002
Lower CL Dif	-2971.0	Prob > t	0.2501
Confidence	0.95	Prob < t	0.7499

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	23240923.2	23240923	0.4630	0.5002
Error	39	1957473092	50191618		
C. Total	40	1980714015			

Table 39. Area Under the Curve LN(CK) analysis
t Test
Placebo-DHA
Assuming equal variances

Difference	1.6937	t Ratio	1.858212
Std Err Dif	0.9115	DF	39
Upper CL Dif	3.5373	Prob > |t|	0.0707
Lower CL Dif	-0.1499	Prob > t	0.0353*
Confidence	0.95	Prob < t	0.9647

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	29.38580	29.3858	3.4530	0.0707
Error	39	331.90323	8.5103		
C. Total	40	361.28903			

Table 40. Area Under the Curve CRP analysis
t Test
Placebo-DHA
Assuming equal variances

Difference	2.4467	t Ratio	1.54047
Std Err Dif	1.5883	DF	39
Upper CL Dif	5.6594	Prob > |t|	0.1315
Lower CL Dif	-0.7659	Prob > t	0.0658
Confidence	0.95	Prob < t	0.9342

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	61.3256	61.3256	2.3730	0.1315
Error	39	1007.8599	25.8426		
C. Total	40	1069.1855			

Table 41. Area Under the Curve IL-1ra analysis

Tests that the Variances are Equal

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	1.6661	1	39	0.2044
Brown-Forsythe	2.1547	1	39	0.1502
Levene	4.7791	1	39	0.0349*
Bartlett	29.0415	1	.	<.0001*
F Test 2-sided	15.9056	19	20	<.0001*

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
2.6313	1	21.271	0.1195

t Test

1.6221

Table 42. Area Under the Curve IL-6 analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	1.66187	t Ratio	2.065076
Std Err Dif	0.80475	DF	39
Upper CL Dif	3.28964	Prob > |t|	0.0456*
Lower CL Dif	0.03411	Prob > t	0.0228*
Confidence	0.95	Prob < t	0.9772

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	28.29182	28.2918	4.2645	0.0456*
Error	39	258.73390	6.6342		
C. Total	40	287.02572			

Table 43. Area Under the Curve PGE2 analysis

t Test

Placebo-DHA

Assuming equal variances

Difference	-399.8	t Ratio	-1.31205
Std Err Dif	304.7	DF	39
Upper CL Dif	216.6	Prob > |t|	0.1972
Lower CL Dif	-1016.2	Prob > t	0.9014
Confidence	0.95	Prob < t	0.0986

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	1637552	1637552	1.7215	0.1972
Error	39	37098972	951256		
C. Total	40	38736523			

Table 44. TUT ANOVA

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	2590.636	235.512	4.6550
Error	234	11838.910	50.594	**Prob > F**
C. Total	245	14429.546		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	537.2757	10.6194	0.0013*
Set	5	5	2004.3988	7.9235	<.0001*
Set*Treatment	5	5	25.4969	0.1008	0.9919

Table 45. Change in Markers (Final – Baseline) Correlation Analysis

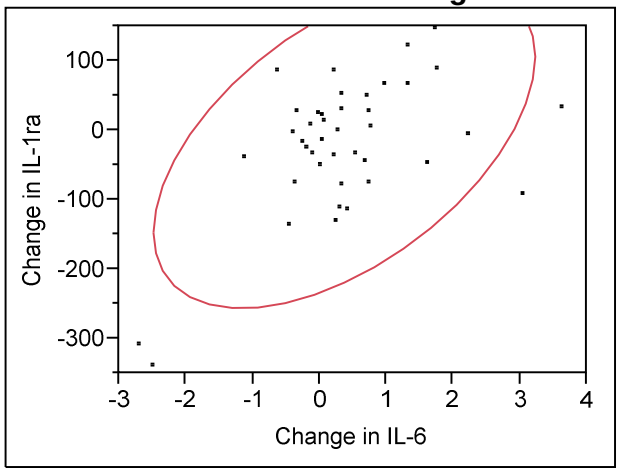
Correlations

	Change in PGE2	Change in IL-6	Change in IL-1ra	Change in CRP	Change in natural Log CK	Change in ISO	Change in DOMS (Day 4 - Day 2)	Change in ROM
Change in PGE2	1.0000	-0.2266	-0.0265	-0.0274	-0.2062	0.2228	-0.1848	0.0834
Change in IL-6	-0.2266	1.0000	0.5370	0.2409	0.5460	-0.4227	0.2496	-0.3872
Change in IL-1ra	-0.0265	0.5370	1.0000	0.1694	0.2018	-0.1372	0.4798	-0.1359
Change in CRP	-0.0274	0.2409	0.1694	1.0000	0.1257	-0.2159	-0.0117	-0.0274
Change in natural Log CK	-0.2062	0.5460	0.2018	0.1257	1.0000	-0.5243	0.3591	-0.4379
Change in ISO	0.2228	-0.4227	-0.1372	-0.2159	-0.5243	1.0000	-0.2828	0.5678
Change in DOMS (Day 4 - Day 2)	-0.1848	0.2496	0.4798	-0.0117	0.3591	-0.2828	1.0000	-0.0267
Change in ROM	0.0834	-0.3872	-0.1359	-0.0274	-0.4379	0.5678	-0.0267	1.0000

CI of Correlation

Variable	by Variable	Correlation	Lower 95%	Upper 95%
Change in IL-6	Change in PGE2	-0.2266	-0.4994	0.0871
Change in IL-1ra	Change in PGE2	-0.0265	-0.3315	0.2834
Change in IL-1ra	Change in IL-6	0.5370	0.2747	0.7249
Change in CRP	Change in PGE2	-0.0274	-0.3322	0.2826
Change in CRP	Change in IL-6	0.2409	-0.0721	0.5107
Change in CRP	Change in IL-1ra	0.1694	-0.1459	0.4534
Change in natural Log CK	Change in PGE2	-0.2062	-0.4832	0.1084
Change in natural Log CK	Change in IL-6	0.5460	0.2865	0.7309
Change in natural Log CK	Change in IL-1ra	0.2018	-0.1128	0.4797
Change in natural Log CK	Change in CRP	0.1257	-0.1893	0.4172
Change in ISO	Change in PGE2	0.2228	-0.0911	0.4964
Change in ISO	Change in IL-6	-0.4227	-0.6463	-0.1322
Change in ISO	Change in IL-1ra	-0.1372	-0.4268	0.1780
Change in ISO	Change in CRP	-0.2159	-0.4910	0.0982
Change in ISO	Change in natural Log CK	-0.5243	-0.7164	-0.2583
Change in DOMS (Day 4 - Day 2)	Change in PGE2	-0.1848	-0.4659	0.1303
Change in DOMS (Day 4 - Day 2)	Change in IL-6	0.2496	-0.0628	0.5175
Change in DOMS (Day 4 - Day 2)	Change in IL-1ra	0.4798	0.2019	0.6861
Change in DOMS (Day 4 - Day 2)	Change in CRP	-0.0117	-0.3182	0.2970
Change in DOMS (Day 4 - Day 2)	Change in natural Log CK	0.3591	0.0579	0.6004
Change in DOMS (Day 4 - Day 2)	Change in ISO	-0.2828	-0.5432	0.0272
Change in ROM	Change in PGE2	0.0834	-0.2301	0.3813
Change in ROM	Change in IL-6	-0.3872	-0.6209	-0.0903
Change in ROM	Change in IL-1ra	-0.1359	-0.4257	0.1793
Change in ROM	Change in CRP	-0.0274	-0.3323	0.2826
Change in ROM	Change in natural Log CK	-0.4379	-0.6570	-0.1505
Change in ROM	Change in ISO	0.5678	0.3152	0.7453
Change in ROM	Change in DOMS (Day 4 - Day 2)	-0.0267	-0.3316	0.2833

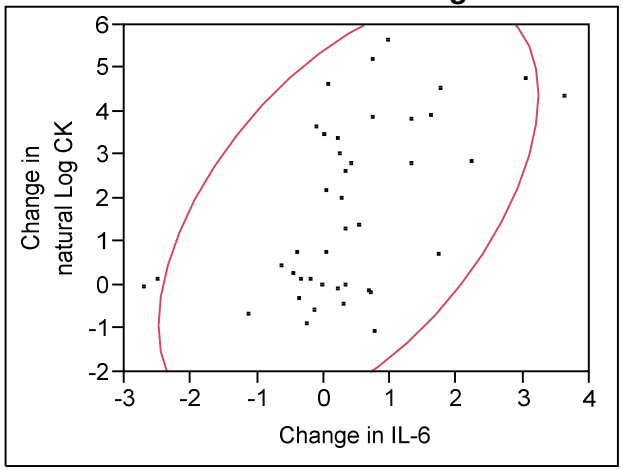
Table 46. Bivariate Fit of Change in IL-1ra By Change in IL-6



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in IL-6	0.36978	1.1676	0.536955	0.0003*	41
Change in IL-1ra	-20.6634	96.32575			

Table 47. Bivariate Fit of Change in natural Log CK By Change in IL-6

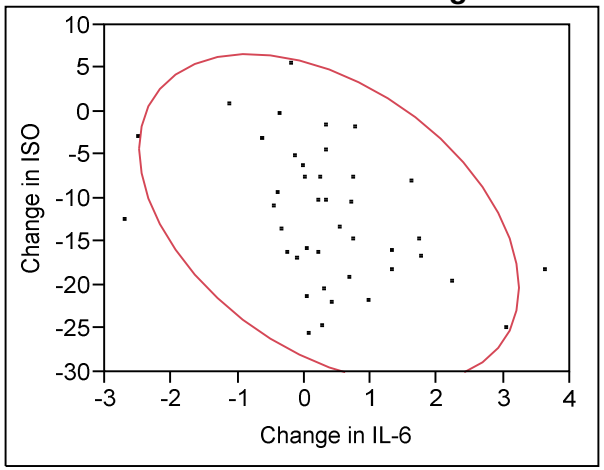


Bivariate Normal Ellipse P=0.950

Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in IL-6	0.36978	1.1676	0.54601	0.0002*	41
Change in natural Log CK	1.735117	1.986471			

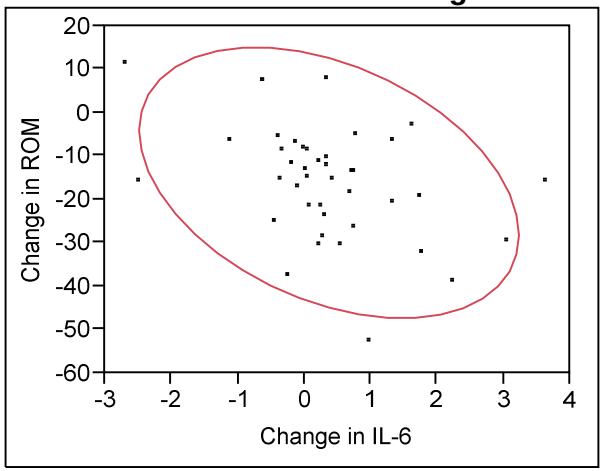
Table 48. Bivariate Fit of Change in ISO By Change in IL-6



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in IL-6	0.36978	1.1676	-0.42267	0.0059*	41
Change in ISO	-12.2659	7.736783			

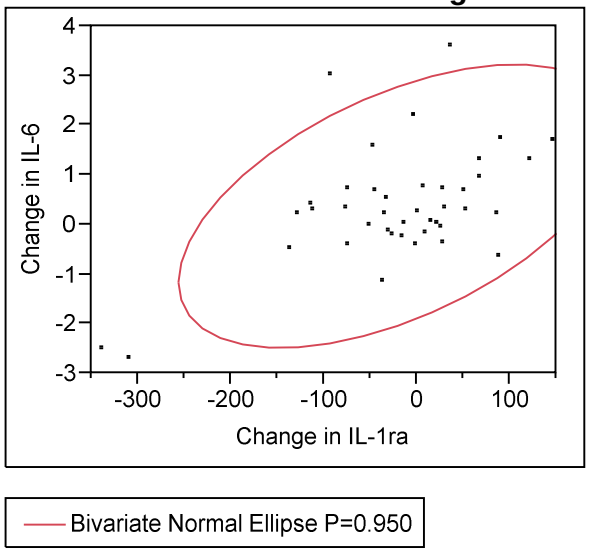
Table 49. Bivariate Fit of Change in ROM By Change in IL-6



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in IL-6	0.36978	1.1676	-0.38716	0.0124*	41
Change in ROM	-16.0976	12.75452			

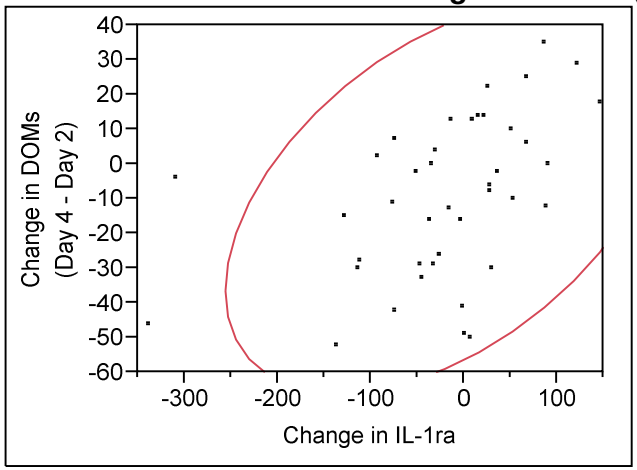
Table 50. Bivariate Fit of Change in IL-6 By Change in IL-1ra



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in IL-1ra	-20.6634	96.32575	0.536955	0.0003*	41
Change in IL-6	0.36978	1.1676			

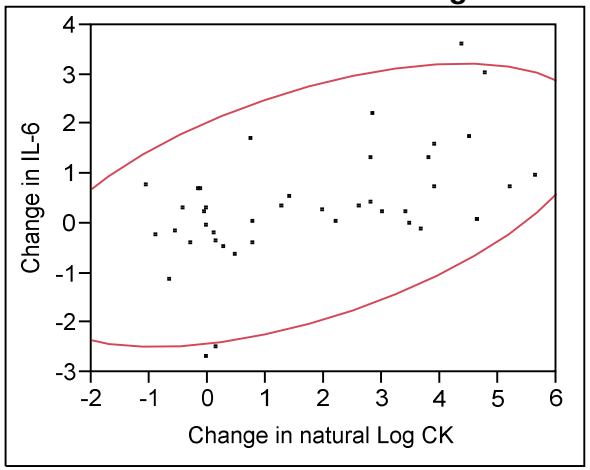
Table 51. Bivariate Fit of Change in DOMS (Day 4 - Day 2) By Change in IL-1ra



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in IL-1ra	-20.6634	96.32575	0.479752	0.0015*	41
Change in DOMS (Day 4 - Day 2)	-9.46341	23.1053			

Table 52. Bivariate Fit of Change in IL-6 By Change in natural Log CK

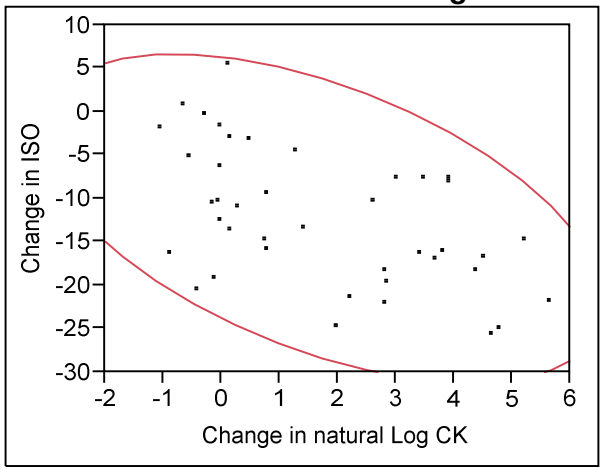


Bivariate Normal Ellipse P=0.950

Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in natural Log CK	1.735117	1.986471	0.54601	0.0002*	41
Change in IL-6	0.36978	1.1676			

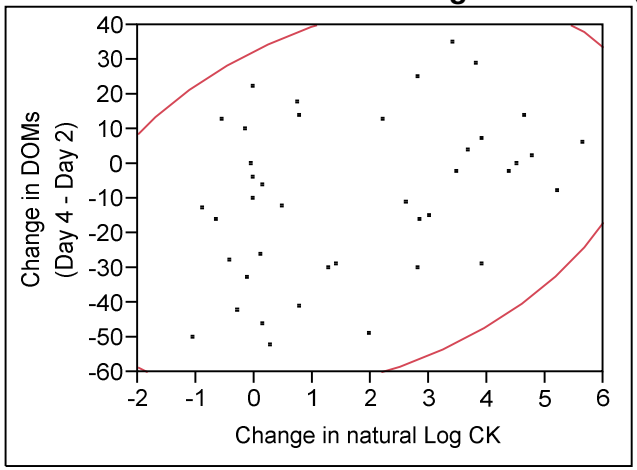
Table 53. Bivariate Fit of Change in ISO By Change in natural Log CK



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in natural Log CK	1.735117	1.986471	-0.52428	0.0004*	41
Change in ISO	-12.2659	7.736783			

Table 54. Bivariate Fit of Change in DOMS (Day 4 - Day 2) By Change in natural Log CK

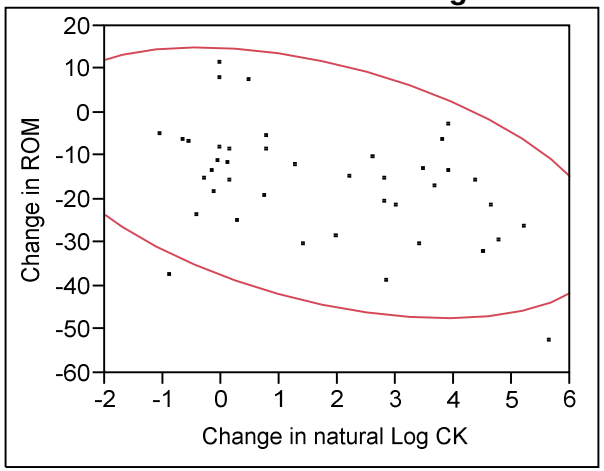


Bivariate Normal Ellipse P=0.950

Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in natural Log CK	1.735117	1.986471	0.359123	0.0211*	41
Change in DOMS (Day 4 - Day 2)	-9.46341	23.1053			

Table 55. Bivariate Fit of Change in ROM By Change in natural Log CK

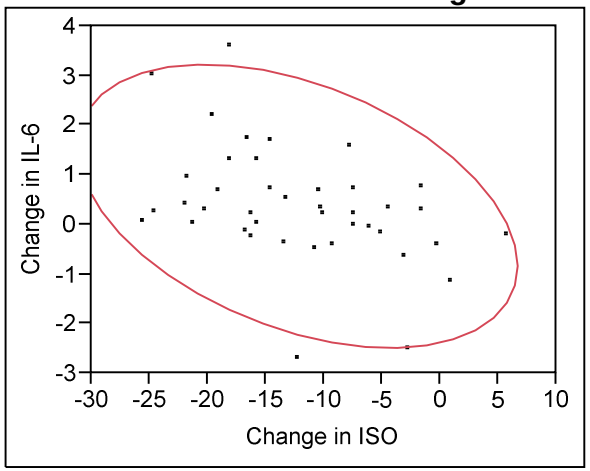


Bivariate Normal Ellipse P=0.950

Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in natural Log CK	1.735117	1.986471	-0.43789	0.0042*	41
Change in ROM	-16.0976	12.75452			

Table 56. Bivariate Fit of Change in IL-6 By Change in ISO

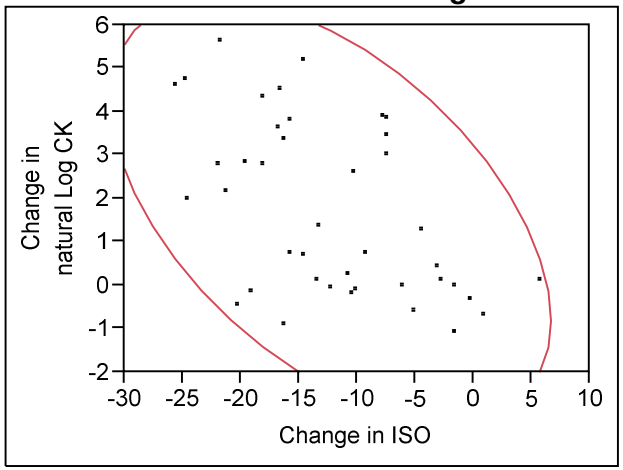


Bivariate Normal Ellipse P=0.950

Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in ISO	-12.2659	7.736783	-0.42267	0.0059*	41
Change in IL-6	0.36978	1.1676			

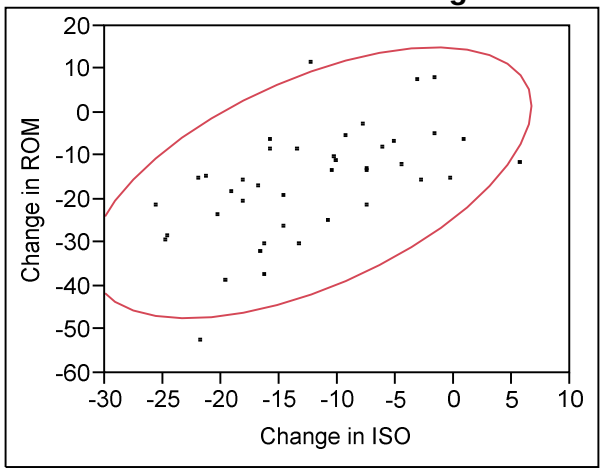
Table 57. Bivariate Fit of Change in natural Log CK By Change in ISO



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in ISO	-12.2659	7.736783	-0.52428	0.0004*	41
Change in natural Log CK	1.735117	1.986471			

Table 58. Bivariate Fit of Change in ROM By Change in ISO

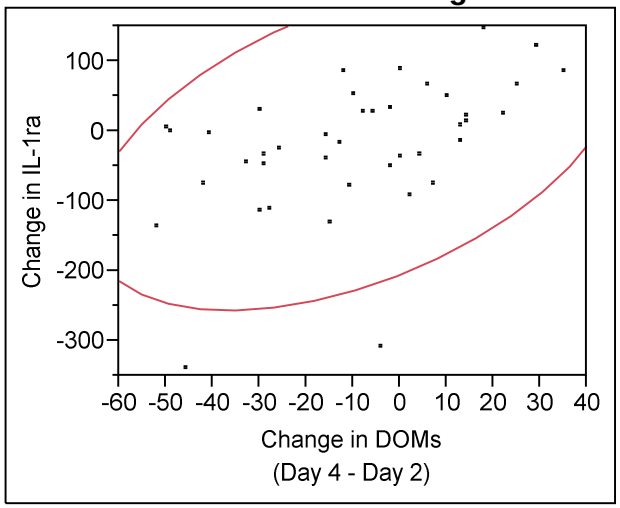


Bivariate Normal Ellipse P=0.950

Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in ISO	-12.2659	7.736783	0.567823	0.0001*	41
Change in ROM	-16.0976	12.75452			

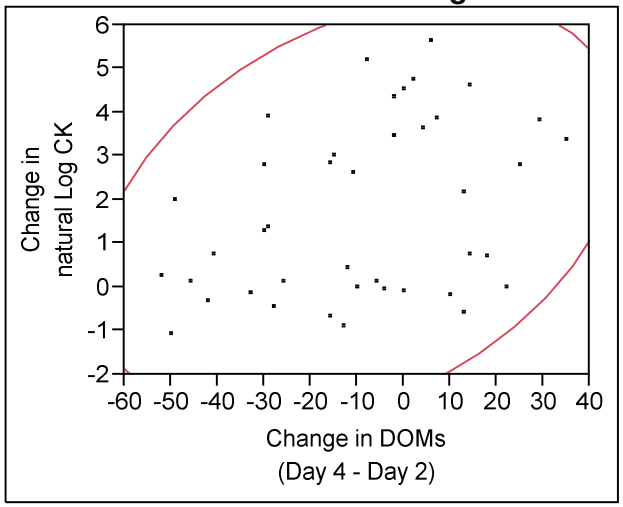
Table 59. Bivariate Fit of Change in IL-1ra By Change in DOMS (Day 4 - Day 2)



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in DOMS (Day 4 - Day 2)	-9.46341	23.1053	0.479752	0.0015*	41
Change in IL-1ra	-20.6634	96.32575			

Table 60. Bivariate Fit of Change in natural Log CK By Change in DOMS (Day 4 - Day 2)

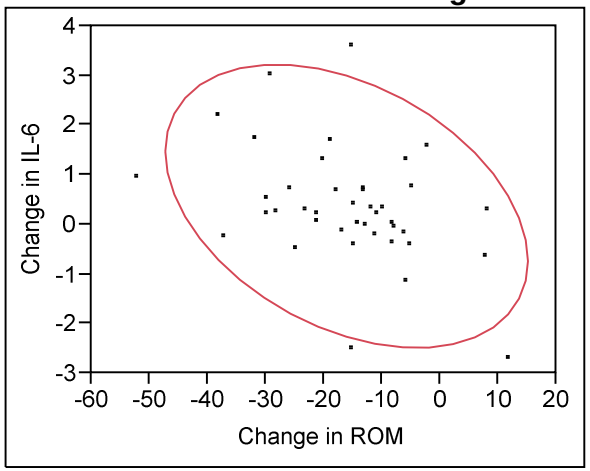


Bivariate Normal Ellipse P=0.950

Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in DOMS (Day 4 - Day 2)	-9.46341	23.1053	0.359123	0.0211*	41
Change in natural Log CK	1.735117	1.986471			

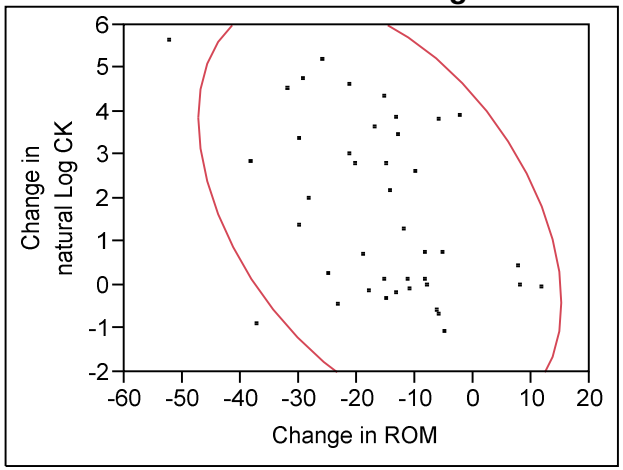
Table 61. Bivariate Fit of Change in IL-6 By Change in ROM



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in ROM	-16.0976	12.75452	-0.38716	0.0124*	41
Change in IL-6	0.36978	1.1676			

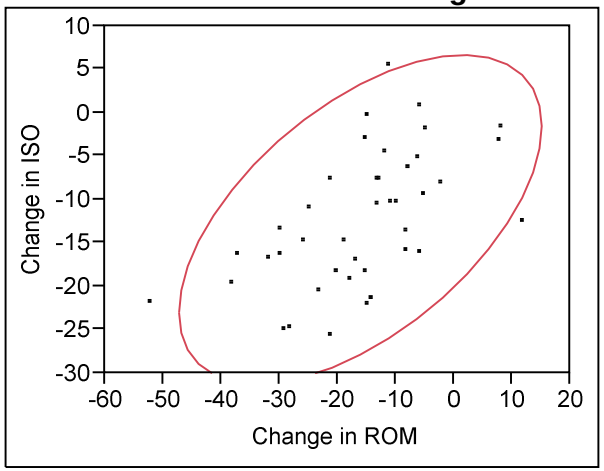
Table 62. Bivariate Fit of Change in natural Log CK By Change in ROM



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in ROM	-16.0976	12.75452	-0.43789	0.0042*	41
Change in natural Log CK	1.735117	1.986471			

Table 63. Bivariate Fit of Change in ISO By Change in ROM



Correlation

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
Change in ROM	-16.0976	12.75452	0.567823	0.0001*	41
Change in ISO	-12.2659	7.736783			