EFFECT OF DHA SUPPLEMENTATION ON MUSCLE DAMAGE AND INFLAMMATION DURING THE FIRST TWO WEEKS OF A NOVICE RESISTANCE TRAINING PROGRAM

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Keywords: Omega-3 fatty acids, strength training, creatine kinase, C-reactive protein

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ABSTRACT

Aim: The purpose of this study was to investigate docosahexaenoic acid (DHA) ingestion on muscle damage and inflammation during the first two weeks of a novice resistance training (RT) program. **Methods:** This study was a placebo-controlled, double-blind design. Forty-one healthy untrained males between the ages of 18 and 28 years consumed 2,000 mg/d of either DHA or corn oil (PCB) for 44 days including a 28 day loading period. Serum fatty acids were analyzed to determine treatment efficacy. During the 17 day training period, an acute eccentric exercise bout was implemented followed by a full-body RT regimen thrice weekly. Six fasted blood draws (days 1, 2, 4, 7, 12, and 17) during this exercise period were analyzed for creatine kinase (CK) and C-reactive protein (CRP). Maximum isometric strength (ISO) of the elbow flexors, delayed onset muscle soreness (DOMS), and range of motion (ROM) were measured on day 1 prior to exercise and also on days 2, 3, 4, 7, 12, and 17. **Results:** The CK response and the area under the curve (AUC) analysis for DOMS trended to decrease in the DHA group in comparison to placebo ($p=0.0925$ and $p=0.0536$, respectively). Treatment showed no effect on CRP levels. DHA supplementation significantly increased serum DHA by 380% as a proportion of total fatty acids ($p<0.0001$). **Conclusion:** This study does not demonstrate convincing benefits of DHA ingestion to recovery from a new resistance exercise program but does suggest a need for further investigation.

Keywords: Omega-3 fatty acids, strength training, creatine kinase, C-reactive protein

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Chapter 1: INTRODUCTION

Introduction

The American Heart Association (AHA) estimates 82 million adults in the United States have one or more types of cardiovascular disease (CVD) and that CVD accounted for 32.8% of all deaths in 2008 (1). These statistics have encouraged government agencies and health associations to provide exercise guidelines to the general public. The AHA and the American College of Sports Medicine (ACSM) recommend resistance training (RT): to increase muscle mass and strength; to improve bone mineral density and lipoprotein profile; and to decrease fat mass and blood pressure (2-5). Another advantage of partaking in resistance training is the apparent inverse relationship between muscular strength and death from all causes including cancer (6, 7).

Although many benefits of resistance training are known amongst the general public, the Centers for Disease Control reported in 2004 that less than 22% of men and 18% of women strength trained at least twice a week (8). A common dilemma associated with beginning a new resistance training program, delayed onset muscle soreness (DOMS), could be, at least in part, the reason for these low statistics. DOMS occurs after eccentric muscular contractions; microtears in the exercised sarcomeres result, caused from both the direct mechanical resistance and subsequent inflammation (9, 10). Relatively untrained individuals who are unaccustomed to RT are more likely to experience greater amounts of muscle damage and muscle soreness due to an absence of muscular adaptation to the exercises (11). After the initial exercise bout, the recovery process rebuilds the damaged fibers and induces protection from future bouts of the same exercise—a process called the repeated bout effect (9). However, the pain and discomfort that accompanies this healing process can deter a beginner from continuing with the exercise regimen and ultimately prohibit them from establishing complete muscular adaptation. To

combat this irritation, people have used anti-inflammatory medications. However, some evidence show negative effects such as gastrointestinal distress, renal and liver injury, and heart failure; inhibition of protein synthesis, muscle regeneration, and hypertrophy; and interference with muscular adaptation (12, 13). For a healthier alternative with minimal side effects, a natural dietary supplement could be utilized to reduce muscle damage and soreness during the initiation of resistance exercise training in order to increase compliance and completion of exercise programs.

Omega-3 fatty acids, especially long chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential nutrients for human health. The importance of these fatty acids, especially in relation to omega-6 fatty acids, is becoming increasingly recognized. Evidence shows omega-3 fatty acids may reduce the risk of CVD through reducing arrhythmias, altering prostaglandin production, and retarding athlerosclerotic plaque growth (14). A study has also shown that omega-3s are inversely associated with markers of systemic inflammation (CRP, IL-6, and TNFα) (15). As a result, recommendations for dietary intake of omega-3 fatty acids have been published. The AHA advises patients with coronary heart disease to consume about 1,000 mg combined EPA+DHA per day and patients with hypertriglyceridemia to consume 2,000 to 4,000 mg/d, under a physician's care (16). A National Heart, Lung, and Blood Institute study suggests that a daily dietary intake of 0.5 to 1.0 g of EPA+DHA reduces the risk of cardiovascular death in middle aged American men by about 40%; other data suggest omega-3 fatty acids may also decrease cancer mortality (17). Founder and president of the Center for Genetics, Nutrition, and Health, Dr. Artemis Simopoulos, recommends omega-3s for athletes as well, suggesting 1,000-2,000 mg/d of EPA+DHA to prevent inflammation in muscles and joints (18). All of these aforementioned recommendations

also suggest low or equal ratios of omega-6:omega-3 fatty acids. About 10,000 years ago prior to agriculture, humans consumed roughly equal amounts of both polyunsaturated fatty acids (PUFA) (18). However, western style diets typically have an estimated ratio of 10:1 to 20:1 and a total omega-3 intake of about 1,600 mg/d, of which ~1,400 mg comes from α-linolenic acid via vegetable oils and only ~100-200 mg comes from EPA and DHA (14, 18). A significant contributor to the high omega-6 fatty acid content in western diets is the abundance of vegetable oils, specifically corn, grapeseed, and sunflower oils.

As a result, the long chain omega-3 intake of EPA and DHA should be carefully considered among nutritional sources and supplements. Because algae produce EPA and DHA, prime sources of these nutrients include shellfish and cold-water fatty fish including salmon (as EPA and DHA become increasingly concentrated high in the food chain), but other sources include grass-fed beef and free-range eggs. On average, farmed Atlantic salmon were found to contain 2.65 g EPA+DHA per 100 g wet weight while wild Pacific salmon were found to contain 1.04 g of EPA+DHA per 100 g wet weight (19). In testing omega-3 fatty acid content in Australian grass-fed cattle, an average 100 g of rump cut was found to contain 47.5 mg of EPA+DHA (20). Lastly, eggs from free-ranging chickens in Greece were found to have 7.8 g EPA+EHA/mg yolk, in comparison to eggs from a supermarket having only 1.1 g/mg yolk (21). While both prove to be beneficial sources of EPA and DHA, aquatic options are greater than terrestrial on average.

Omega-3 fatty acids could potentially benefit resistance training athletes through their known anti-inflammatory capabilities. Evidence suggests that pharmaceutical interventions can target cyclooxygenase (COX) and lipooxygenase (LOX) receptors and reduce muscle soreness after a novel resistance training bout, while other studies show no benefit (22-25). Omega-3s

might be an effective treatment to reduce the inflammatory response and resultant muscular soreness that occurs during the start of a new RT program. These lipids have been shown to alleviate the inflammatory response through EPA and DHA competing as substrates with the omega-6 fatty acid arachidonic acid (ARA) for the COX and LOX enzymes and subsequently producing anti-inflammatory eicosanoids (18, 26). The synthesis of eicosanoids, key mediators and regulators of inflammation, including prostaglandins, thromboxanes, and leukotrienes, is partly influenced by proportions of omega-3 and omega-6 fatty acids within cells. The eicosanoids formed from omega-6 fatty acids are pro-inflammatory, whereas the eicosanoids from omega-3 fatty acids are anti-inflammatory (or less pro-inflammatory) (26). Omega-3 fatty acids are thought also to influence cytokine signaling pathways via altering transcription factors. For example, nuclear factor kappa-β (NFκβ), a transcription factor responsible for inducing several pro-inflammatory cytokines (TNFα, IL-6, etc.) and enzymes which in part can cause chronic inflammatory diseases, can be inhibited by omega-3 fatty acid ingestion (27). Additionally, omega-3s can bind and activate peroxisome proliferator-activated receptors (PPARs) to repress certain targets, including NFκβ (26). Therefore, increased dietary omega-3 fatty acids could result in decreased induction of inflammatory cytokines and increased production of anti-inflammatory eicosanoids, subsequently resulting in a lesser inflammatory state overall. This hypothesis has not been evaluated thoroughly in athletes.

Statement of Problem

Muscular soreness often results from resistance training and is caused, at least in part, by muscle damage and inflammation. A nutritional supplement shown to have the potential to reduce excess inflammation and muscle damage could be beneficial to many populations in the

athletic community, especially the untrained who are beginning a RT program for the first time and are likely to experience great soreness. Few supplements or drugs have been effective in reducing exercise-induced muscle damage and inflammation without unwanted side effects. Omega-3 fatty acids have been demonstrated to reduce the inflammatory response in some clinical conditions. DHA, the longest chain omega-3, has effects on the inflammatory response that may translate to less muscular soreness, faster recovery, and reduced inflammation in individuals initiating a RT program.

Purpose and Significance

The purpose of this study is to investigate whether 28 days of DHA supplementation benefits the muscle damage and inflammatory responses during the beginning of a RT program for untrained people in the healthy population. Limited research exists regarding the effect of omega-3 fats and none has been published on DHA's effect on humans during weight training. This is the first study to examine the effect of DHA supplementation on muscle damage and inflammatory responses during the first few weeks of a full-body RT regimen. If successful, this study could initiate new ideas for future research evaluating the mechanisms underlying the action of DHA during strength training.

Hypotheses

1. Following 28 days of DHA supplementation, blood fatty acid profiles will be modified as compared to the placebo (PCB) group as indicated by:
    a. Increased serum DHA content

2. Throughout the first 14 days of a new resistance training program in untrained young adult men, indirect markers of muscle damage will be less in the DHA group as compared to the PCB as indicated by:
    a. Decreased serum CK concentration
    b. Decreased DOMS
    c. Decreased change in ROM
    d. Decreased change in ISO
3. Throughout the first 14 days of a new resistance training program in untrained young adult men, the inflammatory response will be less in the DHA group as compared to the PCB as indicated by:
    a. Decreased serum CRP concentration

Limitations

- Subjects were free-living
- All treatment group subjects received a standard dose of DHA, regardless of body mass
- No muscle biopsies were collected to confirm extent of muscle damage
- All markers of muscle damage and inflammation (DOMS, ROM, ISO, and serum CK and CRP) were indirect measures
- Workouts 2-6 were not supervised by any investigators
- Subjects performed their first workout soon after a fasted blood draw with only moderate consumption of carbohydrates (G2 Gatorade)
- Some subjects were able to infer which treatment they were on (ex. fish burps)

- Subjects were not restricted to a standardized diet but asked to maintain their usual dietary habits
- Maximum strength for each lift was estimated from a multiple repetition maximum calculation rather than a direct 1RM
- There was no group that performed the eccentric exercise without the subsequent resistance training

Delimitations

- Subjects were males, ages 18-28 years, healthy non-smokers
- Subjects were free of orthopedic injury, illness, or disease that would either negatively affect performance or measurements or present a risk to the subjects or researchers
- Subjects were not resistance-trained for at least 6 months prior to the beginning of the study
- The resistance training regimen was in accordance with ACSM guidelines for untrained subjects
- Subjects were instructed to discontinue other supplementation or medications which could confuse results
- Investigators were blinded to subject treatment assignment
- The independent variable for the treatment group was ingestion of 2 grams of DHA per day every day for the entire study
- The independent variable for the placebo group was ingestion of 2 grams of corn oil per day every day for the entire study

- The dependent variables were levels of serum fatty acids, serum CK, serum CRP, DOMS, ROM, and ISO

Basic Assumptions

- Subjects did not alter dietary habits throughout the duration of the entire study
- Subjects ingested their supplement fully every day of the study
- Subjects fasted for at least 12 hours prior to blood draws
- Subjects did not ingest any other supplements or medications during the study
- Subjects were honest in completing questionnaires
- Subjects completed no other resistance exercise during the study
- Subjects completed all exercises as prescribed
- Subjects gave maximal effort during eccentric and resistance training sessions

Abbreviations and Definitions

- ADP- adenosine diphosphate: important molecule in metabolic processes in living cells
- ATP- adenosine triphosphate: high energy molecule that serves as the energy source for many metabolic processes
- BMI- body mass index: an anthropometric dividing an individual's weight by height squared (kg/m^2)
- CK- creatine kinase: an enzyme in muscle tissue that catalyzes the conversion of phosphocreatine and ADP into creatine and ATP; also an indicator for muscle damage when found in blood

- CRP- C-reactive protein: an acute-phase blood protein synthesized by the liver in response to macrophages; indicates changes in overall inflammation
- CVD- cardiovascular disease: refers to any disease that involves the heart or blood vessels, usually related to atherosclerosis (arterial disease)
- DHA- docosahexaenoic acid: a 22-carbon chain omega-3 fatty acid; a mediator of the inflammatory process
- ECM- extracellular matrix: extracellular part of tissue that provides structural support to cells; connective tissue
- IL-6- interleukin 6: a cytokine secreted by T cells and macrophages to stimulate immune response; has both pro-inflammatory and anti-inflammatory characteristics
- NFκB- nuclear factor kappa-light-chain-enhancer of activated B cells: a protein complex that plays a key role in regulating the immune response
- n-3 PUFA- omega-3 polyunsaturated fatty acids: essential fatty acids found in marine and plant oils that have been shown to possess anti-inflammatory capabilities
- PCB- placebo: purposefully ineffective treatment for a disease or experiment designed to deceive the patient
- PPAR- peroxisome proliferator-activated receptors: ligand-activated nuclear transcription factors that play important roles in inflammation and other important metabolic processes
- ROS- reactive oxygen species: a variety of molecules containing one unpaired electron that is highly reactive and may be damaging on the cellular level if unbalanced by antioxidants

- RT- resistance training: refers to training that uses a resistance (machines, free weights, elastic bands, chains, etc.) to the force of muscular contraction; also termed strength training
- TNF-α- tumor necrosis factor alpha: a pro-inflammatory cytokine involved in systemic inflammation; also contributes to the acute phase reaction

Chapter 2: REVIEW OF LITERATURE

Literature Review

I. Resistance Training

Introduction

Resistance training (RT) refers to any training using a resistance to the force of muscular contraction, commonly achieved through use of bodyweight, machines, free weights, elastic bands, etc. Several styles of RT exist, including but not limited to calisthenics, powerlifting, weightlifting, plyometrics, and gymnastics. Most individuals participate in RT using free weights or machines designed to stress the muscles through concentric and eccentric contraction.

Benefits

Resistance training positively affects multiple systems in the body. The benefits improving the musculoskeletal system are well-known but others that are less recognized enhance function of the circulatory, endocrine, and neuromuscular systems (2, 5, 28-30).

The physical benefits involving the muscular and skeletal systems include increased muscular strength, hypertrophy, power, endurance, protein synthesis, and bone mineral density (3, 5, 28). The magnitude of these adaptations is influenced by RT experience. For example, In a review of over 100 studies, mean muscular strength increased approximately 40% in untrained, 20% in moderately trained, 16% in trained, and 2% in elite participants over training periods ranging from 4 weeks to 2 years (28). Hypertrophy induced by RT is vital for 1) its positive relationship to force production and performance, 2) appearance (i.e., bodybuilding), and 3) increases in lean body mass that may increase total energy expenditure (28).

It is widely understood that resistance training can increase the size of the exercised muscle. Whole-muscle hypertrophy can result from either hyperplasia (increase in fiber numbers) or hypertrophy (enlargement of existing fibers) (31).

Hyperplasia occurs through activation and proliferation of myogenic progenitor cells, also called satellite cells, after muscle fiber damage. These cells are normally quiescent myoblasts located on the periphery of adult muscle fibers (32-34). They then migrate to the site of extensively damaged fibers where they differentiate and fuse together to form a new muscle fiber. The myonuclear domain theory states that a single nucleus can only manage a certain sarcoplasmic volume and that any increases in fiber cross-sectional area require a proportional increase in nuclei. Evidence credits fusion of satellite cells with damaged fibers as the reason for the increase in nuclei with hypertrophy (32). While satellite cells exhibit enormous potential in muscle regeneration, the contribution of fiber hyperplasia is likely small (<5%) (31).

Hypertrophy is credited as the major contributor to muscle repair. Two types of muscular hypertrophy exist: sarcoplasmic and myofibrillar. Fiber sarcoplasmic hypertrophy involves growth of the sarcoplasm and non-contractile material not directly contributive of fiber force production. Cross-sectional area of the fiber increases but with no associated increase in strength (31). Myofibrillar hypertrophy involves enlargement of the fiber due to more myofibrils. RT can stimulate synthesis of contractile proteins (specifically actin and myosin) of the sarcomere, although the magnitude of this effect is partly dependent on amino acid availability and blood flow (31, 35). After a bout of heavy RT, the rate of protein synthesis in human skeletal muscle fibers rapidly increases, more than doubles at 24 hours post-exercise, and declines rapidly to near baseline after 36 hours (36). Myofibrillar hypertrophy then includes linkage of these proteins

into new overlapping contractile filaments. This type of hypertrophy increases both filament density and force production of the muscle fiber (31, 37).

Different training programs induce these two types of hypertrophy in various proportions. Mostly myofibrillar hypertrophy occurs in elite weightlifters while sarcoplasmic hypertrophy typically occurs among bodybuilders. While some lifters train at high intensities primarily for body weight gains, most athletes aim to achieve myofibrillar hypertrophy, in order to gain maximal increases in contractile filament density and muscle force production (31).

Safe and proper prescription of resistance training has been shown to improve the circulatory, endocrine, and neuromuscular systems as well. Some benefits include: decreases in fat mass, blood pressure, cardiovascular demands to exercise, and risk of type 2 diabetes mellitus and breast and colon cancer; increases in basal metabolic rate and number and synchronicity of motor unit recruitment; improvements in blood lipid profiles, glucose tolerance, and insulin sensitivity; and maintenance of long-term independence and functional capacity (2, 5, 28-30).

Non-resistance training adults lose approximately 1.01 lb (0.46 kg) of muscle annually after 50 years of age and experience a 50% reduction in type II muscle fibers (responsible for highest levels of strength) by 80 years of age (2). RT can make the elderly more functional and independent, the untrained leaner and healthier, and the trained more athletic and powerful.

Prescriptions for Resistance Training

Several resistance training prescriptions have been recommended for the general public. For patients without cardiovascular disease (CVD), the American Heart Association recommends a resistance training regimen for disease prevention (such as CVD) including 8 to 10 exercises (preferably machines) exercising the major muscle groups of the body. This

organization recommends that resistance should be set at 30-40% of 1 repetition maximum (the greatest weight able to lift only once; abbreviated 1RM) for upper body exercises and 50-60% of 1RM for lower body exercises. One set of 8 to 12 repetitions for each exercise is recommended and workouts should be performed 2-3 days weekly (38). The primary goal for this exercise prescription by AHA is prevention in chronic diseases. For additional muscular strength and hypertrophy gains, training programs designed for the generally healthy public (especially athletes or experienced resistance trainers) incorporate higher volumes, intensities, and frequencies, as these lifters often integrate more sets, heavier weights, and exercise 4-6 times per week.

For healthy adult beginners, the ACSM recommends the use of both single- and multiple-joint exercises (starting with machines and progressing to free weights with advanced training experience) involving the body's major muscle groups two to three times weekly (39). For volume, one to three sets per exercise with 60-80% of 1RM for 8-12 repetitions is recommended with 1-2 minute rest intervals; repetitions are to be completed with slow to moderate lifting velocity (39).

Some controversy exists as to optimal number of sets that should be performed. In a study comparing volumes of one set versus three sets, training with one set of contractions produced significantly smaller strength increases than training with three sets of contractions. This study suggests that in the early phases of RT three sets of each resistance exercise may generate greater strength gains than single sets (40). Additionally, for individuals aiming for maximum increases in muscular strength, lean body mass, and athletic performance, periodized multiple-set programs with advanced exercises may be more beneficial (4). Periodization incorporates progressive variation of volume, intensity, and other parts of the RT regimen in

attempt to maximize force production and has been utilized by some for both recreational and rehabilitative training (39). Kraemer et al (41) and Feigenbaum et al (42) also prescribe extremely similar (nearly identical) RT regimens for healthy, untrained adults for performance benefits. Recommended RT programs have been shown to provide health benefits; however, intensities and volumes within a program design may be altered in attempt to increase hypertrophy and strength (39).

Muscle Damage

Introduction

The tensions involved in resistance training can cause damage (commonly referred to as "injury") to the muscle fibers involved. This damage can be characterized by loss of muscle function, altered morphology at microscopic levels, altered intracellular protein levels, and loss of intracellular muscle proteins to the surrounding environment (43). Muscle injury can be classified into three types: type I injury includes muscle soreness occurring 24 to 48 hours following unusually difficult exercise; type II injury involves a severe immobilizing pain from a muscle tear, spanning from a few fibers torn to a complete tear of the muscle; and type III is muscle soreness or cramps that occur during or immediately after exercise (44). For the purpose of this literature review, type I muscle damage is the focus; this can be estimated by the loss of muscle function caused by the physical disruption of muscle structures involved in producing or transmitting force (43). Most researchers agree that the reduction in function is related to micro-injury to skeletal muscle fibers and extracellular matrix (44).

Causes of Muscle Damage

Skeletal muscle damage resulting from resistance exercise can be divided into two possible pathways: metabolic and mechanical. The mechanical hypothesis suggests that damage occurs as a direct consequence of the mechanical tension put on myofibers (9). Eccentric muscular contractions are capable of producing more force than isometric and concentric contractions (9, 44, 45). Eccentric lengthening of sarcomeres results in stretched myofilaments, so much so that many no longer overlap within the sarcomere. As a result of this reduction in myofilament overlap, passive structures (desmin, synemin, and titin) of the sarcomere absorb the additional tension and Z-line streaming ensues. The high tension placed upon these passive filaments from repetitive eccentric contractions may cause failure of the overall muscle structure and is evident through reductions in the muscle's maximal force production (9). Also, disruption of sarcomeres can alter the excitation-contraction (E-C) coupling system, which can interfere with ATP generation. The muscle fiber structural damage initiates a process of fiber degeneration and subsequent regeneration (45).

Another theory states that, metabolic changes from resistance training may cause sarcomere disruption. Ischemia and/or hypoxia during resistance exercise may disturb ion concentrations, generate waste accumulations, and cause an ATP deficiency within the muscle fiber (9). These results can potentially disrupt calcium metabolism and stimulate proteolytic pathways. Specifically, inhibition of the Ca^{++}-ATPase in the sarcoplasmic reticulum and sarcolemma occurs, causing elevated cytosolic concentrations of Ca^{++} and activation of several degradative pathways—including the Ca^{++}-dependent proteolytic and phospholipolytic pathways. These pathways degrade structural and contractile proteins and the sarcolemma (9,

46). Thus, damage to muscle fibers occurs from either mechanical or metabolic stress, or likely some combination of both factors.

Effects of Muscle Damage

Muscle damage resulting from resistance exercise can entail physical changes to both inside and outside of the affected muscle fiber. After intense eccentric exercise, histochemical and electron microscope examinations of human muscle tissue show damage or changes to the sarcomeres, myofibrils, myofilaments, sarcolemma, mitochondria, T-tubules, and cytoskeleton (10, 43, 47-51). The muscle experiences excessive strain during lengthening contractions—more tension is placed on passive structures and less on the active. This results in extensive myofibrillar disruptions such as Z-line streaming, loss of thick myofilaments, and disturbed arrangement of myofilaments at the A-band (10, 52). It has been reported that fast glycolytic muscle fibers are more susceptible to damage induced by eccentric contractions than are slow oxidative fibers. This is likely in part due to the low oxidative capacity of fast glycolytic fibers which predisposes them to intense eccentric contractions through depletion of high energy phosphate molecules and subsequently disengagement of actin-myosin connections in the rigor state (43). Slow muscle fibers are also thought to contain higher levels of cytoskeletal and "heat shock" proteins than fast fibers, which aid in structural support and protection from stress (43). Myofibrillar damage is more extensive in days following the eccentric exercise than immediately after the exercise, suggesting additional delayed damage from metabolic or inflammatory processes (10).

Outside the muscle fiber, extracellular matrix (ECM) expansion is evident in damaged muscle tissue and may contain collagens, fibronectins, proteoglycans, and glycoproteins suggesting damage to connective tissue as well (44, 53). Adaptation of collagen, the major

adhesion-promoting component of muscle ECM, has been shown to be associated with mechanical loading; evidence has shown attenuation of contraction-induced muscle damage is associated with increased muscle collagen content in rats (54). An ECM protein, tenascin C, has been reported to dramatically respond to unaccustomed exercise and to be essential in muscle damage repair and possibly a role in the regeneration process (54). The ECM also contains growth factors such as insulin-like growth factors (IGFs) that are activated after onset of muscle damage and have been shown to aid in muscle regeneration and adaptation (54, 55). The ECM of the perimysium and endomysium has only recently begun investigation and exhibits great potential in overall muscle metabolism.

The muscle typically begins repair within days after onset of damage but is not completely regenerated for 2 to 3 weeks after the single bout (53). The direct mechanical force is thought to be the factor that initiates this damage, which is exacerbated later indirectly by metabolic factors (53).

Delayed onset muscle soreness (DOMS) is a symptom of muscle damage induced by unaccustomed, strenuous resistance exercise with an eccentric component. DOMS encompasses physical pain in the exercised muscles beginning at about 24 hours and disappearing at about 3-4 days following the bout (10, 56). Disruption of muscle fibers and changes in peripheral nociceptor sensitivities are thought to be greatly responsible for the perception of pain that is experienced during DOMS (56). Specifically, the concentrations of molecules such as prostaglandins are increased after high-intensity activity and sensitize muscle nociceptors. This lowers the threshold for stimulation as a result, and pain sensations from the damaged muscle tissue to the central nervous system increase (10). Also, DOMS seems to cause the central nervous system to be more susceptible to muscular pain (56). A consequence of DOMS is the

reduced ability of the exercised muscles to contract optimally as a result of this pain, damage, and inflammation induced by damaging eccentric exercise. This sometimes extreme discomfort may dissuade individuals from continuing or adhering to a new exercise program. If recovery-related pain could be alleviated by reducing the DOMS response following unaccustomed training bouts, it is possible that tolerance of soreness and compliance of the exercise regimen would increase.

Assessments of Muscle Damage

Direct assessment of muscle damage proves to be challenging as it only possible in humans through either muscle biopsies or magnetic resonance imaging (MRI). Biopsies provide only a small sample and since the entire muscle is not damaged evenly throughout, estimating the amount of damage can be difficult (10). MRIs can assess damage throughout the entire muscle non-invasively. However, MRI machines are extremely expensive and the examination time for one subject can be upwards of 60 minutes each visit. Due to these challenges, investigators commonly use indirect measures to assess damage.

Indirect assessments of muscle damage include measures of DOMS, muscle function, and several blood markers. Sensations of DOMS are subjective but can be used to diagnose severity of muscle damage through implementation of a visual analog scale (ie., 100 mm line, 0 = no pain, 100 = extremely painful) after palpating, flexing, and/or stretching of the exercised muscle. Another indicator includes using a dynamometer to test subjects' ISO, which have been shown to decrease as much as 50-60% from baseline in days after intense eccentric resistance exercise and not fully return to baseline for 2-3 weeks (44). Decreases in ROM (up to 20° in elbow flexors) and increases in limb circumference (as much as 3-5% in knee extensors) are also often observed

after muscle damaging exercise (44, 57), likely results of tightness and swelling of the exercised muscle.

One blood marker often utilized to quantify muscle damage is the analysis of creatine kinase (CK) in blood. After a muscle has been damaged, the sarcolemmal disruption allows muscle proteins such as CK to be released from the fiber into the blood stream (58, 59). Therefore, plasma CK concentrations reach abnormally high levels after damaging exercise, indicating muscle breakdown. Assessment of muscle damage through MRI corresponds with circulating CK activity for the majority of subjects (r=0.90-0.94); thus, individuals who exhibit the greatest muscle damage will generally have the highest blood CK activity (60). However, in that same study, peak CK activities for ten male subjects ranged from 236-25,244 U/L (60). Intersubject variability in the responses is one of the drawbacks for implementation of this measure (10, 58).

Magnitude and Time Course of Creatine Kinase

The time course of peak CK activity depends on age, gender, race, muscle mass, and the type of exercise being performed (58). After downhill running, for instance, CK typically peaks at 12-24 hours post-exercise and with magnitudes ranging from 100-600 IU (61, 62). On the contrary, after intense resistance-induced eccentric exercise, CK activity peaks later after 2-6 days and at magnitudes reaching 2,000-10,000 IU (10, 58, 63, 64). However, subjects performing intense squat training demonstrated lower and earlier peak CK levels at 6-24 hours post-exercise (65-67). Furthermore, it appears that the longer time course to peak CK activity correlates with a larger increase in CK activity (10), which explains why less damaging downhill running results in peaks earlier than more damaging eccentric exercise. As a result, CK activity is likely more dependent upon exercise intensity than duration or volume (68).

Magnitude and Time Course for Consequences of Muscle Damage

Magnitudes and time courses of muscle functional change vary depending upon intensities and types of exercise involved. Concentric resistance training protocols are normally associated with 10-30% strength loss immediately post-exercise, returning to baseline within a few hours, whereas high-force eccentric resistance training produces the highest degrees of strength loss up to 50-65% compared to pre-exercise values, lasting 7-14 days post-exercise (10). Studies have shown that peaks in CK activity after intense eccentric exercise of the elbow flexors range from 2,000-10,000 U/L and occur about 4 days post-exercise (69-71). Muscle soreness begins hours after exercise and tends to peak 1-2 days post-exercise (10). Maximal eccentric contractions usually produce soreness values of 7-8 on a scale from 1-10. The differences in soreness values have been shown to be consistent with the differences in strength loss and increases in CK activity (10).

Different time courses and magnitudes of changes occur between eccentric exercise-induced muscle damage in the arm and in the leg. Although the elbow and knee are two different types of joints, they flex and extend about one axis, which is typically easier to control than multi-axial joint movements. An analysis of variance was used on CK data from over 80 studies and indicated that elbow flexor injury models yielded a mean blood CK value of 5,440±850 U/L that was five times higher than the 1080±370 U/L mean value induced by the knee extensors. Furthermore, a t test was used with over 60 studies comparing isometric strength loss data between these two muscle groups. Average strength loss from the elbow flexor studies (40% ± 2%) was significantly greater than that observed for the knee extensor studies (27% ± 3%) (72). Although the knee extensors have a considerably greater mass than that of the elbow flexors, these findings suggest that the elbow flexors are substantially more injury-prone. A

likely explanation for this finding is that the legs are used for locomotion and have acquired a protective effect from muscle damage significantly greater than that of the arms. This explains why eccentric exercise of the arm causes greater strength reductions and higher blood CK activity than that of the leg (72, 73).

Table 1: Time Course of Changes After Maximal Eccentric Exercise

				Inflammation	
	Soreness	**CK**	**Strength**	**Acute**	**Chronic**
Exercise Stimulus			↓↓↓	↑	
1-12h post-exercise			↓↓↓	↑↑↑	
24h post-exercise	↑↑↑	↑	↓↓↓	↑	
48h post-exercise	↑↑↑	↑	↓↓		↑
3-5d post-exercise	↑	↑↑↑	↓		↑↑
5-7d post-exercise	↑	↑↑	↓		↑↑↑
7+d post-exercise		↑↑	↓		↑↑

↑ = minor increase/decrease; ↑↑ = moderate increase/decrease; ↑↑↑ = large increase/decrease.

SOR = soreness; CK = creatine kinase; STR = strength.

Modified from the source: Clarkson et al(10)

Repeated Bout Effect

Once an individual experiences DOMS after unaccustomed exercise, the same exercise is no longer unaccustomed. The individual can perform similar exercise a few days after and experience less or no muscle soreness or damage. This protective effect against muscle damage is termed the "repeated bout effect" and can last from several weeks to several months (74).

Factors influencing the magnitude of this effect include time between bouts, number of eccentric contractions, muscle length, and exercise mode (75). Although the exact mechanism for the repeated bout effect has yet to be determined, a few theories exist including the neural, connective tissue, and cellular theories.

The neural theory states that eccentric contractions preferentially recruit fast-twitch motor units and that muscle damage results from high stress on a small number of active fibers. Supporters hypothesize that a neural adaptation would alter motor unit activity (ie., increase synchronicity, increase recruitment of slow-twitch fibers) and distribute the stresses among fibers more evenly, subsequently reducing myofibrillar disruption (74).

The mechanical theory states the exercise-induced muscle damage begins with a mechanical disruption of myofibrils. Increases in both dynamic (active muscles) and passive (relaxed muscles) stiffness have been observed following eccentric training. Cytoskeletal proteins desmin and titin (responsible for orientation of sarcomeres) are viewed using an electron microscope from perfect, parallel alignment in healthy muscle fibers to disrupted alignments in damaged fibers. As a result, an adaptation to the cytoskeleton would seem like the first line of defense against further damage. A protective effect has also been attributed to intramuscular connective tissue for its ability to lessen myofibrillar stresses (74).

Lastly, the cellular theory involves adaptations in the fiber itself and in the inflammatory response to eccentric contractions. The sarcomere strain theory suggests that contractions performed at longer muscle lengths cause muscle damage due to irreversible sarcomere strain, resulting in a longitudinal increase in sarcomere numbers to reduce sarcomere strain and myofibrillar disruption. Because impaired excitation-contraction (E-C) coupling has accounted for 50-75% of strength loss within 5 days of a damaging eccentric bout, adaptations in the

sarcoplasmic reticulum and E-C coupling process may help explain the reduced strength loss following a repeated bout. An adapted and more efficient inflammatory response (explained in a later section) is also credited as a part in muscle adaptation and acquiring a repeated bout effect (74).

While a consensus theory explaining the mechanism has yet to be developed, it is likely that a combination of these theories is responsible for this phenomenon (11). The repeated bout effect is advantageous as it results in faster recovery of muscle strength and range of motion, reduced swelling and muscle soreness, and smaller increases in muscle proteins in the blood following a subsequent bout of resistance exercise (75).

This adaptation from one damaging exercise bout can be evident during the second bout, as early as 3-4 days after the initial session (76, 77). Chen (76) even showed that performing a second bout of exercise 3 days after the initial does not produce further damage or slow recovery, even if the intensity of the second bout is greater. Nosaka et al (64) found that indicators of muscle damage were either less or non-existent after a second bout of eccentric exercise, even 6 to 10 weeks after the first bout. Lastly, results from Clarkson et al (78) suggest that a damage resistant adaptation occurs and any damage that does arise is repaired at a faster rate.

Although adaptation may be present after even the initial unaccustomed exercise session, the repeated bout effect is often most noticeable after multiple bouts. On average, CK activity, maximal isometric force, and soreness tended to return to baseline levels more rapidly after the second and third bouts (70, 79). After these values returned to baseline, they remained steady or even improved slightly, providing evidence for the adaptations for muscle protection and increased strength.

The most damage from a full body resistance training program is likely in the initial workouts as described above. However, there has been limited research to determine the possible effect of more than several bouts on muscle damage if a RT program (ie., 3 d/wk) is initiated immediately after the damaging bout. It would be valuable to know whether continued RT exacerbates the muscle damage or if recovery from the initial damaging bout continues regardless. Jowko et al (80) prescribed a full-body RT regimen consisting of both concentric and eccentric exercises for three weeks, thrice weekly. A significant increase (43 lbs accumulative) in maximum muscle strength for all exercises was observed. Serum CK activity significantly increased from baseline (200 U/L) to week 2 (1100 U/L) but reduced to 400 U/L by the end of the third week. Nissen et al (81) observed even greater CK increases in response to two weeks of RT two to three times per week. Serum CK increased from 245 U/L at baseline to 15,868 U/L at week one to 1,408 U/L at week 2 to 666 U/L at week 3. Two studies that measured serum CK only at baseline and after 4 weeks of a RT program noted only moderate increases in CK activity (332±88 to 429±93 U/L and 205.3±36.2 to 236.7±31.5 U/L) (82, 83). These data suggest that the signs of skeletal muscle damage from the initial resistance exercise bouts increase through at least one to two weeks and drop to near baseline by four weeks after training initiation. The complete time course of this response, however, is not clear since these training studies had wide gaps between blood measures. In other words, it is unclear whether the acute, short-term increase in serum CK after the first exercise bouts was higher or lower than that after 7-14 days since that was not measured.

II. Inflammation

Components and Process

Inflammation is the human body's natural response to injury or infection and an essential component of the recovery process. The innate immune system, also known as the non-specific immune system, is the first line of defense against foreign pathogens and tissue damage. Cells of this immune system, including phagocytes, macrophages, and neutrophils, recognize non-self microbials or missing tissues and remove apoptotic cells (84-86). If necessary, the innate immune system can activate the adaptive immune system, also called the specific immune system. This immune system is comprised of highly specialized, systemic lymphocytes (ie., B and T cells) that generate immunity through recognition and remembrance of specific pathogens, followed by elimination of them (84). It is thought that innate immunity responds to acute muscle damage while a combination of innate and adaptive immunity repairs chronic muscle damage.

Cytokines are a group of regulatory proteins secreted by white blood cells and other body cells in response to stimuli such as mechanical stresses, reactive oxygen products, and stress hormones (87, 88). As a result, these molecules can be measured to gauge the magnitude of an inflammatory response. One of the cytokines, IL-6, is substantially elevated (notably more than others) after exercise and generally peaks 0.5-2 hours after (89). Although it was thought that increased IL-6 concentrations after muscle damage were derived from the immune system only, it is now thought that the contracting skeletal muscle is also a source of the circulating IL-6 post-exercise (90). This cytokine has been shown to produce both acute pro-inflammatory and anti-inflammatory effects. For pro-inflammation, IL-6 can signal transcription of CRP (87, 91, 92) and can up-regulate gene expression of TNFα (93). Regarding anti-inflammation, IL-6 has been

shown to increase levels of interleukin-1 receptor antagonist (IL-1ra), which blocks IL-1 receptors and prevents IL-1 from inducing its pro-inflammatory effects (90). Chronic elevations of IL-6, however, seem to be detrimental and associated with higher incidence of type II diabetes mellitus, cardiovascular disease, sarcopenia, and chronic inflammation (91, 94-96). As a result, the literature suggests that IL-6 should be controlled for optimal balance and overall health.

CRP, an acute phase protein, is an important blood marker of inflammation often monitored in clinical trials. This acute-phase protein is a reliable indicator of systemic inflammation whose blood concentrations increase from less than 0.001 mg/mL to upper limits of 6-10 mg/mL during the acute-phase response (97, 98). IL-6 and IL-1 are the major stimuli that induce CRP synthesis (97). CRP levels generally reflect the circulating IL-6 levels and correlate with inflammation. Furthermore, CRP concentrations rise and fall more suddenly than most acute-phase proteins. This establishes CRP as a useful long-term marker of inflammation to follow clinical response to treatments (97).

Evidence that Resistance Training Induces Inflammation

Muscle damage from an acute bout can induce an inflammatory process. Many studies have observed that heavy RT increases the common cytokine interleukin-6 (IL-6) and others (IL-10, IL-1β and IL-1ra), usually occurring between 2 and 48 hours post-exercise (57, 99, 100). MacIntyre et al (99) also noted an increase in neutrophils in the exercised muscle—suggesting a relationship between damage to the fiber proteins and inflammation.

The intensity and characteristics of the resistance exercise protocol are likely to influence the individual cytokine responses (101). If exercise is intense enough to initiate an inflammatory response, pro-inflammatory cytokines (tumor necrosis factor-alpha {TNFα}, IL-6, etc.) are

released first, followed by anti-inflammatory cytokines (IL-1ra, etc.). Investigation into cytokine activity after high intensity/short duration exercise, anaerobic exercise, and concentric and eccentric exercise has grown, and it is known that this inflammatory response varies with the type of exercise performed (102).

Several studies have examined the association between exercise and inflammation. Some incorporated 45 minutes of downhill running and observed significant increases in peak mRNA expression of IL-6 (~10%) and IL-8 (~10%), TNFα (2.8-fold) and IL-6 (3.6-fold) transcripts, muscle IL-1β (2.5-fold), and leukocyte (10.1-fold) and neutrophil (2-fold) infiltration (103-105). Buford et al (106) observed a significant increase (~10%) of IL-6 mRNA expression after 3 sets x 10 repetitions at 80% 1RM on three lower body exercises. Smith et al (107) measured cytokine activity after 4 sets of 12 repetitions at 100% 1RM on eccentric-phase bench press and leg curl. They observed that IL-6 significantly increased from baseline to 12 hours (~1.4-fold), 24 hours (~2.7-fold), and 72 hours (~2.4-fold) post-exercise; a trending increase was seen at 48 hours as well.

Peake et al (108) tested cytokine levels in untrained subjects after training with submaximal (10% maximal isometric force, MIF) eccentric contractions of the elbow flexors of one arm and then maximal (100% MIF) eccentric contractions of the elbow flexors of the opposite arm no less than two weeks later. Serum IL-6 concentrations significantly increased ($p<0.05$) for both trials (between 0.5 and 1.0 pg/mL), while the submaximal trial also showed an interaction effect ($p=0.044$) and the maximal trial did not. In other words, the IL-6 response to exercise was reduced in the second arm following submaximal isometric contraction suggesting a second bout effect even when different limbs are used (108). Bilateral deficit and repeated bout effect may possibly have lessened the inflammatory response for the maximal trial.

Uchida et al (109) observed significant increases ($p<0.05$) in serum prostaglandin E_2 (PGE_2)concentrations in four different groups of bench pressing with varying intensities (50%, 75%, 90%, and 110% of 1RM), with a significantly greater increase in the 110% of 1RM group. After an acute bout of resistance training (3 sets x 10 reps at 80% 1RM on machine squat, leg press, and leg extension exercises), Buford et al (106) found significant increases in skeletal muscle mRNA expression of IL-6 ($p<0.001$) and TNFα ($p=0.007$), but no significant changes in serum concentrations of these cytokines were observed.

Antagonistic effects have also been discovered, however. Hirose et al (110) found significant decreases ($p<0.05$) in plasma TNFα concentrations (~15%) after two bouts of eccentric exercise of the elbow flexors separated by four weeks. It appears intense resistance exercise, especially when unaccustomed, evokes an inflammatory response evident in both muscle tissue and the bloodstream; however, further investigation is necessary in identifying specific time courses and magnitudes of cytokine concentrations after resistance exercise.

While acute, unaccustomed exercise can increase inflammation, regular resistance training may lessen the inflammatory response and indirectly reduce the risk of inflammatory diseases including cardiovascular disease and type II diabetes mellitus (95). Long-term RT appears to reduce inflammation, but this is based on limited research and requires confirmation. Stewart et al (111) tested CRP levels in both young and old physically inactive people during 12 weeks of both RT and aerobic exercise thrice weekly. Researchers found that both young and old physically inactive people experienced significant decreases ($p<0.01$) in CRP concentrations (~58% combined) from pre-training to after 12 weeks. Physically inactive people of all ages also showed a trend to have increased baseline serum CRP concentrations compared to active, suggesting the incorporation of aerobic and resistance training combined has more potential to

lessen systemic inflammation in untrained people than trained. These data suggest that regular, prolonged RT has the capabilities of reducing levels of pro-inflammatory cytokines in healthy adults.

Chronic resistance training can increase an anti-inflammatory cytokine, IL-1ra (100, 101, 112). Izquierdo et al (100) reported significant increases ($p<0.05$) in serum IL-1ra concentrations (from ~50 to ~60 ng/mL) immediately post-exercise throughout 7 weeks of bi-weekly RT. Nieman et al (112) put strength-trained athletes through a RT workout and observed modest but significantly increased levels ($p<0.05$) of plasma IL-1ra concentrations (197±32 pg/mL pre-exercise to 226±22 pg/mL post-exercise to 209±21 pg/mL 1 hr post-exercise). Although this remains a topic with limited research available, RT seems to improve inflammation by dampening pro-inflammatory cytokines (i.e., IL-6) and possibly increasing anti-inflammatory cytokines (i.e., IL-1ra). The specific time course for these changes has not been clarified from baseline to after multiple weeks of training.

According to Gleeson et al (113), chronic periods of heavy training can depress both innate immunity and the ability to adapt—and thus possibly reduce performance. The implications of this exercise-induced immune dysfunction as well as the definition of overly heavy training are unclear. However, it suggests that an individual might experience negative consequences on immunity and inflammation from excessive RT. In contrast, after a period of tapering or deloading (training at lower intensities and volumes), immune function and performance both improve.

Acute and Chronic Inflammatory Responses to Resistance Training

After muscle damaging exercise, a sequence of interactions between muscle and immune cells ensues. A T helper 1 (Th1) inflammatory response begins as neutrophils and classically activated M1 macrophages invade the damaged muscle roughly 2 hours after initial injury and reach peak concentrations 2-4 days after (86, 114-118). The combined action of these neutrophils and macrophages promotes the increase of pro-inflammatory cytokines including TNFα and IL-6, which, in addition to CRP produced and secreted by the liver, communicate and regulate with immune cells to cause further damage to myofibers (116, 118). After 2 to 4 days post-initial muscle damage, the first wave of classically activated M1 macrophages is replaced by a T helper 2 (Th2) inflammatory response and a second wave of alternatively activated M2 macrophages, which are responsible for attenuation of the inflammatory response (114, 116, 118). These macrophages secrete anti-inflammatory cytokines including IL-1ra, IL-4, IL-10, and IL-13 that stabilize the present inflammation and help promote tissue growth and regeneration (89, 90, 116, 118). Absence or lack of these anti-inflammatory cytokines can slow muscle growth post-exercise and inhibit muscle differentiation and regeneration (116).

Although differences in inflammation between acute and chronic resistance exercise have not been thoroughly studied, some literature exists. According to Tidball et al (116), chronic muscle damage causes an altered response of macrophage invasion and function that differs from acute damage. For example, mice with *mdx* muscular dystrophy (extremely similar to Duchenne's muscular dystrophy in humans) have mutations of the dystrophin gene, which result in progressive muscle wasting, necrosis, and ultimately high levels of inflammation; this disease can be used to simulate chronic muscle damage. While these mice experience a relatively normal initial invasion of classically activated M1 macrophages, a second wave of alternatively

activated M2a macrophages invades earlier in the process and overlaps the first wave for a longer period of time. Thus, because the M1 macrophages are not the predominant phagocytes in the muscle as long as compared to the acute injury model, the cytotoxicity of M1 macrophages is reduced and excess damage due to inflammation is decreased. Additionally, it is thought that chronic muscle damage involves not only an innate immune response but also an adaptive (specific) immune response involving various types of T cells (116). Furthermore, McHugh et al (11) note that cellular adaptations (strengthening of sarcolemma, sarcoplasmic reticulum, and sarcomere) following the initial resistance bout(s) account for reduced muscle fiber damage and subsequently decreased inflammation, thus reducing cytokine and immune response. Further research is necessary to strengthen this area of knowledge.

Negative Implications of Inflammation on Muscle

Recent literature suggests a cause and effect relationship between pro-inflammatory cytokines and muscle atrophy, although it is unknown if these effects are direct or indirect. For example, studies have observed reductions in muscle protein synthesis (or total net loss) during extended periods of high inflammation (TNFα, IL-1, and/or IL-6 as markers) (119-121). Signaling cascades (ie., extracellular response kinases, mammalian target of rapamycin) and insulin-like growth factors (ie., IGF-1) are largely responsible for hypertrophic increases in human skeletal muscle through regulating protein synthesis, mobilization of satellite cells, and entrance into the cell cycle (120). Prolonged and high levels of these cytokines decrease the activity of these hypertrophic signaling cascades by disrupting phosphorylation of important proteins involved in these pathways. Excess cytokine levels also reduce the anabolic actions of IGF-1 through increasing resistance of growth hormone (119, 120). The reduced anabolic

response and muscle protein net loss hinders the synthesis of myofibrillar and sarcoplasmic proteins, ultimately hindering muscle hypertrophy and strength (119, 120). Literature suggests that chronic periods of high inflammation should be reduced to prevent hindrances in muscular hypertrophy and strength and to optimize healthy function of muscle fibers.

Chronic inflammation is thought to be associated with muscle loss in aged individuals or those with diseases such as cancer (119). Sarcopenia, often related to aging and poor lifestyle decisions, is characterized by low muscle mass, low muscle strength, and decreased physical performance (122, 123). Factors influencing sarcopenia include loss of alpha-motor neuron input, changes in anabolic hormones, decreased dietary protein intake, and reduced physical activity. Additionally, research suggests that sarcopenia is at least partially a chronic inflammatory state controlled by cytokines and oxidative stress (123, 124). Specifically, IL-6, TNFα, and IL-1β significantly influence this muscle wasting condition (125).

Visser et al (126) observed that healthy older humans with higher plasma concentrations of IL-6 and TNFα had lower muscle mass (3.3% to 6.5% less) and muscle strength (5.5% to 8.8% less) compared to elderly with low cytokine or acute-phase protein levels. Schaap et al (96) showed that high levels of IL-6 (>5 pg/mL) and CRP (>6.1 μg/mL) were associated with a 2- to 3-fold greater risk of losing at least 40% of muscle strength over 3 years. Logically, reductions in these pro-inflammatory proteins would likely result in muscular improvements (or at least lack of muscular impairments) including increased protein synthesis rates, greater muscle mass, and greater strength, as well as overall positive health development. This hypothesis is supported by the study by Trappe et al (127) that demonstrated superior gains in muscle size and strength over a 12 week RT program in older subjects who consumed COX-inhibiting drugs each day compared to placebo.

The evidence for beneficial effect of non-steroidal anti-inflammatory drugs on muscle strength and hypertrophy, soreness, and inflammation has been seen in some studies (127-129) but not others (23, 130). Adverse muscular effects have been observed in some cases, such as suppression in protein synthesis response, increased CK activity, reductions in satellite cells (13, 24, 131). Some evidence also shows other adverse effects such as GI distress, renal and liver injury, and heart failure (12, 13).

Evidence That Diet Can Influence Inflammatory Response to Exercise

The pain associated with DOMS after intense exercise has caused investigators to search for ways to reduce this discomfort. Some dietary changes and nutritional supplements have been shown to affect the body's inflammatory response following exercise and subsequently additional muscle damage. Some of these supplements demonstrating reductions in DOMS or inflammation include vitamins C and E, flavonoids, carnitine, β-hydroxy-β-methylbutyrate, creatine, branched-chain amino acids, and essential fatty acids (132).

Howatson et al (9) reports that long-term supplementation of anti-oxidants (vitamins C or E) or β-hydroxy-β-methylbutyrate (HMB) or co-ingestion of protein and carbohydrates can reduce muscle damage following exercise. A combination of tocopherols, flavonoids, and DHA was observed to reduce elevations in post-exercise inflammation through IL-6 (medians (IQR): 7.1±22.7 placebo versus 0.0±7.2 treatment) and CRP (medians (IQR): 0.39±0.95 placebo versus -0.004±0.66 treatment) (133). Creatine supplementation reduced elevations in TNFα (64%) and PGE_2 (91%) (134). In addition, some specific foods have been evaluated for an effect on muscle damage or inflammation. For example, sweet cherries were found to reduce CRP activity (25%) in healthy adults at rest and to decrease strength loss (20±16% placebo versus 5±18% treatment)

and pain perception (3.2±1.1 placebo versus 2.4±0.7 treatment) in college males following eccentric exercise (135, 136).

Although many studies show certain diets and supplements can influence the muscle damage or inflammatory response, this trend has not been entirely consistent. Some studies showed dissimilar and even detrimental results (137, 138). For instance, vitamin C has been shown to have no effect or even increase CK activity post-exercise (138, 139). Rawson et al (67) observed no reduction of skeletal muscle damage or enhancement of recovery after resistance exercise with creatine supplementation. Prolonged, large doses of vitamin E (α-tocopherol) were found to have no effect on plasma homocysteine and cortisol concentrations after exhaustive exercise (140). While unaccustomed exercise is bound to produce inflammation, it is possible that the response can be modified by the ingestion of certain nutrients or supplements.

III. Omega-3 Fatty Acids

Introduction

Omega-3 fatty acids are polyunsaturated fatty acids capable of alleviating inflammation. α-Linolenic acid (ALA), the smallest n-3 fatty acid, is found in rapeseed, walnuts, and flaxseed. The most important omega-3s to health, however, are the longest chain molecules in the family, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two fatty acids are produced by algae and commonly found in coldwater fish, in addition to lesser sources such as grass-fed beef and free range eggs. The metabolism of omega-3s produces cardio-protective eicosanoids (3-series thromboxanes and prostaglandins and 5-series leukotrienes) that reduce inflammation (26).

Conversely, omega-6 fatty acids produce inflammatory eicosanoids (2-series thromboxanes and prostaglandins and 4-series leukotrienes). Both omega-6 and omega-3 are considered essential fatty acids (EFAs) because mammals are unable to synthesize them and must consume them through diet. Both EFAs can be converted to longer chain fatty acids; linoleic acid (LA) can be converted into arachidonic acid (AA) and ALA into EPA and DHA (17).

Prior to the spread of agriculture 10,000 years ago, humans likely consumed omega-6 and omega-3 fatty acids in ratios of 1:1 or 2:1. However, this ratio has recently been disturbed by increased farming that has led to production of vegetable oils high in omega-6 fatty acids, ultimately resulting in Western diets with high omega-6:omega-3 ratios of 10:1 or 20:1 (17). A balanced intake of both classes of EFAs is critical for health, and guidelines for omega-3 fatty acid intake are similar for patients with CVD and for athletes. The AHA recommends 1,000 mg/d of EPA+DHA for patients with documented coronary heart disease and 2,000 to 4,000 mg/d EPA+DHA for individuals with hypertriglyceridemia (16). For healthy athletes at the leisure level, Simopoulos (18) recommends about 1,000 to 2,000 mg/d EPA+DHA at a ratio of 2:1 (EPA:DHA).

Connection of Omega-3 Fats to Inflammation

The anti-inflammatory effects of omega-3 fats may result from several different mechanisms. First, omega-3 and omega-6 fatty acids compete for COX and LOX enzymes. EPA and ARA compete for the production of anti- or pro-inflammatory eicosanoids, respectively, such as prostaglandins, thromboxanes, and leukotrienes. DHA can also act as a substrate for COX and LOX enzymes and be metabolized into neuroprotectins and resolvins,

which are mediators possessing anti-inflammatory and immunoregulatory characteristics (26). Secondly, omega-3 fatty acids are thought to influence cytokine signaling pathways via altering transcription factors. For example, omega-3s can bind and suppress NF-κβ, a transcription factor responsible for the production of several pro-inflammatory cytokines (TNFα, IL-6, IL-1β, etc.) which in part can cause chronic inflammatory diseases (26, 27, 141). Omega-3s can also bind and activate PPARs, transcription factors that regulate inflammatory signaling pathways. One of the actions of PPARs includes suppressing NF-κβ (26). Therefore, increased dietary omega-3 fatty acids can cause decreased circulatory inflammatory cytokines and increased production of anti-inflammatory eicosanoids, subsequently resulting in an inflammatory state of lesser magnitude overall.

In a study by Pischon et al (142), Americans with higher intakes of omega-3s reported lower levels of inflammatory markers. In the category with the highest intake of EPA+DHA (90-100th percentiles), soluble TNF receptor 1 levels were 14% lower in men and 6% lower in women than in the lowest quintile, while soluble TNF receptor 2 levels were 19% lower in men and 5% lower in women than the lowest quintile. Men in the 90-100th percentile range of omega-3 intake also had the lowest concentrations of IL-6 and CRP in comparison to all other percentiles. Significant inverse relationships have been observed between omega-3 ingestion and blood TNFα, IL-6, and CRP levels (143-146). Those individuals in the highest CRP tertile (>3.0 mg/L) showed significantly lower concentrations of total omega-3 fatty acids, EPA, and docosapentaenoic acid (DPA) in comparison to the other tertiles (146). Plasma DHA levels alone were inversely associated with both IL-6 and CRP (143). Additionally, strong positive correlations were found between plasma levels of TNFα, IL-6, and CRP and the ratio of omega-6 to omega-3 (143). Kelley et al (147) showed that 3,000 mg/d DHA supplementation for 90 days

decreased blood inflammatory markers CRP (15%) and IL-6 (23%) in hypertriglyceridemic men. Studies showed that cancer cells treated with omega-3s significantly decreased colon inflammation via lower activity of NF-κβ (148, 149). These studies show that omega-3s possess the ability to reduce inflammation through either competition with COX and LOX enzymes or inhibiting cytokine signaling pathways.

While the vast majority of evidence supports anti-inflammatory effects of omega-3 fats, limited data suggest a pro-inflammatory effect. Omega-3 fatty acids can induce inflammation by decreasing production of PGE_2, a TNFα suppressor. In addition, EPA and DHA may indirectly enhance TNFα and IL-6 expression. The increased TNFα and IL-6 production is inversely related to the PGE_2 concentration, suggesting that omega-3 fatty acids increase these pro-inflammatory mediators through regulation of PGE_2 (141, 150, 151). However, increased production of TNFα and IL-6 through decreased PGE_2 (pro-inflammatory effects) is much less in magnitude compared to the decreased production of TNFα and IL-6 through suppressed NF-κβ (anti-inflammatory effects). Thus, on balance, it is appropriate to classify omega-3 fatty acids as anti-inflammatory (or less pro-inflammatory).

Issues of Dose and Type of Fatty Acid Used in Interventions

In examining the effect of omega-3 fatty acid supplementation, studies have incorporated many different supplementation periods, dosages, and type of fatty acid(s). While a couple studies used only a 7-day supplementation period (133, 152), most studies used a supplementation period of 4 to 6 weeks (153-157) and others included longer periods of 12 or more weeks (158, 159). In terms of issued doses, some studies administered relatively smaller doses of 0.5 to 0.7g of DHA+EPA (154, 155). However, other studies assigned doses of

EPA+DHA upwards of 2,400 to 4,400 mg/d (153, 156-159). All of the studies examined both EPA and DHA, while some of these trials also included other smaller omega-3s. Most studies designed their supplementation interventions with EPA+DHA as the prime ingredient (133, 153, 155-158) but some included other non-omega-3 compounds, making conclusions as to the specific role of omega-3 fats in the outcome more difficult to discern (133, 154, 155).

Some studies measured increases in levels of either serum omega-3s (153, 154, 156-158) or cellular incorporation of omega-3s (160, 161) before and after the supplementation periods (153, 154, 156-158, 160, 161). The dose and duration (1800 mg/d of total omega-3s and 30 days) prescribed by Lenn et al (154) was successful at significantly increasing both serum EPA (475%) and DHA (425%) concentrations. Toft et al (160) supplemented male runners with 3,600 mg/d of omega-3s for 6 weeks prior to a marathon run and found that blood mononuclear cell (BMNC) fatty acid compositions of these omega-3s in the treatment group were significantly greater than the control group (12.4% vs. 8.4%, respectively) and showed less incorporation of AA. Similar results were seen by Nieman et al (157) who supplemented 2000 mg EPA + 400 mg DHA per day for 6 weeks and observed serum concentrations of EPA and DHA significantly increase (311% and 40%, respectively) in the treatment group. Lastly, Trebble et al (161) assigned subjects with EPA/DHA (in a 2:1, wt:wt) for a total of 12 weeks—300 mg/d total for the first four weeks, 1000 mg/d total for the second four weeks, and 2000 mg/d total for the last four weeks. Results indicated that EPA and DHA incorporation into plasma phosphatidylcholine and erythrocyte phosphatidylethanolamine demonstrated a positive-dose dependent response to increasing omega-3 fatty acid intake, showing a plateau effect at higher levels of ingestion. This study concluded that supplemental omega-3 fatty acid ingestion between 300 and 2000 mg/d in healthy subjects results in increased blood levels of these fatty acids, with 2000 mg/d being near

the maximally needed dose for optimal blood levels (161). Although an optimal dose remains unknown, the results from all aforementioned studies suggest that ~2000 mg/d of long chain omega-3 fatty acids supplemented for 4-6 weeks can significantly increase blood levels of these fatty acids.

While some studies have recorded increased levels of blood omega-3 fatty acids after supplementation, the association between omega-3 ingestion and exercise adaptation remains unclear. The optimal dose and duration are still unknown as well. However, the present literature suggests a dose of 2,000 mg/d DHA for 28 days would be an appropriate quantity and duration to increase blood DHA levels and potentially reduce inflammatory measures.

Table 2: Outline of Studies Incorporating Omega-3 Fatty Acid Ingestion

Study	Dose	Duration	Measures	Outcome
Bloomer (153)	2224mg EPA + 2208mg DHA	6 weeks	Blood EPA, DHA, CK, CRP, TNFα, & TEAC; muscle soreness	↑ blood levels of EPA (~690%) and DHA (~130%) pre-exercise; ↓ resting levels of CRP (~50%) and TNFα (~28%)
Burke (152)	3000mg n-3FA	7 days	Soreness ratings; arm swelling; temperature	↓ elevations of soreness after eccentric exercise (15% less than placebo)
Lenn (154)	1800mg n-3 FA (22.11% EPA & 14.95% DHA) + 100IU d-α tocopherol/dL-α tocopheryl acetate	30 days	Blood EPA, DHA, CK, & IL-6; muscle soreness, circumference, range of motion, isokinetic strength, & isometric strength	↑ blood EPA (~450%) and DHA (~110%) pre-exercise
McAnulty (156)	2000mg EPA + 400mg DHA	6 weeks	Blood EPA, DHA, & F-2 isoprostanes	↑ blood EPA (~300%) and DHA (~50%)
Nieman (157)	2000mg EPA + 400mg DHA	6 weeks	Blood EPA and DHA; immune function and inflammation measures in endurance athletes	↑ blood EPA (~310%) and DHA (~40%)
Phillips (133)	800mg docosahexaenoate + 300mg mixed tocopherols + 300mg flavonoids	14 days	Blood CK, CRP, & IL-6; pain and range of motion measurements	↓ elevations in IL-6 and CRP than placebo at day 10 post-exercise
Tartibian (155)	324mg EPA + 216mg DHA + 100IU d-α-tocopherol/dl-α-tocopherol acetate	30 days + 2 days	Pain, circumference, and range of motion	↓ mean variation of perceived pain at 48 hours post-exercise ↓ mean variation of ROM at 48 hours post-exercise ↑ mean variation of thigh circumference at 24 and 48 hours post-exercise
Toft (160)	3,600 mg/d omega-3 PUFA + 21.6mg tocopherol	6 weeks	FA composition in blood mononuclear cells (BMNC); TNFα, IL-1ra, & IL-6 plasma levels	↑ levels of EPA(2.8±0.7% vs 0.9±0.3%) & total n-3 PUFA (12.4±1.5% vs 8.4±1.6%) in blood mononuclear cells compared to control group
Trebble (161)	EPA:DHA (2:1, wt:wt); Weeks 1-4: 1,000 mg/d FO; Weeks 5-8: 3,000 mg/d FO; Weeks 9-12: 6,000 mg/d FO	12 weeks	Phosphatidylcholine and phosphatidylethanolamine; TNFα and IL-6 production	EPA and DHA incorporation into phosphatidylcholine & phosphatidylethanolamine demonstrated dose-dependent responses to ↑ omega-3 intake ↓TNFα and IL-6 with ↑ omega-3 ingestion
Zabel (158)	3000mg of n-3 FA: 2000mg EPA, 500mg DHA, & 500mg other n-3 FA	12 weeks	Blood EPA, DHA, CRP, & IL-6	↑ plasma levels of EPA (1.1±0.8% to 4.1±2.2%) and DHA (4.1±1.3% to 5.3±1.6%) from baseline to week 12

Evidence for Anti-Inflammatory Effect of Omega-3 Fats following Exercise

Some studies examined indirect measures following exercise that could support efficacy of the dose and duration of omega-3 supplementation (see Table 1). A study by Burke et al (152) investigated omega-3 supplementation with two eccentric bouts of the elbow flexors, the initial after 14 days of omega-3 restriction (control) and the second after 7 days of 3,000 mg/d omega-3 supplementation. Arm soreness was significantly less (15%) after the second bout. Although this initially suggests a reduction in damage or inflammation by omega-3 fats, this may simply be the result of a repeated bout effect. Phillips et al (133) assigned 800 mg/d docosahexaenoate + 300 mg/d mixed tocopherols + 300 mg/d flavonoids for two weeks and showed attenuation of CRP and IL-6 concentrations in treatment subjects compared to those consuming placebo. Efficacy of supplements was observed; however, these observed effects cannot be completely credited to DHA as tocopherols and flavonoids were also incorporated into the treatment and may have potentially played a role. Jouris et al (162) observed subjects perform eccentric biceps curls on two occasions, once after 14 days of dietary omega-3 restriction (control trial) and again after 7 days of 3000 mg/d omega-3 supplementation (omega-3 trial). Arm soreness increases were significantly reduced in the omega-3 trial in comparison to the control trial. Lastly, Tartibian et al (155) examined the effect of 30 days of modest omega-3 ingestion on perceived pain and DOMS symptoms after eccentric exercise of knee extensors. Subjects were supplemented with 324 mg/d EPA + 216 mg/d DHA for 30 days prior to 40 minutes of eccentric bench stepping. Significant differences were observed in perceived pain and range of motion at 48 hours post-exercise and thigh circumference at 24 and 48 hours post-exercise. In summary, several studies reported beneficial effects of omega-3 fats in doses from

540 to 3000 mg/d and supplementation durations from 7 days to 30 days, but not all reported an influence of ingestion of these fats on muscle damage or inflammation.

Although additional research can help solidify omega-3 fatty acids' anti-inflammatory effects, some studies have suggested that these fatty acids can decrease indices of inflammation such as pain, range of motion, limb circumference, and blood biomarkers such as CRP and IL-6 after exercise (133, 152, 155). While it is theoretically possible that omega-3s have effects on inflammation after damaging exercise, further investigation is necessary to verify an effect and to discover the optimal dose and duration of supplementation as well as the mechanism for any change.

In conclusion, unaccustomed eccentric exercise from resistance training causes muscle damage which later stimulates an inflammatory response. Reduced physical performance, soreness, and other symptoms are often resultants of this bodily response. In multiple studies of individuals with chronic high inflammation (e.g. arthritis, obesity, diabetes), omega-3 fatty acids have demonstrated anti-inflammatory effects. This evidence as well as a few studies using eccentric exercise supports the notion that these fats have the potential to reduce exercise-induced muscle damage and inflammation. However, the few studies that have attempted to test this hypothesis are contradictory and some did not measure the factors that would be most indicative of inflammation. Two studies have proposed DHA has superior anti-inflammatory effects in comparison to EPA (163, 164). Further investigation is needed into whether long chain omega-3 fatty acid ingestion, in concurrence with the initiation of a new full-body resistance training program, can enhance muscle recovery, decrease overall inflammation, and reduce risk of inflammatory diseases.

Chapter 3: JOURNAL MANUSCRIPT

Effect of DHA Supplementation on Markers of Muscle Damage and Inflammation During The First Two Weeks of a Novice Resistance Training Program

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ABSTRACT

Aim: The purpose of this study was to investigate docosahexaenoic acid (DHA) ingestion on muscle damage and inflammation during the first two weeks of a novice resistance training (RT) program. **Methods:** This study was a placebo-controlled, double-blind design. Forty-one healthy untrained males between the ages of 18 and 28 years consumed 2,000 mg/d of either DHA or corn oil (PCB) for 44 days including a 28 day loading period. Serum fatty acids were analyzed to determine treatment efficacy. During the 17 day training period, an acute eccentric exercise bout was implemented followed by a full-body RT regimen thrice weekly. Six fasted blood draws (days 1, 2, 4, 7, 12, and 17) during this exercise period were analyzed for creatine kinase (CK) and C-reactive protein (CRP). Maximum isometric strength (ISO) of the elbow flexors, delayed onset muscle soreness (DOMS), and range of motion (ROM) were measured on day 1 prior to exercise and also on days 2, 3, 4, 7, 12, and 17. **Results:** The CK response and the area under the curve (AUC) analysis for DOMS trended to decrease in the DHA group in comparison to placebo ($p=0.0925$ and $p=0.0536$, respectively). Treatment showed no effect on CRP levels. DHA supplementation significantly increased serum DHA by 380% as a proportion of total fatty acids ($p<0.0001$). **Conclusion:** This study does not demonstrate convincing benefits of DHA ingestion to recovery from a new resistance exercise program but does suggest a need for further investigation.

Keywords: Omega-3 fatty acids, strength training, creatine kinase, C-reactive protein

INTRODUCTION

Increased ingestion of omega-3 fatty acids has been associated with reductions in inflammation and cardiovascular disease risk factors (1). The anti-inflammatory effects of omega-3 fatty acids occur through reductions of inflammatory eicosanoids produced by cyclooxygenase (COX) and lipooxygenase (LOX) enzymes (2). These fats can compete with and replace arachidonic acid as substrate for these enzymes and thus reduce production of inflammatory prostaglandins. Pharmaceuticals (ie., aspirin, celecoxib) targeting COX and LOX enzymes have been investigated in clinical trials with resistance training (RT) to attenuate delayed onset muscle soreness and improve muscle recovery (3, 4). Some of these investigations, however, have demonstrated unfavorable side effects, including gastrointestinal distress, renal and liver injury, and muscle adaptation interference (5, 6). Omega-3 fatty acids may mitigate muscle damage and inflammation following RT similarly to pharmacological interventions and with the exclusion of harmful side effects, due to their nature of functioning as substrates rather than inhibitors to these enzymes.

Resistance training has many benefits on the musculoskeletal system (7, 8). High force contractions, particularly eccentric, can cause myofibrillar damage that is demonstrated by elevated blood CK, reduced range of motion (ROM) and peak isometric strength (ISO), and increased delayed onset muscle soreness (DOMS) (9-11). Few studies have observed the inflammatory response to resistance exercise beyond a few days but Jowko et al (12) observed an over five-fold increase in serum CK after two weeks of a new RT program in men. Damage can also stimulate the inflammatory response that is important for repair but can negatively impact muscle protein metabolism if it is overly excessive or prolonged (13). This exercise-related soreness can discourage athletes to continue exercise programs. Therefore, reducing muscle

damage and inflammation could provide exercise performance advantages by enabling athletes to more easily adhere to RT programs and have superior adaptations.

Inflammation, the mammalian natural response to injury and an essential component of the healing process, induced from resistance exercise may stimulate even more damage after the exercise bout (14). A delayed anti-inflammatory response, however, replaces the inflammatory process 2-4 days later as the damage is cleared and adaptation ensues (15). We are not aware of any studies that have followed the response of inflammatory indicators beyond a few days of a resistance exercise bout. However, the evidence that damage can be present for at least two weeks suggests that inflammation may continue (12).

No studies to date have investigated DHA's effects on muscle damage and inflammation during the initiation of a new RT program in untrained men. Rodacki et al (16) studied the effects of fish-oil supplementation in elderly women during a 90 or 150 day strength training program and observed greater improvements in muscle strength and functional capacity compared to the control group. While the mechanisms for these muscle improvements are unclear, it is possible that they may have been derived from a dampening of the inflammatory response to muscle damage that occurs subsequent to RT. However, that study did not perform any measures of inflammation. Phillips et al (17) investigated short-term supplementation of a combination of tocopherols, flavonoids, and docosahexaenoate in healthy untrained males during one eccentric arm exercise bout and found significantly less elevations in plasma IL-6 and CRP. This suggests that DHA, at least in combination with other supplements, has the ability to reduce inflammation within the recovery period after resistance exercise.

Accordingly, this study investigated the effects of DHA in healthy and untrained men on muscle damage and inflammation caused by 17 days of RT similar to that which is

recommended by the American College of Sports Medicine (ACSM) for beginners (18). The value of this study is to provide information on the practical value of ingestion of DHA supplements on post-exercise muscle damage and inflammation in those beginning a resistance exercise program.

Methods

Subjects

Fifty healthy, non-smoking males between the ages of 18-28 were recruited for participation. Subjects who had participated in strenuous resistance exercise (defined as two or more training bouts per week using either weights or intense bodyweight resistance) within the previous six months were excluded. Only males were selected to limit variation due to hormone levels and resulting differences in muscle damage by gender (19). Exclusion criteria included subjects who smoked or had history of inflammatory disease, illness, or a limiting orthopedic injury. The study protocol was approved by the Virginia Tech Institutional Review Board prior to subject recruitment.

Design

The entire randomized, placebo-controlled experiment lasted 45 days, which included a supplementation-only period of 28 days (supplement was taken throughout the entire duration of the experiment) and a training period of 17 days. Group assignment and bottle labeling was done randomly by the principal investigator who was not involved with daily testing and thus could not bias results. Martek Biosciences Corporation (Columbia, MD) provided the treatment and placebo supplements. Subjects were asked to ingest 2 grams daily (two capsules in the morning and two later in the day, each with a meal) of a dietary supplement for 28 days (day -28

to 1). Subjects in the treatment group were given DHA capsules (source is algae, see www.martek.com), and subjects in the placebo (PCB) group were given corn oil capsules. Weekly reminders via email were delivered in attempt to increase compliance. Bottles contained more supplements than required to fulfill the prescription, so compliance to the supplementation-only period was determined by counting the remaining supplements when subjects returned their bottles on day 1. A new supply was distributed for the duration of the experiment, and the remaining pills in those bottles after return were counted for compliance to the 17 day training period. Throughout the course of the study, subjects were asked to refrain from any other vitamin, mineral, and performance-enhancing supplement use and to avoid considerable changes in their regular diets, although this was uncontrolled.

Immediately after the baseline measures on day 1, the acute eccentric exercise protocol was performed. Data from the first four days of the exercise period that included the acute eccentric exercise bout were examined separately in the thesis of another graduate student (DiLorenzo, 2012). On day 4, the RT regimen began after the blood measures and physical assessments. The RT program lasted until the end of the study. This manuscript will focus on the data over the entire experiment from days 1-17 with special focus on the RT period on days 4-17. As summarized in Figure 1, blood measures (serum CK and CRP) and physical assessments (ISO, DOMS, ROM, and bodyweight) were performed on days 1, 2, 4, 7, 12, and 17. ISO, DOMS, and ROM were also measured on day 3, and bodyweight was measured on day -28.

Generally, all testing occurred in the morning hours between 6:30AM-12:00PM. Subjects participated in follow-up measurements at approximately the same times as previous

ROM, DOMS, and ISO measurements, although there was some deviation in time due to schedules. The average blood collection time was 8:31AM ± 43 minutes.

Anthropometric Measurements

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 ACSM procedure (20). The sums of the skinfold measurements were used to estimate body compositions from the relevant Jackson-Pollock equations (21).

Range of Motion (ROM)

Upon arrival to the laboratory on days 1, 2, 3, 4, 7, 12, and 17, subjects had their elbow joint angles measured on their non-dominant arm using a technique similar to that of Chen et al (22) and Lavender et al (23). To measure the relaxed arm angle, the subject was instructed to let his non-dominant arm hang freely by his side. The base of the goniometer was then placed over the lateral epicondyle. One arm of the goniometer was aligned in the center of the humorus 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 his hand to his shoulder joint without lifting his elbow. 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 on day -28.

Delayed Onset Muscle Soreness (DOMS)

DOMS was assessed prior to the ISO test on measurement days. Subjects were asked to complete 10 repetitions using a 5 lb weight with the exercised arm. During the elbow flexion, subjects were asked to think about the levels of perceived soreness in the exercised limbs. After completing this set, subjects indicated the levels of arm soreness on a visual analog scale (VAS) measuring 100 mm in length by making a vertical mark. The VAS was anchored by the designations "No Soreness" at 0 mm and "Unbearable Soreness" at 100 mm.

Maximum Isometric Strength (ISO)

On day 1, subjects had their baseline ISO tested at 90° of elbow flexion in their non-dominant arm using a Biodex System 3 Pro Isokinetic Dynamometer. In preparation 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 set with a dumbbell weighing 15 lbs for 4-6 repetitions. After a 2-3 minute rest, the subjects completed an ISO test. For this test, subjects were positioned so the non-dominant elbow angles were bent at 90° of flexion and the shoulder angles were bent at 45° of flexion. Subjects were instructed to exert maximal effort and force to the attached lever arm of the dynamometer for three seconds. Following this three-second contraction, subjects rested for 30 seconds prior to the next contraction. In total, subjects performed three contractions. Measures of peak isometric strength and average peak strength were recorded. The maximum peak isometric strength was defined as the greatest torque produced during any of the three contractions and the average peak isometric strength as the mean torque applied over the course of the three contractions. For strength tests completed after

day 1, only the 5 lb weight was used as a warm-up to prevent heavier loading from reducing maximal effort production by subjects.

Acute Eccentric Exercise Protocol

On day 1 after the 28 day supplementation-only period, subjects performed an acute eccentric exercise protocol of the non-dominant arm. Following a warm-up, subjects performed 6 sets of 10 repetitions of biceps extension on a preacher bench at 140% of the predetermined 1RM. The speed of the contraction was aimed to be 5 seconds from top of movement (distal arm perpendicular to floor) to bottom of movement (elbow angle of ~10 degrees). Investigators counted 1 to 5 audibly to inform the subject during the repetitions but did not attempt to provide motivation.

Resistance Training Protocol

Multiple Repetition Maximum Testing

On day 4, three days after the eccentric exercise bout, subjects began a RT program stressing all major muscle groups. This program was designed for the purpose of increasing strength and hypertrophy and was compliant with the recommendations by the ACSM (18). Six total exercises (leg press, machine chest press, cable row, machine shoulder press, lat pulldown, and cable biceps curl) were utilized after a warm-up period (Table 1). For the first resistance training day (day 4), a multiple repetition maximum test was completed for each subject in order to yield an estimated 1 repetition maximum (1RM). After an opening 3-5 minute full-body warm-up session (ie., elliptical, treadmill), subjects performed necessary warm-up sets for each of the six exercises, beginning with lighter weights and progressing with increasing intensities.

Investigators instructed proper technique for each lift prior to subject participation. Investigators then predicted an intensity that could be performed for no less than 4 and no more than 6 repetitions, as the ACSM does not recommend one-repetition maximums for untrained individuals. Rest intervals were 60 seconds between warm-up sets and 2-3 minutes between max-out sets. Additional max-out sets were sometimes necessary if subjects over- or underperformed relative to predictions of investigators. In some circumstances when multiple max-out sets began to fatigue subjects, a 3RM or 7RM was recorded and used in order to obtain the most accurate data value. However, a 4-6RM was desired for both safety and accuracy parameters. These multiple repetition maximum sets were used to calculate an estimated 1RM. The Wathen equation was used for this calculation: 1RM = mass of submaximal load (100 / (48.8 + 53.8 exp [-0.075 x number of repetitions]))—shown to be accurate for healthy people of all ages for up to 10 repetitions (24-26).

Submaximal Training Bouts

After the calculated 1RM estimate, the intensities and weights for the following five bouts were prescribed at 70% 1RM for multi-joint (leg press, machine chest press, cable row, machine shoulder press, and lat pulldown) and 60% 1RM for the single-joint exercise (cable biceps curl) for the first week (workouts 2 and 3); these intensities increased to 75%1RM and 65%1RM, respectively, for the second week (workouts 4,5, and 6) (Table 1).

Six total training days were performed on non-consecutive days of the week (days 4, 6, 9, 11, 13, and 16). Each training day consisted of 3-4 sets of 8 repetitions for each exercise. Leg press, the only lower body exercise, comprised 4 sets for additional volume while the remaining upper body exercises comprised 3 sets each. Subjects began each workout with a light warm-up (ie., jump rope, elliptical) and then continued into the RT regimen. Rest periods consisted of

about 60 seconds between warm-up sets and 2 minutes between working sets. Only the initial session was supervised by a researcher. Subjects were asked to complete workout forms legibly to indicate the numbers of repetitions, sets, and lifts performed each session and to submit the forms to the experimenters. After the workout, subjects needed a signature by a gym supervisor to validate that they had done a workout. This does not guarantee compliance.

Blood Collection

A certified phlebotomist withdrew blood samples from subjects' antecubital veins into a serum separator tube on days -28, 1, 2, 4, 7, 12, and 17 following a 10-hour overnight fast. Blood was centrifuged at a speed of 3000 g at 4 °C for 15 minutes. Serum was then aliquoted into separate vials for blood marker organization and stored at -80 °C until analysis.

Serum Analysis

Serum was collected and analyzed for CK and CRP on days 1, 2, 4, 7, 12, and 17. Serum DHA was also measured on days -28 and 1.

Serum DHA was analyzed using a lipid extraction and methylation procedure originally performed by Hara et al (27) but modified by Corl et al (28). 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 duplicates. 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 CRP was conducted via enzyme linked immunosorbent assay kits from R&D Systems (Minneapolis, MN). 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. Intra- and inter-assay coefficients of variation were 7.1% and 22.0% for CRP, respectively.

Statistics

Data are provided as averages with standard deviations. All baseline data on subject characteristics, blood values, ROM, DOMS, and ISO were compared using a two-sided t test to determine if there was uniformity between groups. A repeated measures analysis of variance (ANOVA) was used to detect differences by treatment and time for DHA, CK, CRP, DOMS, ROM, and ISO with significance set to $p<0.05$. Natural log transformation of CK was implemented since these data were not normally distributed; appropriate statistical tests were then able to be performed. Area under the curve (AUC) was also calculated for CK, CRP, DOMS, ROM, and ISO. AUC was approximated by summing the products of the differences of adjacent time points by the mean values of each time point using the following equation: $[T1*(M1+M2)/2] + [T2*(M2+M3)/2]\ldots + [Tx*(Mx+My)/2]$, where M is the measurement and T is time. For missing data points, the average value of the points before and after the missing point was used in place. For missing data on day 17, the same value on day 12 was used in

place. Correlation analysis of total AUC for dependent measures was performed using Pearson's correlation coefficients. All AUC measurements were based on absolute values and not percentages. Like baseline data, estimated 1RM data were analyzed using a two-sided pooled t test in conjunction with Bartlett, Levene, O'Brien, and Brown-Forsythe tests to verify equal variance between treatments ($p<0.05$) and to determine if differences in testing variables occurred between groups. All statistical analyses were performed using JMP® 9.0 (SAS Institute Inc. 2011).

RESULTS

Subjects

Fifty subjects were recruited to participate in this investigation, but only 41 completed the entire study and are included in the data analysis. Three subjects from the DHA group and six from the PCB group did not complete the study for reasons unrelated to supplementation and were excluded in the analysis. Specifically, six subjects had scheduling difficulties arise, two no longer desired to participate, and one began using a new medication which could have potentially interfered with the inflammatory processes being measured. Overall, 21 subjects participated in the DHA group and 20 in the PCB group. Baseline characteristics did not differ between groups (Table 2). No differences in bodyweight over the experiment between groups were observed. The average bodyweight change from day 1 to day 17 was 0.6 ± 0.3 kg for treatment and -0.1 ± 0.3 kg for placebo ($p=0.1020$).

Compliance

The exit survey was completed by 40 of the 41 subjects, 20 in the DHA group and 20 in the PCB group. Most subjects (65%) believed they were in the treatment group. In the DHA group, 14 of 20 subjects (70%) correctly guessed their treatment. In the PCB group, 6 of 20 subjects (30%) correctly guessed their treatment. Fifty percent of subjects answered "yes" for the perception of the supplement's capability to improve recovery time following exercise. Regarding frequency of consumption of assigned supplements, four subjects claimed 75% compliance (two for each group) while the remaining subjects claimed 90-100% compliance. Throughout the duration of the study, 176 total pills should have been ingested by each subject (none consumed on the last day of study since the final measurements were taken in a fasted state). The DHA group subjects consumed 164 ± 4 pills; the PCB group subjects consumed 164 ± 5 pills. Among the DHA group, 8 of 21 subjects believed that they were receiving the treatment due to experiencing a fish-like aftertaste or as one subject put it, "gas like fish oil." Treatment supplementations resulted in no other adverse effects. Analysis of serum DHA showed 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 2). Serum DHA increased by approximately 380% over this supplementation period for the DHA group while remaining unchanged for the PCB group.

Resistance Training Rating

The difficulty of the RT program on a 1-10 scale (0 being not difficult and 10 being the most difficult) was also determined through exit surveys. The average perceived difficulty rating from the DHA group was 6.5 ± 0.3 while the PCB group rated the difficulty similarly as 6.6 ±

0.3 (p=0.8026). The estimated 1RM for each of the six exercises was added for each subject. There was no difference in the sums of estimated 1RMs for the six lifts between groups (p=0.9463) (Table 3).

Range of Motion (ROM)

The largest reduction in ROM was experienced on the day after the eccentric exercise bout (day 2). Subjects in the DHA group had an average decline of 21 degrees in their total ROM on this day while subjects in the PCB group had an average decline of 25 degrees (Figure 3). ROM gradually improved but was still reduced below baseline for both groups on day 7 after the eccentric exercise bout. By day 17, both groups had values return to ~100% of baseline. Statistical analysis displayed a significant time effect ($p<0.0001$) but no significant effects due to treatment or treatment*time. Differences in ROM AUC between treatment groups were not significant. AUC of ROM correlated positively to AUC of maximum ISO ($r= 0.3629$, $p=0.0197$) (Table 6).

Delayed Onset Muscle Soreness (DOMS)

Maximum perception of peak muscle soreness occurred two days after eccentric exercise (day 3), corresponding to scores of 44 ± 21 mm and 51 ± 17 mm on a 100 mm scale for DHA and PCB groups, respectively (Figure 4). By day 4, both groups continued the decrease with the most rapid reduction between days 4 and 7. By day 17, subject soreness perception had decreased to 1 mm and 2 mm for DHA and PCB groups, respectively. Statistical analysis displayed a significant time effect ($p<0.0001$) but no significant effects due to treatment or

treatment*time. Differences in DOMS AUC for days 1-17 showed a trend to be lower for the DHA group than the PCB group (p=0.0536).

Maximum Isometric Strength (ISO)

The acute eccentric exercise bout resulted in substantial reductions in ISO on the day following (day 2) of 43% and 41% for the DHA and PCB groups, respectively (Figure 5). ISO had modest improvements toward baseline by day 4 with a more robust improvement but still ~80% of baseline at day 7. ISO had almost fully recovered by day 17 but still remained significantly lower than baseline at 92% of initial. Statistical analysis displayed a significant time effect (p<0.0001) but no significant effects due to treatment or treatment*time. Differences between groups in ISO AUC were not significant. AUC of maximum ISO correlated positively to AUC of ROM (r= 0.3629, p=0.0197) (Table 6).

Creatine Kinase

There was a significant time effect for lnCK (p<0.0001), with both DHA and PCB groups peaking on day 7 (Table 4). The lnCK was still 29% elevated on day 12 and while it fell close to baseline by day 17, the average was still modestly higher than baseline (Figure 6). For days 1-17, there was a statistical trend for a treatment*time effect in that lnCK tended to be lower for the DHA group (p=0.0925) (Figure 6). In absolute concentrations, the DHA group peaked at a ~33-fold increase from baseline at day 7 whereas the PCB group peaked at an ~18-fold increase from baseline at day 4 (Table 4). Differences between groups in lnCK AUC were not statistically significant.

C-Reactive Protein

Analysis of serum CRP on day 1, after the loading supplementation period but before the exercise, revealed a trend for the DHA group to have a lower average CRP value than PCB ($p=0.0938$). There was a significant time effect for serum CRP ($p=0.0019$), with concentrations peaking on day 7 for both DHA and PCB groups with ~2.5-fold and ~1.5-fold increases, respectively. However, analysis of serum CRP revealed no significant treatment or treatment*day effects (Table 5). Average serum CRP was higher than baseline on day 7 but was no different on days 12 and 17. Differences in CRP AUC between groups were not statistically significant.

DISCUSSION

The primary focus of this investigation was to examine the effects of DHA supplementation for 44 days on muscle damage and inflammation before and during two weeks of a novice RT program. It was hypothesized that DHA could function similarly to pharmaceutical interventions (COX inhibitors) reducing inflammatory responses and subsequent muscle damage resulting from exercise. This investigation was designed to evaluate whether DHA is a useful supplement to support recovery during a new RT program.

The resistance training period elicited muscle damage as demonstrated through substantial increases in serum CK. Although many studies have examined muscle damage for a few days after an eccentric exercise bout, no studies to date have monitored CK throughout the beginning of a new RT program as often as the present study. However, a few have measured CK levels for several days after an acute bout of eccentric exercise of the elbow flexors. Lee et al (29) observed a CK curve peaking at 4 days post-exercise between 2,000 and 2,500 U/L and

then slowly decreasing at 5 days post-exercise. Newham et al (30) demonstrated a peak CK range of 735-10,904 U/L 4 days post-exercise with a slow decrease following. Clarkson et al (31) showed a CK peak of 7,713 U/L at day 4, followed by a decrease to 2,603 U/L at day 7, and a return to near baseline levels at day 10. The changes we observed in serum CK for the first week following the eccentric exercise bout demonstrated later peak times by about 2-3 days but were comparable in magnitude to those observed by others. The initiation of the RT program on day 4 may have exacerbated initial muscle damage caused on day 1, resulting in later peak serum CK values on day 7 in our study compared to those only using one eccentric bout,.

A couple of studies examined indicators of muscle damage over several weeks of initiation of full-body resistance training. Jowko et al (12) observed CK increases of over 5-fold at week 2 and over 2-fold on the third week of the resistance training program. Nissen et al (32) observed CK increases of 64-fold at week 1, 5-fold at week 2, and 2-fold at week 3. The present study also showed peak concentrations after week 1 (~16-fold) and significantly reduced concentrations after week 2 (near baseline levels). Both comparative RT programs included more overall exercises and incorporated either higher intensities (up to 90% 1RM) or volumes (inclusion of 2-3 warm-up sets for each exercise and up to 15-20 repetitions per set) than in the present study. These more challenging training programs could have stimulated more total damage and slowed recovery, evident by the higher serum CK levels through the second week of RT in those studies. Overall, the RT programs, given moderate differences in design, caused similar patterns, though with differing magnitudes, in muscle damage.

Other indicators of muscle damage, reductions in ISO and ROM and increases in DOMS, were highest 24-48 hours after the eccentric exercise and recovered towards baseline throughout the training period. In the present study for all subjects, reductions in ISO were maximal on day

2 with an average 42% decrease; DOMS peaked on day 3 at an average 46 mm score, and ROM peaked on day 2 with a 23 degree decrease. Bryer and Goldfarb (33) showed a slightly less decrease of 30% in ISO at 24 hours after a similar acute eccentric exercise; a peak of DOMS at 48 hours post-exercise as well but with a significantly higher average value at 66 mm; and a maximal decrease of 15% in ROM at 48 hours post-exercise. Two studies observed these measurements for longer periods of time post-exercise than most others. Lenn et al (34) observed peak changes in ISO (~20% decrease), DOMS (~40 mm increase), and relaxed arm angle (~10 degree decrease) at 2 days post-exercise. ISO and DOMS then steadily returned to baseline levels by 7 days post-exercise while relaxed arm angle recovered at a slower pace and never fully returned to baseline levels. In young men, Lavender and Nosaka (23) measured damage indicators for 10 days after the eccentric bout. They observed 50-60% decreases in ISO, 30-35 mm DOMS scores, and ROM decreases of ~20 degrees from immediately to 2 days post-exercise. ROM further decreased another 10 degrees 4 days post-exercise. DOMS scores returned to baseline levels 7 days post-exercise while ISO and ROM steadily but slowly increased without reaching baseline by 10 days post-exercise. Our functional measures compared similarly but not identically to those observed by others, with peaks typically being shown at 24-48 hours post-exercise and at similar magnitudes. However, the returns to baseline values (not until day 17 or later) for measures in the present study were shown to recover at a slower rate than shown in other studies (23, 34). Thus, we assume The RT program in our study slowed the rate of recovery of these measures, but a control group that did not perform the resistance training program would be required for verification.

Acute-phase proteins and cytokines are released in response to increased inflammatory states and can be measured to estimate the magnitude of the muscle inflammatory response.

Specifically, CRP, an acute-phase protein, is a reliable indicator of systemic inflammation (35). There is research demonstrating impairment of muscle protein synthesis and reduction in muscle mass and strength when IL-6, an acute-phase response cytokine and stimulator of CRP synthesis, is elevated (35-38). Excessive CRP and IL-6 levels can thus bring about poor body composition and reduced muscular strength if left unchecked. As a result, regulating these acute-phase proteins during RT can help establish healthy protein metabolism and lean body mass maintenance.

Although no studies that we are aware of have tracked changes in serum CRP during the first few weeks of resistance training, some studies have looked at the short-term changes following one eccentric exercise bout. Phillips et al (17) found CRP peaked earlier (72 hours) to a lower magnitude (~1.1 mg/L) after a similar acute eccentric exercise of the elbow flexors, compared to our peak on day 7 at ~2.5 mg/L. This suggests but does not prove that initiation of RT after the eccentric exercise bout in our study exacerbated existing muscle damage and induced further inflammation to cause a later peak in CRP.

In the present study, DHA tended to reduce the responses of several markers of damage (serum CK and DOMS) but not of others (ROM and ISO) during the first two weeks of a resistance training program compared to PCB. DHA ingestion tended to dampen the overall magnitude of the CK response over two weeks, suggesting less myofibrillar or sarcolemmal disturbances than the PCB group. Because measurement of serum CK is an indirect assessment, it is difficult to determine with certainty the degree of myofibrillar damage and sarcolemmal disruption following exercise, both of which influence the CK response in the bloodstream. However, CK is one of the most consistently measured blood markers for damage and these results suggest that DHA could have a protective effect from muscle damage. It is possible that

incorporation of omega-3 fats into the sarcolemma provided greater resistance to damage from the RT program. Fiaccavento et al (39) found that dystrophic hamsters fed a diet enriched with α-linolenic acid demonstrated improvements in the histological appearance of their muscular tissue and a superior ability to repair injured fibers. Also, the myocytes of these treated rats were larger with preservation of cell membrane integrity. The modulation of the sarcolemma lipid profile was shown, at least in part, to prevent skeletal muscle lesions in these diseased animals. Liu et al (40) demonstrated that the sarcolemmal fatty acid composition is significantly related to the dietary composition. It appears that this increased incorporation of omega-3 fatty acids into the sarcolemmal membrane may create a greater resistance to muscle damage, or at least a greater resistance to CK leakage, during a RT program. It is acknowledged that these effects on serum CK were statistical trends and did not reach statistical significance. However, the pattern of trends is provocative and suggests value in additional research to verify the influence of DHA on muscle damage.

A trend for lower DOMS ratings over the two weeks of resistance training is also supportive of a benefit to DHA. This trend might also be credited to stabilization of the sarcolemma with increased omega-3 content. With less ruptures or disturbances to the sarcolemma from the RT, it could be possible that less sensory nerves were affected and thus less pain signals were sent to the CNS, resulting in less pain sensations and lower ratings. However, these potential benefits of DHA on muscle damage need to be further studied for confirmation, as significance at the 10% level is not scientifically convincing. Also, with ROM and ISO not being statistically different between groups, the argument for a protective effect on muscle damage remains weak. Another alternative interpretation to the trend for benefit of DHA could

be that random assignment resulted in genetically different groups more or less resistant to exercise-induced muscle damage due to variation in IGF-II alleles (41).

However, studies have evaluated the effect of omega-3 fats alone or with other ingredients on the muscle damage response to one acute eccentric exercise bout. Phillips et al (17) reported that 7 days of ingestion of a multiple-ingredient supplement that included DHA did not influence markers of muscle damage (serum CK) following an eccentric exercise protocol. In an abstract by Burke et al (42) that we were unable to find as a fully published paper, 3,000 mg/d of omega-3 fatty acid supplementation for 7 days reduced soreness but not arm swelling in response to a bout of eccentric biceps curls. Jouris et al (43) also observed significantly smaller increases in soreness after eccentric biceps curls for the omega-3 trial compared to control. Thus, these studies support the value of omega-3 fats on at least one indicator of muscle damage but the lack of consistency in all the measures makes it difficult to infer that omega-3 fats reduce muscle damage. Other measurements, such as magnetic resonance imaging (MRI) or muscle biopsies, would provide more direct assessments of muscle damage and provide more tangible and conclusive results.

Evidence for an effect of omega-3 fats on inflammation caused by exercise is also conflicting. Analysis of serum CRP on day 1, after the loading supplementation period but before the exercise, revealed a trend for the DHA group to have a lower average CRP value than PCB. This suggests that chronic DHA ingestion could have an anti-inflammatory effect independent of resistance exercise. Bloomer et al (44) observed similar results.

Phillips et al (17) reported that 7 days of ingestion of a multiple-ingredient supplement including DHA resulted in significantly smaller elevations of IL-6 and CRP after an acute eccentric exercise bout in comparison to placebo. The difference in outcome for CRP compared

to our study could be related to the greater exercise intensity and volume in our study than that in that by Phillips et al (17). Lower peak CRP values of 0.6 mg/L and 1.2 mg/L (treatment and placebo, respectively) observed in their study suggest lower inflammatory response that may be more susceptible to nutritional intervention. Lenn et al (34) measured serum IL-6 and TNFα after an eccentric exercise bout in subjects who supplemented fish oil but found no differences between groups in these inflammatory markers. No studies are available for comparison to ours that have continued resistance exercise beyond one bout to investigate the short-term effect on inflammatory markers.

In the present study, the AUC of ISO was positively correlated with the AUC of ROM. This finding seems to suggest that muscular tightness was related to decreases in force production. This was the only significant association among dependent measures found. Thus, we did not observe an association between overall damage response and inflammation or damage with strength impairment, for example.

The lack of significant change in markers of muscle damage and inflammation by DHA ingestion in our study is similar to the outcomes of studies using COX inhibitors with acute or chronic resistance exercise. Three isoforms of COX exist (COX-1, COX-2, and COX-3), and all of these essentially carry out the same enzymatic reactions. However, COX-2 appears to be primarily responsible for the inducible, inflammatory effects (45). Paulsen et al (46) observed no differences of muscle function recovery or markers of inflammation and regeneration after 400 mg/d of celecoxib following an acute eccentric exercise bout. Trappe et al (47) found no differences in serum CK or perceived muscle soreness ratings when maximal over-the-counter doses of ibuprofen or acetaminophen were administered after an eccentric exercise bout. Similarly, the same lab reported no effect of these drug regimens on muscle inflammatory cells

gathered from muscle biopsies after an eccentric bout (48). In a longer term study, Krentz et al (3) found no benefits of ibuprofen administration on muscle strength, hypertrophy, or soreness from RT bouts of the biceps flexors five times weekly for six weeks. The lower muscle protein synthesis observed by Trappe et al (47) following an acute resistance exercise bout when ibuprofen or acetaminophen was ingested suggested a negative effect of dampening of the inflammatory response. However, a subsequent training study by that same lab found ibuprofen and acetaminophen enhanced rather than inhibited muscle hypertrophy and strength gains in older adults (4). This suggests that regulation of the COX pathway and reduction in the inflammatory response to exercise can influence muscle protein turnover and muscle mass in humans. In the present study, multiple trends for lower indicators of muscle damage (DOMS and CK) were found, but the lack of significance of these differences limits the ability to conclude that DHA can be useful in enhancing recovery from resistance training.

Other research has demonstrated effectiveness of omega-3 fats as anti-inflammatory agents. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) account for most of the anti-inflammatory effects of omega-3 fatty acids (2). Thus, long-chain omega-3 fatty acid consumption should be emphasized in contrast to short-chain α-linolenic acid (ALA) due in part to its poor conversion efficiency to EPA and DHA (49). While EPA is the primary omega-3 substrate for COX and LOX enzymes, DHA has been shown to provide superior anti-inflammatory benefits compared to EPA in some cases (50, 51). Groeger et al (52) confirmed through mass spectrometry the anti-inflammatory effect of DHA through their nature of acting as PPAR agonists and inhibiting pro-inflammatory cytokine production. In cancer patients, omega-3 fats have been shown to suppress COX-2 and NFκβ activity in tumors (thus decreasing proliferation and allowing apoptosis of cancer cells) and to reduce cachexia, ultimately

improving their quality of life (53). Higher consumption of omega-3 fats among women was associated with lower risk of coronary heart disease (CHD) and particularly CHD deaths, possibly through an anti-inflammatory mechanism (54). Greater reported consumption of EPA and DHA is associated with greater gray matter volume in the brain, improving memory, mood, and emotional arousal (55). Evidence for anti-inflammatory effects of omega-3 fats exist for many populations in both healthy and diseased patients and for many different tissues and systems within the body. This supports the argument for additional research using omega-3 fats related to muscle damage and inflammation during a RT program.

Our supplementation protocol was effective in substantially boosting serum DHA to expected levels similar to other studies (44, 56, 57). Although we did not directly measure membrane composition of DHA, we expect that there were similar increases since other studies show directly proportional associations between the quantity of omega-3 administration (both parenteral and enteral) and amount of omega-3 incorporation into membranes (56-58). We would expect the cell membrane DHA levels to increase even further if the duration of supplements were longer but the rate of change would begin to lessen. However, other studies have observed reducing effects of either muscle damage or inflammation after omega-3 fat ingestion of similar (or even lesser) doses and durations as our study (17, 42, 59), so we do not think that the supplementation protocol limited the outcomes of the intervention.

Because no significant effects and only trends were found in the present study, it would be beneficial to use more subjects in the research to increase the statistical power. This would then better determine if these trends were indeed true differences. It is also possible that the effects of DHA can be observed only after multiple weeks of a new RT program, after a more robust adaptation to the exercise. Rodacki et al (16) and Smith et al (60) both found in older

adults that long-term omega-3 fatty acid supplementation (8-21 weeks) improved muscle protein synthesis, strength, and functional capacity. These effects may be credited to the anti-inflammatory nature of omega-3 fats. It can be inferred that the most noticeable effects of omega-3 fatty acids can be observed through a long-term investigation, supporting the need for longer durations and further exploration into this topic.

This investigation was the first to monitor the short-term effects of DHA on markers of muscle damage and inflammation with untrained men beyond a single bout. While there were no statistical differences observed between groups and our hypothesis of DHA's ability to reduce these markers has been rejected, multiple trends for effects on muscle damage suggest value for further investigation into this topic.

CONCLUSION

Supplementation of DHA at 2,000 mg/d for 4 weeks dramatically increases serum levels of these fatty acids and tends to decrease resting serum concentration of CRP. DHA supplementation did not significantly decrease markers of inflammation and muscle damage during the beginning weeks of a novice resistance training protocol in untrained young men. Individuals in the DHA group, however, tended to have lower DOMS and serum CK, both markers of muscle damage. Future studies investigating the effects of DHA on resistance training programs should consider using a protocol of longer duration with more subjects to determine the possibility of any long-term effects. Additional research into the efficacy of DHA as a non-pharmaceutical anti-inflammatory agent during resistance training is suggested.

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MANUSCRIPT FIGURES

Figure 1: Study Timeline

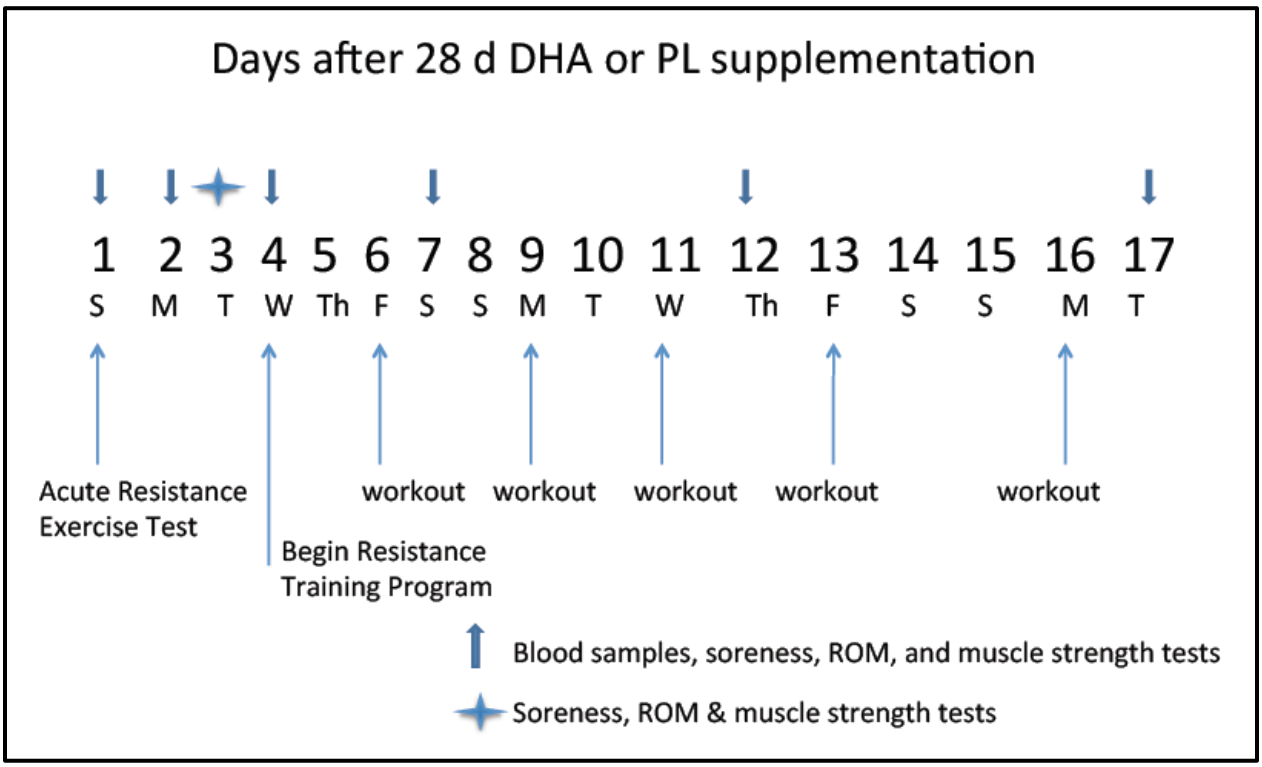
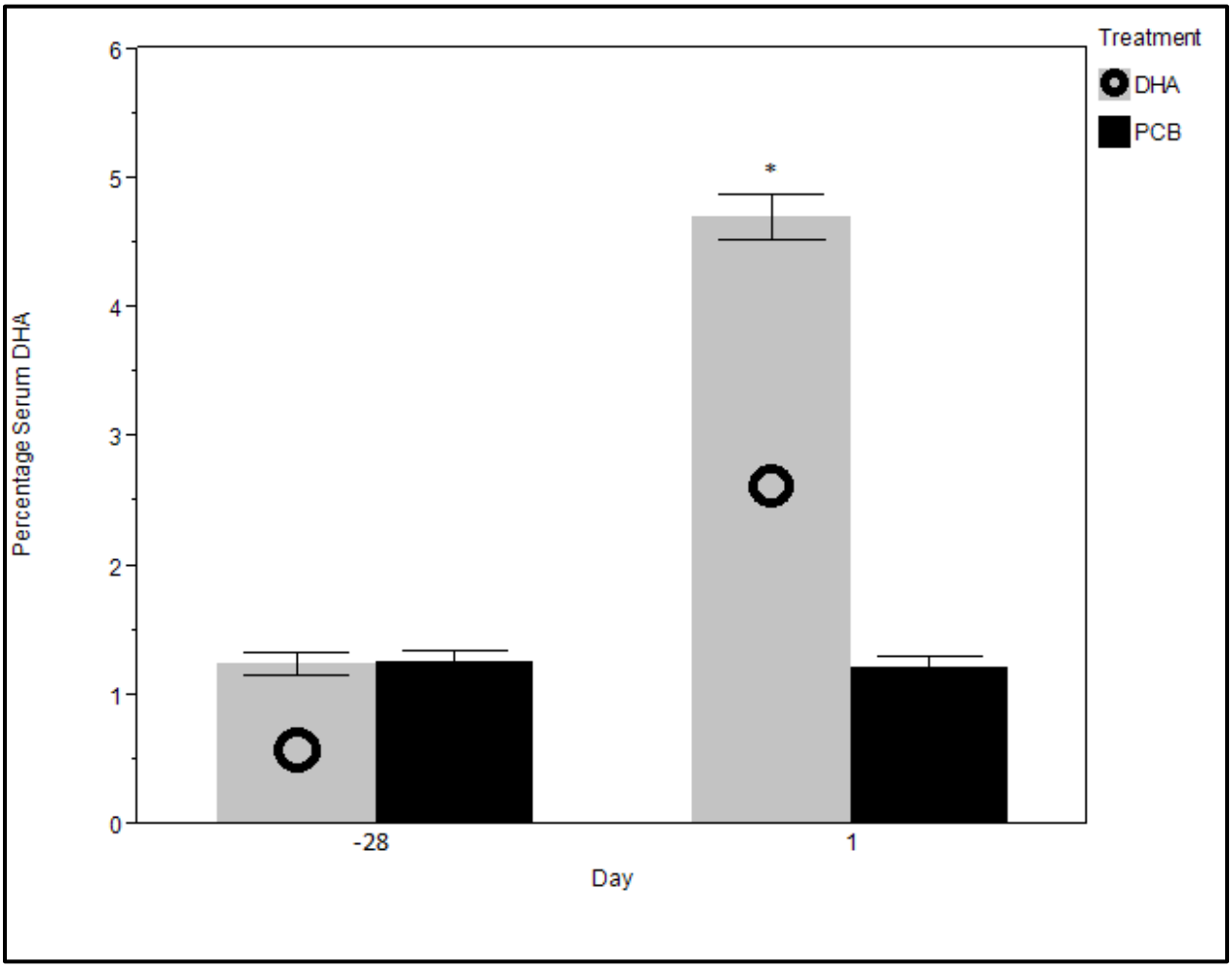


Figure 1 represents the timeline of the entire exercise training period from day 1 to day 17.

Figure 2: Serum DHA Percentage at Days -28 and 1



In Figure 2, * indicates significant difference ($p<0.0001$) between treatments from day -28. Day -28 represents the time point 28 days before day 1. DHA, docosahexaenoic acid.

Figure 3: Range of Motion by Day

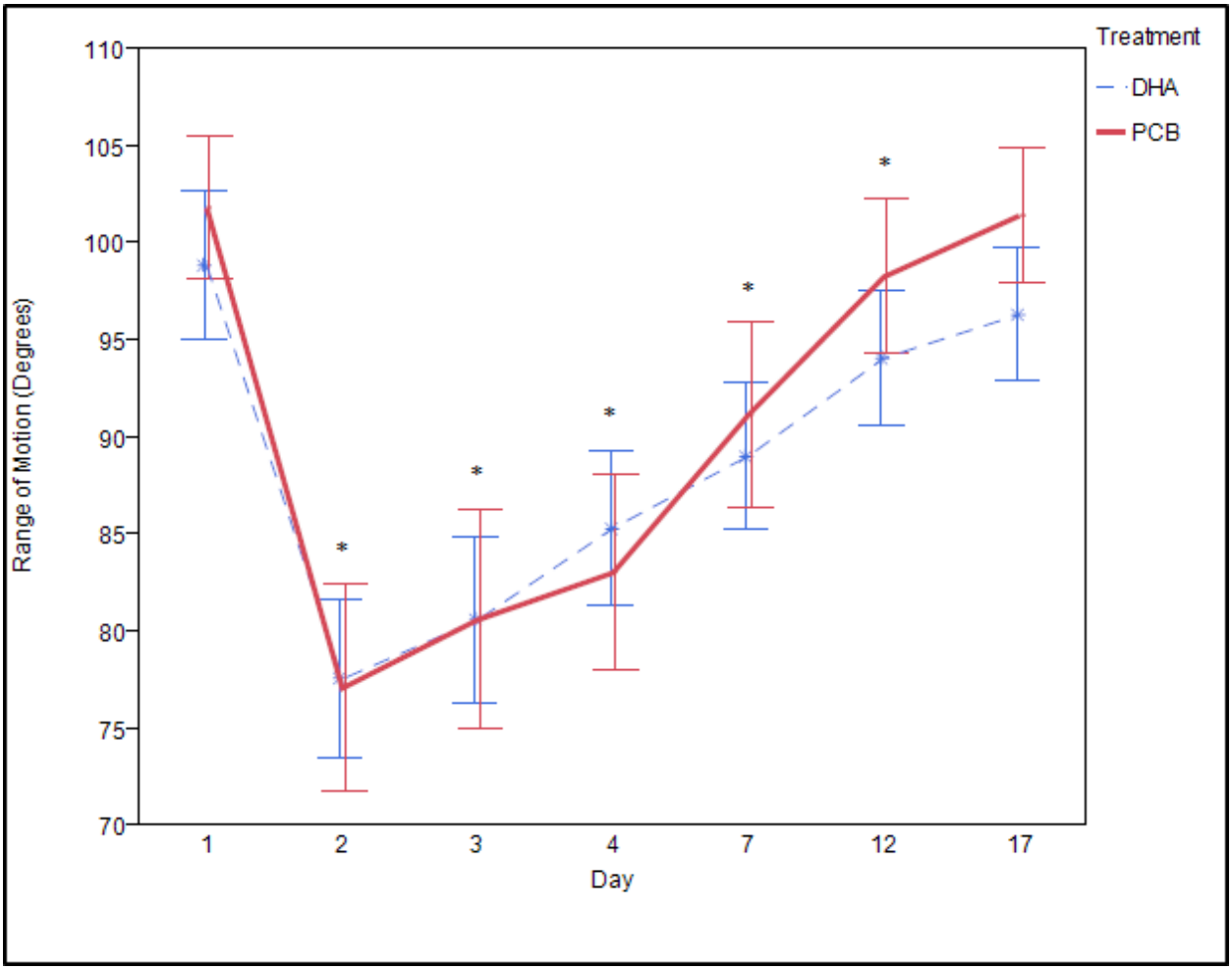


Figure 3 indicates that there were no treatment differences in mean range of motion (ROM) at any time point. * Indicates a time difference from day 1.

Figure 4: Delayed Onset Muscle Soreness by Day

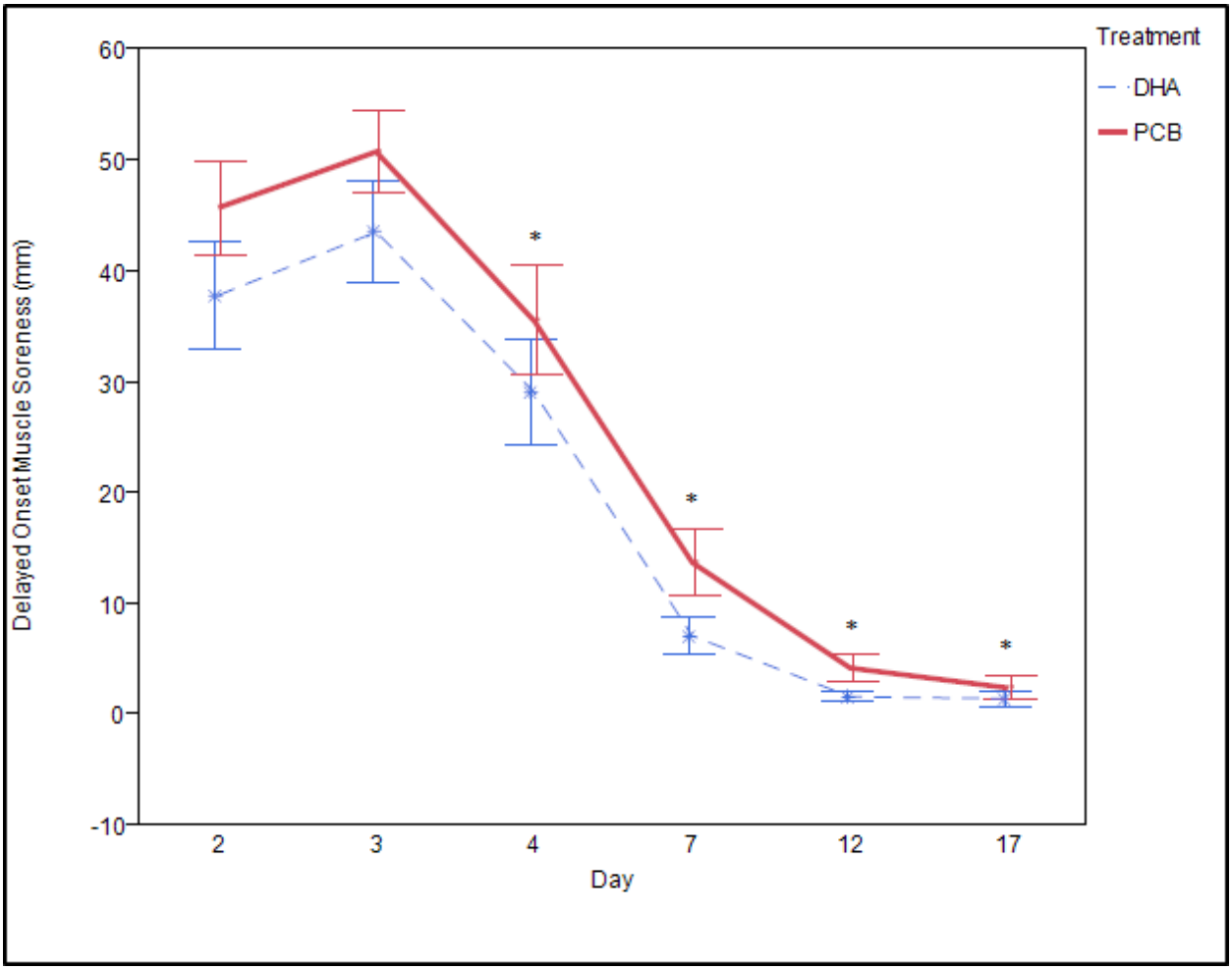


Figure 4 indicates that there were no treatment differences in delayed onset muscle soreness (DOMS) at any time point. * Indicates a time difference from day 2.

Figure 5: Maximum Isometric Strength by Day

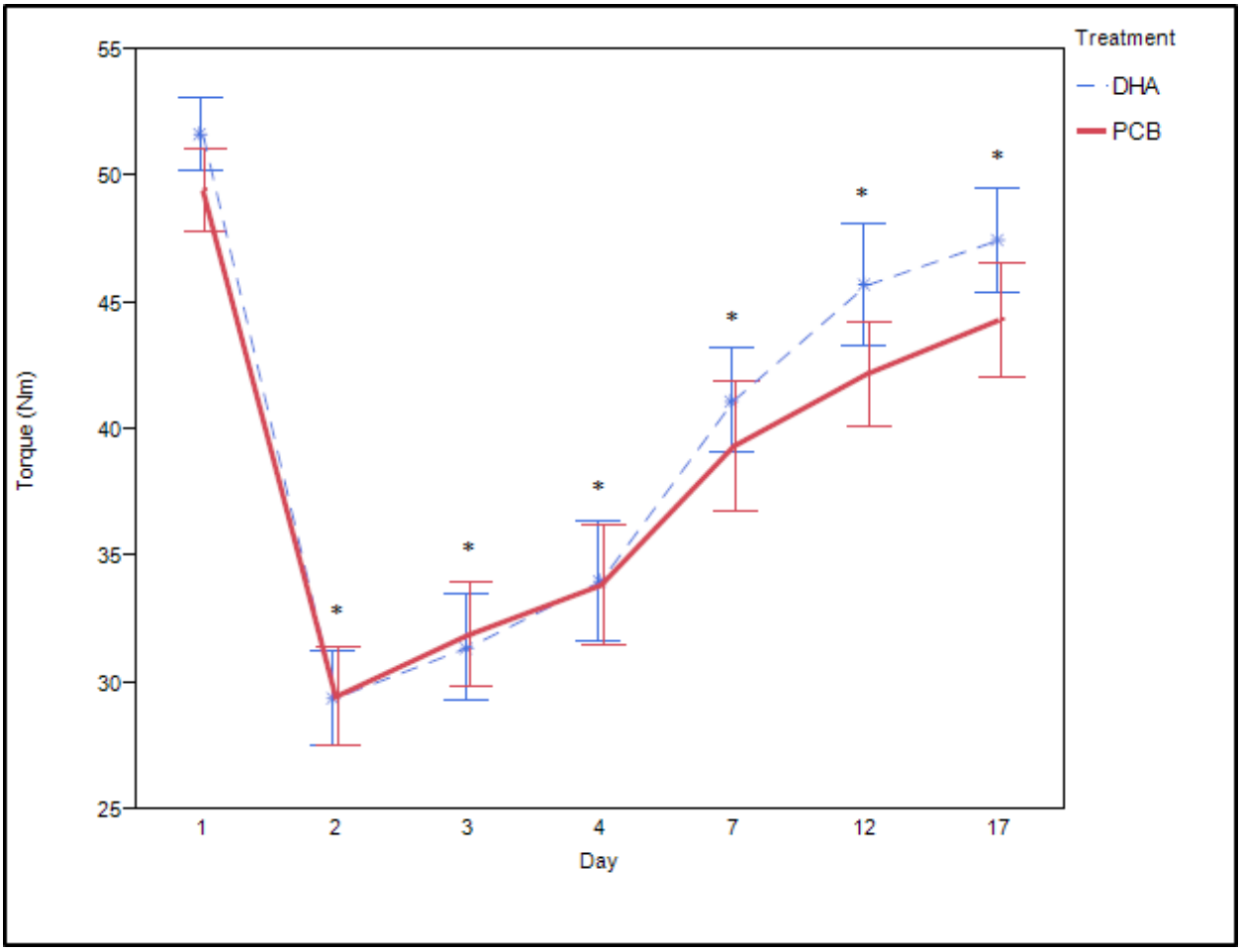


Figure 5 indicates that there were no treatment differences in maximum mean isometric strength (ISO) at any time point. * Indicates a time difference from day 1.

Figure 6: Natural Log of Serum Creatine Kinase Activity by Day

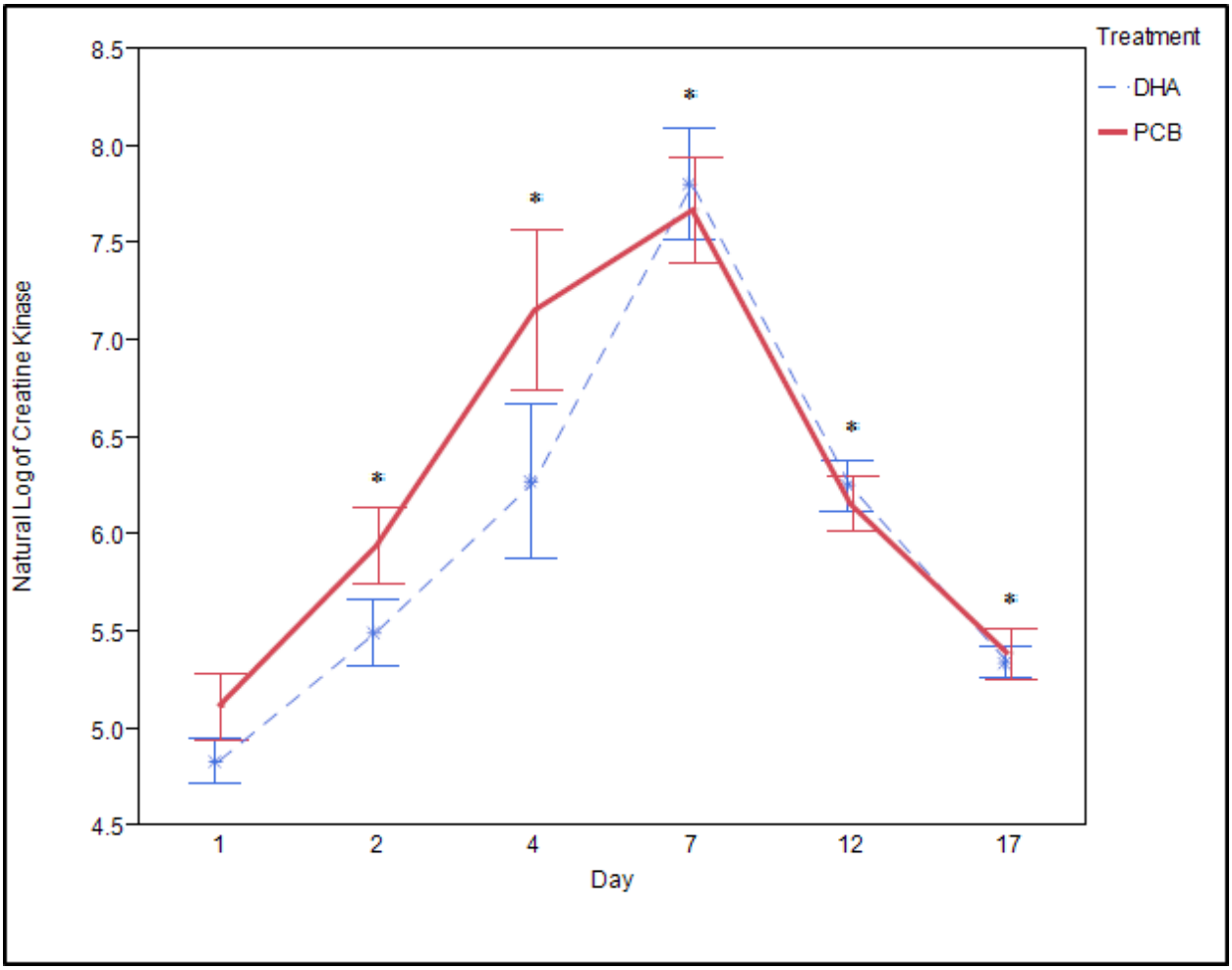


Figure 6 indicates that there were no treatment differences in the natural log of creatine kinase (CK) at any time point. * Indicates a time difference from day 1.

MANUSCRIPT TABLES

Table 1: Resistance Training Program

Exercise	Sets	Repetitions	Intensity for Workouts 2-3	Intensity for Workouts 4-6
Leg Press	4	8	70%1RM	75%1RM
Machine Bench Press	3	8	70%1RM	75%1RM
Cable Row	3	8	70%1RM	75%1RM
Machine Shoulder Press	3	8	70%1RM	75%1RM
Lat Pulldown	3	8	70%1RM	75%1RM
Cable Biceps Curl	3	8	60%1RM	65%1RM

Table 2: Subject Baseline Characteristics

	Age (years)	**Body Weight (kg)**	**Body Fat (%)**	**BMI**
DHA n=21	22.2 (2.8)	76.3 (12.6)	13.8 (5.6)	24.4 (4.6)
PCB n=20	21.3 (2.5)	80.6 (18.6)	15.9 (5.0)	25.4 (5.4)
Values are the means with standard deviations in parentheses. There were no statistical differences between groups ($p<0.05$).				

Table 3: Resistance Training Estimated 1RM

	Leg Press (kg)	Machine Bench Press (kg)	Cable Row (kg)	Machine Shoulder Press (kg)	Lat Pulldown (kg)	Cable Biceps Curl (kg)	Sum (kg)
DHA	177.5 (66.9)	78.4 (14.3)	74.4 (9.2)	73.6 (16.4)	73.7 (8.3)	43.0 (7.8)	520.5 (91.6)
PCB	180.4 (48.6)	78.5 (9.6)	74.0 (10.8)	77.6 (15.7)	71.1 (9.6)	40.9 (7.5)	522.4 (78.2)
Values are means with standard deviations in parentheses. There were no statistical differences between groups ($p<0.05$).							

Table 4: Serum Creatine Kinase Activity (U/L)

	Day 1	**Day 2**	**Day 4**	**Day 7**	**Day 12**	**Day 17**
Absolute Concentration (U/L)			*	*		
DHA	146 (96)	346 (353)	3152 (7621)	4804 (5223)	614 (344)	223 (88)
PCB	238 (296)	563 (555)	4286 (5433)	3827 (3815)	573 (350)	270 (242)
Log Transformation		*	*	*	*	*
DHA	4.8 (0.5)	5.5 (0.8)	6.3 (1.8)	7.8 (1.3)	6.3 (0.6)	5.3 (0.4)
PCB	5.1 (0.8)	5.9 (0.9)	7.2 (1.9)	7.7 (1.2)	6.2 (0.6)	5.4 (0.6)

Values are means with standard deviations in parentheses. There were no statistical differences between groups ($p<0.05$). * Indicates statistically significant difference at time point from day 1 ($p<0.05$).

Table 5: Serum C-Reactive Protein Concentration (mg/L)

	Day 1	**Day 2**	**Day 4**	**Day 7**	**Day 12**	**Day 17**
Absolute Concentration (mg/L)	#			*		
DHA	0.90 (1.01)	1.26 (1.70)	1.36 (2.19)	2.24 (2.60)	1.35 (1.87)	1.38 (2.30)
PCB	1.69 (1.81)	2.29 (2.17)	1.87 (1.46)	2.73 (2.15)	1.65 (1.56)	1.81 (2.62)
Values are means with standard deviations in parentheses. There were no statistical differences between groups ($p<0.05$). * Indicates statistically significant difference at time point from day 1 ($p<0.05$). # Indicates a trend for a treatment effect for lower average CRP than PCB ($p<0.10$).						

Table 6: Associations Between Changes in Selected Dependent Measures

Measure	Statistic	lnCK AUC	CRP AUC	ROM AUC	DOMS AUC	ISO AUC
lnCK AUC	Correlation	1	-0.0498	-0.1176	-0.0201	0.0387
	p-value	0	0.7572	0.4641	0.9008	0.8099
CRP AUC	Correlation	-0.0498	1	-0.0728	-0.0220	0.1165
	p-value	0.7572	0	0.6512	0.8912	0.4682
ROM AUC	Correlation	-0.1176	-0.0728	1	-0.0015	0.3629
	p-value	0.4641	0.6512	0	0.9927	0.0197*
DOMS AUC	Correlation	-0.0201	-0.0220	-0.0015	1	-0.0292
	p-value	0.9008	0.8912	0.9927	0	0.8560
ISO AUC	Correlation	0.0387	0.1165	0.3629	-0.0292	1
	p-value	0.8099	0.4682	0.0197*	0.8560	0

* indicates statistical significance ($p<0.05$). lnCK, natural log of CK; CRP, C-reactive protein; ROM, range of motion; DOMS, delayed onset muscle soreness; ISO, peak isometric force

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Chapter 4: SUMMARY AND RECOMMENDATIONS

SUMMARY

The purpose of this study was to examine the effect of docosahexaenoic acid (DHA) on markers of muscle damage (serum CK, ISO, DOMS, ROM) and inflammation (serum CRP). DHA has demonstrated anti-inflammatory effects through binding the cyclooxygenase (COX) enzyme (21, 26), the primary target of pharmaceutical interventions attempting to reduce post-exercise muscular soreness (23, 24, 106, 127, 165). Similar studies have attempted to reduce muscle damage using omega-3 fatty acid supplementation. However, these have implemented aerobic exercise or have included a short loading period or small dosage of omega-3 fatty acids prior to testing (133, 152, 155).

The hypothesis of the present study stated that the ingestion of 2,000 mg/d of DHA for 44 days would reduce markers of inflammation (serum CRP) and muscle damage (serum CK, ΔISO, ΔDOMS, ΔROM) that result after an acute eccentric resistance exercise test and during the first two weeks of a novice resistance training program in comparison to placebo. The dosage was determined in part because of recommendations from the American Heart Association (14). It was also selected because the quantity of omega-3 intake has been shown to be positively correlated with overall changes in membrane composition of omega-3 fatty acids and has been shown to be inversely proportional to baseline levels (166, 167).

Fifty healthy and untrained male subjects between the ages of 18-28 years were recruited for the present study but only forty-one completed the entire study (45 days total). Investigators assessed baseline measurements on subjects for anthropometric measurements and serum DHA concentrations (percentage of total fatty acids) on day -28 of the study. Following baseline measurements, subjects ingested 2,000 mg/d of either DHA or PCB (corn oil) for a period of 44 days without any restrictions or alterations to diet or activity. After the 28 day supplementation-

only period, subjects submitted fasted blood samples on days 1, 2, 4, 7, 12, and 17 of the study for analysis of serum CK and CRP. Subjects completed functional assessments of ISO, DOMS, and ROM on days 1, 2, 3, 4, 7, 12, and 17 of the study. On day 1, subjects completed an acute maximal eccentric resistance exercise bout with their non-dominant elbow flexors. Moreover, on day 4, subjects began the first of six workouts of a novice resistance training program. The program incorporated all major muscle groups. Workouts took place on days 4, 6, 9, 11, 13, and 16 of the study. The first workout on day 4 served as a maximum session, as subjects performed 4-6 repetitions of each exercise with the greatest resistance possible in order to estimate a 1RM, which was then used to prescribe intensities for the following five workouts. Submaximal workouts consisted of 70-75% 1RM for all multi-joint exercises and 60-65% 1RM for the single-joint exercise. These workouts included 4 sets of 8 repetitions for the leg press and 3 sets of 8 repetitions for all other exercises.

All baseline data on subject characteristics, blood values, and functional measures were compared using a two-sided t test. A repeated measures analysis of variance (ANOVA) was used to detect differences by treatment and time for CK, CRP, ISO, DOMS, and ROM with significance set at $p<0.05$. Natural log transformation of CK was implemented because these data were not normally distributed. Area under the curve (AUC) was calculated for CK, CRP, ISO, DOMS, and ROM. Correlation analysis of AUC for dependent measures was performed using Pearson's correlation coefficients. All AUC measurements were based on total values and not percentages. Post-hoc analyses of CK, CRP, ISO, DOMS, and ROM were performed using student t tests to determine differences in treatment at individual time points.

Like baseline data, resistance training data were analyzed using two-sided pooled t tests in conjunction with Bartlett, Levene, O'Brien, and Brown-Forsythe tests to verify equal variance

between treatments ($p<0.05$) and to determine if differences in testing occurred between groups. All statistical analysis was performed using JMP® 9.0 (SAS Institute Inc. 2011).

Performance:

There was a trend in CK analysis with the DHA group having lower responses than PCB ($p<0.10$). There was also a trend for DOMS AUC to be lower in the DHA group ($p<0.10$). There were no differences in CRP, ROM, or ISO.

Interpretation:

Muscle Damage:

It is difficult to verify the benefits of DHA considering the rather weak and varying results. Lower trends were observed in two out of the four markers (CK and DOMS) of muscle damage; however, these trends were not significant. Bivariate correlation confirmed that the AUC of ISO was associated with positive changes in ROM. This suggests that muscular tightness can impair maximum isometric force production. An appropriate conclusion states that DHA did not reduce muscle damage but might be able to produce significant results under designs with longer duration or greater statistical power.

Inflammation:

Because a trend for CRP was found to be lower for the DHA group at day 1 after the supplementation but not over the 17 day training period, the treatment tended to reduce inflammation independent of but not during a resistance training program in comparison to PCB. Further research is necessary, however, since this was observed by another study and could be beneficial for health.

Resistance Training:

The estimated 1RMs between the DHA and PCB groups were not different. While some studies suggest that ingestion of omega-3 fats can improve gains in muscle strength and size, muscular strength and hypertrophy were not measured throughout the resistance training period of this study. However, a tendency for the DHA group to gain bodyweight versus the PCB group over the 17 day training period suggests treatment subjects may have accrued more muscle mass as a result of this training. This finding needs to be investigated further as one eccentric exercise and six RT bouts over 17 days does not provide an appropriate time frame for significant gains in lean body mass; longer programs are necessary for investigation into the accuracy of this tendency of greater weight gain. It may be possible that the observational effects of DHA ingestion are difficult to demonstrate over a short exercise period. Omega-3 fatty acids have been shown to stimulate muscle protein synthesis over a time course of 8 weeks (168). DHA has been shown to be an ergogenic aid in strength training over the course of 90-150 days (169). These effects might be credited to the anti-inflammatory role of these fats. As a result, the study of omega-3 fatty acids as an anti-inflammatory supplement and ergogenic aid during resistance training should be continued further. Observable effects of DHA on muscle damage and inflammation may not be evident until multiple weeks or even months after initiation of the RT program.

Future investigation into DHA's effects on muscle damage and inflammation during the beginning of a novice RT program is necessary. Greater subject sample sizes would increase statistical power and show if the weak trends in the present study were true differences. More direct measurements, such as biopsies to assess infiltration of immune cells and magnetic resonance imaging (MRI), can be implemented to increase accuracy of quantification of muscle

damage and inflammation, rather than relying on indirect blood and functional measures. It would also be interesting to take these measurements at different time points, possibly immediately after the first RT workout to test DHA's effect on primary muscle damage or after many weeks or months of RT. It may be that DHA has more specific benefits during the timeline of muscle regeneration, such as alleviating primary or secondary muscle damage or inflammation. Then, it might be easier to pinpoint the reasons for efficacy, including omega-3 incorporation into myofiber membranes, regulation of the COX enzymes, etc.

Implications:

Based on the evidence of this investigation, it is inconclusive if DHA supplementation reduced muscle damage or inflammation in the resistance training setting. Trends existed for a benefit of DHA on several measures of muscle damage suggesting additional study could be done to clarify. It may be possible that the observational benefits of omega-3 fatty acid ingestion are difficult to demonstrate over a short exercise period. Ergogenic effects of strength, protein synthesis, and functional capacity were found over multiple months of simultaneous omega-3 fat ingestion (168, 169); thus, it may be appropriate to study these supplements over a longer duration to confirm effects. The present study suggested that DHA supplementation has the potential to reduce muscle damage but not inflammation after an acute eccentric exercise bout and the first two weeks of a resistance training program. Because some of these measures were not significantly different, further research is needed to confirm these findings.

RECOMMENDATIONS

Although DHA did not display any protective or anti-inflammatory benefits in the present study, investigation of this omega-3 fatty acid should be continued. Future investigations should

examine the effect of omega-3 supplementation on a resistance training program over the course of multiple weeks or even months. Long-term benefits of omega-3s would entice athletes and exercise enthusiasts who are seeking optimal performance capabilities to supplement these nutrients. Also, greater subject sample sizes and more direct markers of muscle damage (MRI, muscle biopsy, etc.) can be used to verify statistical differences in indirect markers, thus providing a firmer conclusion.

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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 conducted 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.

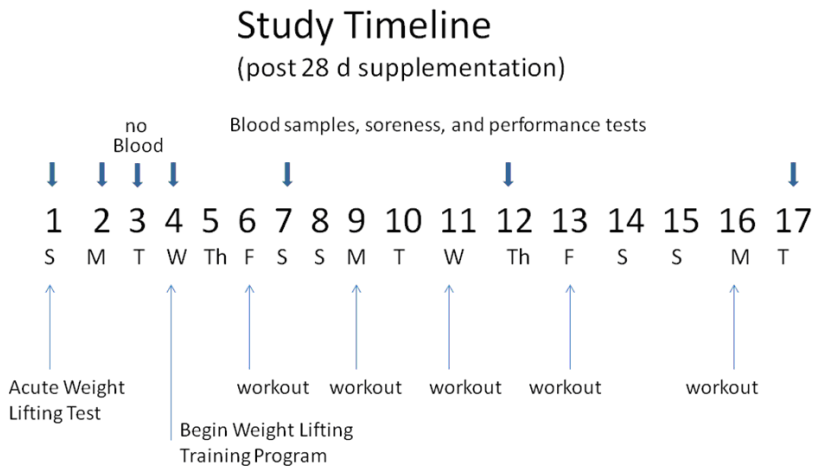
To start your involvement in the study, you will come to our laboratory to have your body composition (estimated by measuring fat fold thickness on three sites of the body with calipers), body weight and height measured. You'll also provide a blood sample to the investigators. Following this visit, you 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 5lb weight. Next have the subject perform 1 set of 4 repetitions using a 15lb 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 1RM.
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 1RM 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			

Rep Max Record

Subject ID#_____ Date:_____

Supervisor:_____ Bodyweight:_____

Exercise	**Warm-Up (Weight x Reps) *2 Sets**	**Max Attempt #1 (Weight x Reps)**	**Max Attempt #2 (Weight x Reps)**	**Max Attempt #3 (Weight x Reps)**
Leg Press				
Vertical Chest Press				
Cable Row				
Shoulder Press				
Lat Pulldown				
Bicep Cable Curl				

Notes:

Resistance Training Program Completion Record (EXAMPLE)

Subject #: _____xxxx_____

Workout 1 Date: Supervisor Signature:

Exercise	Sets	Repetitions	Intensity	Weight	Sets Completed	Repetitions Completed	Weight Used
Leg Press	4	8	70%1RM	250			
Machine Chest Press	3	8	70%1RM	130			
Cable Row	3	8	70%1RM	110			
Machine Shoulder Press	3	8	70%1RM	120			
Lat Pulldown	3	8	70%1RM	110			
Cable Bicep Curl	3	8	60%1RM	45			

Workout 2 Date: Supervisor Signature:

Exercise	Sets	Repetitions	Intensity	Weight	Sets Completed	Repetitions Completed	Weight Used
Leg Press	4	8	70%1RM	250			
Machine Chest Press	3	8	70%1RM	130			
Cable Row	3	8	70%1RM	110			
Machine Shoulder Press	3	8	70%1RM	120			
Lat Pulldown	3	8	70%1RM	110			
Cable Bicep Curl	3	8	60%1RM	45			

Subject #: _____xxxx_____

Workout 3 Date: Supervisor Signature:

Exercise	Sets	Repetitions	Intensity	Weight	Sets Completed	Repetitions Completed	Weight Used
Leg Press	4	8	75%1RM	265			
Machine Chest Press	3	8	75%1RM	140			
Cable Row	3	8	75%1RM	110			
Machine Shoulder Press	3	8	75%1RM	130			
Lat Pulldown	3	8	75%1RM	110			
Cable Bicep Curl	3	8	65%1RM	50			

Workout 4 Date: Supervisor Signature:

Exercise	Sets	Repetitions	Intensity	Weight	Sets Completed	Repetitions Completed	Weight Used
Leg Press	4	8	75%1RM	265			
Machine Chest Press	3	8	75%1RM	140			
Cable Row	3	8	75%1RM	110			
Machine Shoulder Press	3	8	75%1RM	130			
Lat Pulldown	3	8	75%1RM	110			
Cable Bicep Curl	3	8	65%1RM	50			

Workout 5 Date: Supervisor Signature:

Exercise	Sets	Repetitions	Intensity	Weight	Sets Completed	Repetitions Completed	Weight Used
Leg Press	4	8	75%1RM	265			
Machine Chest Press	3	8	75%1RM	140			
Cable Row	3	8	75%1RM	110			
Machine Shoulder Press	3	8	75%1RM	130			
Lat Pulldown	3	8	75%1RM	110			
Cable Bicep Curl	3	8	65%1RM	50			

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

Steps for blood collection on Day 7 and 12 (Serum CRP and 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:

4. 1- 5mL serum separator tube (SST)

Immediately invert SST tube 5 times

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

4) 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) CRP: ~50µL (.05mL)
      ii) CK: ~50µL (.05mL)
      iii) 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:

8. 1- 5mL SST
9. Labeled subject tubes
10. 3 plastic tubes
11. Kim wipes
12. Disposable pipet
13. Tube racks
14. Subject bag

MEASURE	**SAMPLE**
Serum CRP	50µL
Serum CK	50µL

Steps for blood collection on Day 17 (Serum CRP, CK, SOD and Catalase)

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:
5. 1- 5mL serum separator tube (SST)

Immediately invert SST tube 5 times

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

5) 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) Catalase: ~100µL (.1mL)
      ii) SOD: ~50µL (.05mL)
      iii) CRP: ~50µL (.05mL)
      iv) CK: ~50µL (.05mL)
      v) 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:
15. 1- 5mL SST
16. Labeled subject tubes
17. 3 plastic tubes
18. Kim wipes
19. Disposable pipet
20. Tube racks
21. Subject bag

MEASURE	SAMPLE
Serum Catalase	100µL
Serum SOD	50µL
Serum CRP	50µL
Serum CK	50µL

Appendix D: Raw Data

Table 1: Weight Measurements- DHA Group

Subject	Day -28 (kg)	Day 1 (kg)	Day 2 (kg)	Day 4 (kg)	Day 7 (kg)	Day 12 (kg)	Day 17 (kg)	Weight Change (Day 17 - Day -28; kg)	Weight Change (Day 17 - Day 1; kg)
201	67.27	66.25	66.25	67.27	67.50	66.93	66.70	-0.57	0.45
202	52.95	54.43	55.00	54.20	54.55	53.98	53.75	0.80	-0.68
204	78.75	77.61	77.39	78.30	79.09	78.86	78.52	-0.23	0.91
205	73.75	75.57	74.89	75.80	76.25	77.16	78.52	4.77	2.95
207	88.98	87.73	87.61	87.05	87.95	88.30	87.61	-1.36	-0.11
208	68.86	70.34	70.68	70.57	71.14	69.43	69.55	0.68	-0.80
209	84.89	84.09	84.32	85.00	85.34	85.00	82.39	-2.50	-1.70
213	66.14	63.75	64.55	63.64	64.89	63.86	63.98	-2.16	0.23
217	109.77	108.86	110.68	109.09	111.36	110.23	111.14	1.36	2.27
219	65.57	66.48	66.02	66.36	67.16	67.84	66.59	1.02	0.11
224	85.91	89.66	89.32	89.89	88.98	89.09	89.20	3.30	-0.45
226	68.75	68.07	69.09	67.95	69.43	68.07	68.30	-0.45	0.23
227	66.48	65.91	64.55	66.70	66.25	66.25	66.36	-0.11	0.45
228	79.89	78.52	82.16	83.75	84.20	83.75	83.07	3.18	4.55
229	74.77	74.77	76.25	75.57	77.05	76.48	77.50	2.73	2.73
234	94.89	96.59	96.82	96.70	97.95	97.73	96.82	1.93	0.23
239	71.93	71.93	71.48	71.59	71.59	72.16	72.16	0.23	0.23
240	82.50	85.45	87.05	86.14	86.14	85.11	85.11	2.61	-0.34
242	76.82	77.27	77.27	76.70	76.82	77.95	76.82	0.00	-0.45
247	81.14	82.39	82.73	81.82	80.00	83.18	82.95	1.82	0.57
249	61.82	62.50	62.84	62.05	60.45	61.14	63.07	1.25	0.57
MEAN	**76.28**	**76.58**	**77.00**	**76.96**	**77.34**	**77.26**	**77.15**	**0.87**	**0.57**
SD	**12.58**	**12.70**	**12.99**	**12.83**	**13.13**	**13.11**	**13.00**	**1.85**	**1.45**

n=21

Table 2: Weight Measurements- PCB Group

Subject	Day -28 (kg)	Day 1 (kg)	Day 2 (kg)	Day 4 (kg)	Day 7 (kg)	Day 12 (kg)	Day 17 (kg)	Weight Change (Day 17 - Day -28; kg)	Weight Change (Day 17 - Day 1; kg)
203	79.77	79.32	79.09	79.09	80.34	80.11	80.00	0.23	0.68
206	90.91	92.84	93.30	92.73	94.66	93.41	92.16	1.25	-0.68
210	127.61	130.23	130.45	130.00	131.25	132.16	130.45	2.84	0.23
211	90.45	89.20	88.86	88.75	89.43	89.32	89.55	-0.91	0.34
212	77.84	79.55	79.43	78.41	80.80	77.84	77.73	-0.11	-1.82
214	78.30	78.64	78.30	77.50	78.45	76.82	77.39	-0.91	-1.25
215	76.14	77.27	76.70	75.91	76.36	75.68	76.14	0.00	-1.14
216	66.59	67.50	68.07	67.27	67.73	67.39	67.39	0.80	-0.11
218	81.02	80.68	80.80	81.36	81.25	79.20	79.77	-1.25	-0.91
220	62.61	63.52	62.73	62.73	63.64	63.18	63.64	1.02	0.11
222	66.93	65.80	66.82	68.30	69.20	69.09	69.32	2.39	3.52
225	78.52	78.64	77.95	78.86	77.95	78.30	79.09	0.57	0.45
230	124.89	125.57	125.00	125.23	125.34	125.63	125.91	1.02	0.34
232	56.70	56.59	56.36	56.48	56.93	59.77	56.93	0.23	0.34
233	95.45	95.68	95.00	96.36	96.25	95.34	94.32	-1.14	-1.36
241	67.61	66.93	66.93	67.16	66.48	66.25	65.91	-1.70	-1.02
243	63.64	64.43	65.34	63.98	64.55	65.00	63.86	0.23	-0.57
244	85.23	85.57	85.57	84.77	84.09	84.66	84.66	-0.57	-0.91
246	71.36	70.34	70.80	70.34	70.45	70.68	71.48	0.11	1.14
250	70.11	70.68	70.80	70.34	70.91	70.11	70.91	0.80	0.23
MEAN	**80.59**	**80.95**	**80.91**	**80.78**	**81.30**	**81.00**	**80.83**	**0.24**	**-0.12**
SD	**18.62**	**19.04**	**18.94**	**18.99**	**19.08**	**19.07**	**18.95**	**1.16**	**1.17**

n=20

Table 3: Baseline Subject Characteristics- DHA Group

Subject	BF (%)	BW (kg)	BMI (kg/m^2)	AGE (years)
201	10.74	67.27	21.80	23
202	8.30	52.95	18.60	26
204	12.31	78.73	22.40	25
205	15.90	73.73	21.40	22
207	18.16	89.00	26.30	21
208	12.24	68.90	24.60	23
209	21.97	84.90	28.20	25
213	10.30	66.14	22.83	19
217	24.78	109.80	33.60	24
219	5.51	65.57	20.60	22
224	18.91	85.90	26.00	18
226	8.42	68.75	21.30	20
227	8.95	66.50	21.30	22
228	10.30	79.90	23.20	19
229	11.64	74.80	25.30	19
234	22.04	94.89	37.65	20
239	5.73	71.93	20.09	27
240	16.61	82.50	25.01	22
242	18.99	76.82	24.83	23
247	17.99	81.14	27.60	28
249	10.30	61.80	20.60	19
MEAN	**13.81**	**76.28**	**24.44**	**22.24**
SD	**5.61**	**12.58**	**4.56**	**2.84**

n=21

Table 4: Baseline Subject Characteristics- PCB Group

Subject	BF (%)	BW (kg)	BMI (kg/m^2)	AGE (years)
203	16.70	79.73	25.60	24
206	20.48	90.91	27.70	27
210	19.50	127.61	36.12	22
211	15.45	90.45	24.92	23
212	18.07	77.84	24.79	19
214	15.62	78.30	23.40	22
215	17.31	76.10	24.20	22
216	13.26	66.60	20.50	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	78.50	27.10	20
230	23.94	124.90	40.96	20
232	7.80	56.70	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	21.30	19
250	11.61	70.11	23.50	20
MEAN	**15.91**	**80.58**	**25.35**	**21.25**
SD	**4.98**	**18.62**	**5.40**	**2.51**

n=20

Table 5: Supplement Compliance- DHA Group

Subject	Starting (Bottle 1)	Ending (Bottle 1)	Removed from 1st Bottle	Starting (Bottle 2)	Ending (Bottle 2)	Removed from 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**	**18.48**	**18.48**	**0.00**	**12.40**	**12.40**	**18.46**

n=21

DNR- did not return

Table 6: Supplement Compliance- PCB Group

Subject	Starting (Bottle 1)	Ending (Bottle 1)	Removed from 1st Bottle	Starting (Bottle 2)	Ending (Bottle 2)	Removed from 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
250	130	10	120	130	27	103	223
MEAN	**130.00**	**17.35**	**112.65**	**130.00**	**76.29**	**53.71**	**163.94**
SD	**0.00**	**13.08**	**13.08**	**0.00**	**15.16**	**15.16**	**20.60**

n=20

DNR- did not return

Table 7: Serum DHA Data- DHA Group Day -28

Percentage of Total Fatty Acids in Serum (%)						
Subject	**Treatment**	**Day**	**Linoleic Acid**	**Arachidonic Acid**	**EPA**	**DHA**
201	DHA	-28	33.9263	5.7779	0.2635	0.8139
202	DHA	-28	39.1606	7.4670	0.3530	0.8028
204	DHA	-28	33.5394	7.0263	0.2857	0.8355
205	DHA	-28	27.4990	4.2123	0.4510	0.7231
207	DHA	-28	28.7555	5.1267	1.0595	2.0096
208	DHA	-28	37.5895	5.7117	0.2615	1.2363
209	DHA	-28	36.5480	8.6086	0.5833	1.9573
213	DHA	-28	39.0856	7.2431	0.3744	0.9592
217	DHA	-28	35.4427	8.1475	0.2024	1.6171
219	DHA	-28	31.0759	5.2840	0.3864	1.0067
224	DHA	-28	29.5525	6.1974	0.4211	0.9058
226	DHA	-28	38.6991	8.7404	0.4406	1.7063
227	DHA	-28	33.1459	7.0175	0.3876	1.3523
228	DHA	-28	36.2688	8.8556	0.2726	1.4825
229	DHA	-28	36.1399	9.7539	0.4896	1.6808
234	DHA	-28	33.4579	4.5438	0.2843	0.6963
239	DHA	-28	37.3617	6.1697	0.2169	1.5279
240	DHA	-28	30.2654	8.5783	0.5459	0.8069
242	DHA	-28	34.1062	7.6607	0.3981	1.1979
247	DHA	-28	34.1485	5.9279	0.3483	1.6152
249	DHA	-28	31.4174	6.0034	0.6508	1.1579
MEAN			**34.15**	**6.86**	**0.41**	**1.24**
SD			**3.44**	**1.54**	**0.19**	**0.42**

n=21

Table 8: Serum DHA Data- DHA Group Day 1

Percentage of Total Fatty Acids in Serum (%)						
Subject	**Treatment**	**Day**	**Linoleic Acid**	**Arachidonic Acid**	**EPA**	**DHA**
201	DHA	1	35.3357	5.4483	0.5969	4.9107
202	DHA	1	38.8954	4.9045	1.1207	5.3627
204	DHA	1	35.2486	5.8040	0.4860	4.3214
205	DHA	1	34.2691	6.2399	0.6222	4.4283
207	DHA	1	29.8996	6.0322	0.8951	5.5104
208	DHA	1	35.8147	4.8090	0.6524	5.0521
209	DHA	1	34.4425	7.2237	0.8280	5.2837
213	DHA	1	35.6028	6.2125	0.7658	4.2188
217	DHA	1	32.8643	6.5478	0.5469	5.5728
219	DHA	1	33.9784	3.8887	0.7489	5.2128
224	DHA	1	31.8924	4.7989	0.3413	3.0525
226	DHA	1	39.8122	8.3899	0.6247	4.3995
227	DHA	1	35.1812	4.3935	0.6456	5.9540
228	DHA	1	36.9759	6.1519	0.6842	4.5975
229	DHA	1	34.5189	7.2452	0.7396	5.3393
234	DHA	1	32.6904	7.6331	0.4721	5.1925
239	DHA	1	40.6670	4.8579	0.6040	4.3343
240	DHA	1	36.6655	6.6371	0.6935	2.5898
242	DHA	1	33.4829	7.5248	0.5044	5.0756
247	DHA	1	36.4187	5.9973	0.2379	4.8616
249	DHA	1	36.2430	4.7765	0.8384	4.3170
MEAN			**35.28**	**5.98**	**0.65**	**4.74**
SD			**2.55**	**1.20**	**0.19**	**0.81**

n=21

Table 9: Serum DHA Data- PCB Group Day -28

Percentage of Total Fatty Acids in Serum (%)						
Subject	**Treatment**	**Day**	**Linoleic Acid**	**Arachidonic Acid**	**EPA**	**DHA**
203	Placebo	-28	30.5455	6.3574	0.6096	1.9790
206	Placebo	-28	26.1264	7.0526	0.2280	0.8658
210	Placebo	-28	35.7134	6.1689	0.2733	0.8683
211	Placebo	-28	31.7393	8.8550	0.3855	0.9098
212	Placebo	-28	33.3667	5.3338	0.3050	0.9866
214	Placebo	-28	34.1360	10.9922	0.6010	1.8759
215	Placebo	-28	30.7475	5.3077	0.3622	0.8623
216	Placebo	-28	31.4863	7.9276	0.4833	1.3351
218	Placebo	-28	35.9960	9.6136	0.4322	1.5029
220	Placebo	-28	32.5558	8.2643	0.2552	1.1356
222	Placebo	-28	33.5631	7.5323	0.5436	1.4062
225	Placebo	-28	36.7638	4.6942	0.3644	1.3374
230	Placebo	-28	27.7719	6.0722	0.4546	1.2532
232	Placebo	-28	37.0204	7.9845	0.2751	0.6619
233	Placebo	-28	37.4138	5.4855	0.2080	1.1942
241	Placebo	-28	32.4156	7.9731	0.5301	2.1773
243	Placebo	-28	36.8890	7.9618	0.2984	1.3077
244	Placebo	-28	35.3755	8.6198	0.5762	1.2778
246	Placebo	-28	25.5763	7.6732	0.6268	1.0578
250	Placebo	-28	33.9545	6.3349	0.4709	1.1173
MEAN			**32.96**	**7.31**	**0.41**	**1.26**
SD			**3.51**	**1.60**	**0.14**	**0.39**

n=20

Table 10: Serum DHA Data- PCB Group Day 1

Percentage of Total Fatty Acids in Serum (%)						
Subject	**Treatment**	**Day**	**Linoleic Acid**	**Arachidonic Acid**	**EPA**	**DHA**
203	Placebo	1	29.8923	6.9033	0.5352	1.8593
206	Placebo	1	31.1174	7.6354	0.3120	0.8556
210	Placebo	1	26.1120	5.0191	0.2788	0.7902
211	Placebo	1	29.4873	9.7900	0.6502	1.0301
212	Placebo	1	33.9587	5.1368	0.2874	0.7758
214	Placebo	1	33.3855	9.5858	0.4134	1.5736
215	Placebo	1	37.0541	6.6678	0.3127	0.8751
216	Placebo	1	36.5347	8.3926	0.3260	1.2095
218	Placebo	1	36.7554	9.0335	0.5651	1.4054
220	Placebo	1	34.0481	7.9634	0.2576	1.1417
222	Placebo	1	33.1274	10.2301	0.4533	1.6981
225	Placebo	1	32.4833	5.1651	0.6078	1.6211
230	Placebo	1	32.2051	6.2296	0.2959	1.1116
232	Placebo	1	40.2775	5.0682	0.1966	0.7233
233	Placebo	1	33.2243	4.2092	0.3227	1.3891
241	Placebo	1	28.4727	5.0839	0.5426	2.2245
243	Placebo	1	37.4108	6.3070	0.6546	0.9563
244	Placebo	1	35.3817	8.2172	0.4528	1.1545
246	Placebo	1	27.1509	8.9984	0.5939	1.0954
250	Placebo	1	40.6189	6.2767	0.6223	0.8537
MEAN			**33.43**	**7.10**	**0.43**	**1.22**
SD			**4.01**	**1.85**	**0.15**	**0.41**

n=20

Table 11: Resistance Training Estimated 1RM Data- DHA Group

Subject	Total Sum (kg)	Leg Press (kg)	Machine Bench Press (kg)	Cable Row (kg)	Machine Shoulder Press (kg)	Lat Pulldown (kg)	Cable Biceps Curl (kg)
201	461	134	67	80	72	71	38
202	296	35	53	61	56	60	30
204	556	123	92	88	111	90	52
205	414	113	51	67	71	69	42
207	543	185	74	72	92	77	44
208	604	256	87	65	84	70	41
209	580	246	72	69	71	69	54
213	477	150	76	74	64	73	41
217	602	280	76	74	61	67	44
219	421	98	74	71	69	67	42
224	489	167	75	67	59	82	40
226	485	172	75	71	51	77	40
227	467	162	72	65	61	72	35
228	560	189	90	77	79	85	41
229	579	260	79	65	53	76	45
234	657	310	92	85	67	64	40
239	631	183	107	99	95	87	59
240	593	200	90	76	92	79	55
242	628	204	104	87	97	82	53
247	476	156	67	77	77	61	38
249	410	105	72	71	61	71	30
MEAN	**520.6**	**177.5**	**78.4**	**74.4**	**73.6**	**73.7**	**43.0**
SD	**91.6**	**66.9**	**14.3**	**9.2**	**16.4**	**8.3**	**7.8**

n=21

Table 12: Resistance Training Estimated 1RM Data- PCB Group

Subject	Total Sum (kg)	Leg Press (kg)	Machine Bench Press (kg)	Cable Row (kg)	Machine Shoulder Press (kg)	Lat Pulldown (kg)	Cable Biceps Curl (kg)
203	579	215	87	67	90	77	44
206	492	161	64	74	76	72	45
210	559	213	72	80	82	72	41
211	497	161	82	69	80	69	36
212	448	183	69	64	48	56	28
214	545	161	95	82	95	73	37
215	489	145	74	71	90	73	36
216	486	123	85	67	90	79	42
218	515	194	71	69	71	67	44
220	424	138	69	61	71	61	23
222	670	241	90	88	116	82	53
225	661	271	82	99	67	101	42
230	560	232	79	77	56	69	46
232	359	85	62	56	62	60	35
233	545	228	76	64	73	64	41
241	513	167	80	76	72	67	52
243	436	115	77	65	76	64	38
244	615	233	93	76	92	69	51
246	580	200	90	88	82	77	44
250	472	141	72	88	61	71	40
MEAN	**522.4**	**180.3**	**78.4**	**74.0**	**77.6**	**71.1**	**40.9**
SD	**78.2**	**48.6**	**9.6**	**10.8**	**15.7**	**9.6**	**7.5**

n=20

Table 13: Resistance Training Difficulty Rating: DHA Group

Subject	RT Difficulty Rating
201	7
202	4
204	9
205	8
207	4
208	6
209	4
213	8
217	6
219	7
224	7
226	7
227	7
228	7
229	7
234	6
239	8
240	N/A
242	7
247	5
249	5
MEAN	**6.45**
SD	**1.43**

n=20

Table 14: Resistance Training Difficulty Rating: PCB Group

Subject	RT Difficulty Rating
203	7
206	7
210	7
211	8
212	6
214	7
215	8
216	7
218	7
220	6
222	5
225	6
230	7
232	8
233	6
241	5
243	7
244	6
246	7
250	4
MEAN	**6.55**
SD	**1.05**

n=20

Table 15: Serum Creatine Kinase (CK) Data- DHA Group

Creatine Kinase (U/L) for DHA Treatment						
	Day					
Subject	**1**	**2**	**4**	**7**	**12**	**17**
201	110	106	237	4265	448	168
202	153	111	86	1935	593	204
204	165	169	122	831	574	316
205	314	337	159	571	398	224
207	100	149	157	320	204	307
208	135	1269	2189	15293	1106	405
209	48	125	2177	9229	577	135
213	160	318	66	4031	740	162
217	75	126	73	9579	821	114
219	147	855	2423	18326	1327	235
224	456	329	297	5999	679	228
226	274	200	256	223	167	229
227	87	126	187	1529	614	235
228	71	242	635	680	720	129
229	185	1253	33900	11783	1446	396
234	97	455	11379	3848	582	148
239	80	133	284	1809	363	363
240	84	158	3247	2394	238	141
242	115	142	132	569	178	223
247	103	508	8042	6386	539	167
249	106	148	140	1274	588	162
MEAN	**146**	**346**	**3152**	**4804**	**614**	**223**
SD	**96**	**353**	**7621**	**5223**	**344**	**88**

n=21

Table 16: Serum Creatine Kinase (CK) Data- PCB Group

Creatine Kinase (U/L) for Placebo Treatment						
	Day					
Subject	**1**	**2**	**4**	**7**	**12**	**17**
203	74	677	3708	4825	733	147
206	140	250	124	577	391	204
210	259	734	4463	3052	516	233
211	74	472	7663	2690	233	124
212	211	779	10308	13015	1421	254
214	180	1550	16464	2143	902	247
215	143	141	140	346	356	132
216	224	160	76	561	167	142
218	256	219	251	351	252	513
220	54	450	14956	11404	420	359
222	261	770	540	5777	847	248
225	156	192	3156	10271	657	254
230	122	384	882	667	418	169
232	68	102	923	5199	708	237
233	1424	2178	5760	3988	305	110
241	97	237	111	396	254	109
243	290	273	246	4820	481	148
244	457	1371	13604	3972	1188	1188
246	66	119	2131	1558	221	134
250	195	202	219	931	999	448
MEAN	**238**	**563**	**4286**	**3827**	**573**	**270**
SD	**296**	**555**	**5433**	**3815**	**350**	**242**

n=20

Table 17: Serum C-Reactive Protein (CRP) Data: DHA Group

C-Reactive Protein Data (mg/L) for DHA Treatment						
	Day					
Subject	**1**	**2**	**4**	**7**	**12**	**17**
201	0.184	0.152	0.111	0.340	0.155	0.305
202	0.527	0.282	0.186	7.791	0.758	1.025
204	0.351	0.594	0.351	0.432	0.524	0.616
205	0.540	0.696	0.628	1.003	1.158	1.313
207	0.107	0.112	0.075	0.146	0.168	0.173
208	0.085	0.123	0.500	0.986	0.146	0.122
209	0.176	0.250	0.221	0.826	0.292	0.252
213	0.801	0.588	0.282	2.859	0.603	0.180
217	3.450	5.694	6.285	3.336	1.578	0.866
219	0.176	0.235	0.217	0.519	0.098	0.267
224	2.260	2.093	1.931	2.801	4.199	7.684
226	1.444	1.045	0.603	0.419	0.673	0.434
227	0.127	0.101	0.110	0.285	0.708	0.195
228	0.316	1.986	1.071	0.447	0.524	0.275
229	0.434	0.451	1.305	2.240	0.705	0.541
234	3.151	5.850	8.635	5.970	5.985	7.899
239	0.743	0.734	0.576	1.291	1.128	1.128
240	0.552	0.545	0.580	1.005	0.518	0.306
242	1.974	2.916	2.700	8.716	6.590	3.995
247	1.325	1.994	2.042	5.477	1.783	1.316
249	0.076	0.071	0.059	0.131	0.105	0.065
MEAN	**0.895**	**1.262**	**1.356**	**2.239**	**1.352**	**1.379**
SD	**1.0114322**	**1.696**	**2.186**	**2.604**	**1.873**	**2.295**

n=21

Table 18: Serum C-Reactive Protein (CRP) Data: PCB Group

C-Reactive Protein Data (mg/L) for Placebo Treatment						
	Day					
Subject	**1**	**2**	**4**	**7**	**12**	**17**
203	2.693	4.249	4.071	7.242	2.376	2.641
206	1.573	3.262	3.406	2.887	2.688	2.488
210	1.484	1.603	1.733	6.174	2.628	1.886
211	2.466	2.644	2.480	4.601	1.653	2.213
212	0.357	0.384	0.285	1.979	0.391	0.244
214	1.235	2.275	1.799	6.175	3.789	1.295
215	7.908	5.244	2.197	3.792	4.220	12.224
216	0.283	0.473	0.567	0.367	0.325	0.413
218	1.373	1.671	3.672	1.506	5.278	2.418
220	0.661	0.422	0.285	0.358	0.161	1.435
222	0.262	1.029	2.904	1.744	0.802	0.559
225	0.912	1.592	0.946	2.087	0.719	0.709
230	4.322	5.541	4.554	3.305	3.096	2.886
232	0.635	0.648	0.501	1.554	0.751	0.928
233	3.104	2.602	1.985	3.664	2.436	1.745
241	1.629	1.079	0.439	0.113	0.089	0.023
243	0.671	0.868	0.537	1.426	0.495	0.328
244	1.025	1.200	0.801	0.799	0.560	0.560
246	0.920	8.658	3.906	4.566	0.343	1.122
250	0.335	0.305	0.260	0.184	0.180	0.116
MEAN	**1.692**	**2.287**	**1.866**	**2.726**	**1.649**	**1.812**
SD	**1.807**	**2.171**	**1.458**	**2.154**	**1.563**	**2.615**

n=20

Appendix E: Statistical Summary

Table 1: Resistance Training Estimated 1RM Analysis

t Test

PCB-DHA

Assuming equal variances

Difference	3.96	t Ratio	0.067532
Std Err Dif	58.67	DF	39
Upper CL Dif	122.63	Prob > |t|	0.9465
Lower CL Dif	-114.70	Prob > t	0.4733
Confidence	0.95	Prob < t	0.5267

Table 2: Serum DHA Analysis

t Test at Day -28
PCB-DHA
Assuming equal variances

Difference	0.01316	t Ratio	0.103741
Std Err Dif	0.12687	DF	39
Upper CL Dif	0.26979	Prob > |t|	0.9179
Lower CL Dif	-0.24347	Prob > t	0.4590
Confidence	0.95	Prob < t	0.5410

Repeated Measures ANOVA
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 3: Maximum Isometric Strength (ISO) Analysis

t Test at Day 1

PCB-DHA

Assuming equal variances

Difference	-1.4660	t Ratio	-1.04195
Std Err Dif	1.4070	DF	285
Upper CL Dif	1.3034	Prob > |t|	0.2983
Lower CL Dif	-4.2354	Prob > t	0.8508
Confidence	0.95	Prob < t	0.1492

Repeated Measures ANOVA

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	0.3444	0.5607
Day	6	6	234	82.1966	<.0001*
Treatment*Day	6	6	234	0.8462	0.5354

Table 4: Delayed Onset Muscle Soreness (DOMS) Analysis

t Test at Day 2

PCB-DHA

Assuming equal variances

Difference	7.893	t Ratio	1.22756
Std Err Dif	6.430	DF	39
Upper CL Dif	20.898	Prob > |t|	0.2270
Lower CL Dif	-5.112	Prob > t	0.1135
Confidence	0.95	Prob < t	0.8865

Repeated Measures ANOVA

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	3.0937	0.0864
Day	5	5	195	96.5046	<.0001*
Treatment*Day	5	5	195	0.4711	0.7975

Table 5: Range of Motion (ROM) Analysis

t Test at Day 1
PCB-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

Repeated Measures ANOVA
Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	0.0869	0.7697
Day	6	6	234	65.3756	<.0001*
Treatment*Day	6	6	234	1.3904	0.2192

Table 6: Natural Log of Creatine Kinase (CK) Analysis

t Test at Day 1
PCB-DHA
Assuming equal variances

Difference	0.28313	t Ratio	1.36988128
Std Err Dif	0.20668	DF	39
Upper CL Dif	0.70118	Prob > |t|	0.1786
Lower CL Dif	-0.13492	Prob > t	0.0893
Confidence	0.95	Prob < t	0.9107

Repeated Measures ANOVA
Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	1.2722	0.2663
Day	5	5	195	51.7892	<.0001*
Treatment*Day	5	5	195	1.9211	0.0925

Table 7: C-Reactive Protein (CRP) Analysis

t Test at Day 1
PCB-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

Repeated Measures ANOVA
Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	1	1	39	1.3932	0.2450
Day	5	5	195	3.9653	0.0019*
Treatment*Day	5	5	195	0.4240	0.8317

Table 8: Resistance Training Rating Analysis

t Test

PCB-DHA

Assuming equal variances

Difference	0.10000	t Ratio	0.251871
Std Err Dif	0.39703	DF	38
Upper CL Dif	0.90374	Prob > |t|	0.8025
Lower CL Dif	-0.70374	Prob > t	0.4012
Confidence	0.95	Prob < t	0.5988

Table 9: Subject Age Analysis

t Test
PCB-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

Table 10: Body Fat Percentage Analysis

t Test
PCB-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

Table 11: Body Mass Index Analysis

t Test

PCB-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

Table 12: Baseline Weight Analysis

t Test
PCB-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

Table 13: Weight Change Analysis

t Test
PCB-DHA
Assuming equal variances

Difference	-1.5125	t Ratio	-1.6672
Std Err Dif	0.9072	DF	39
Upper CL Dif	0.3225	Prob > |t|	0.1035
Lower CL Dif	-3.3475	Prob > t	0.9483
Confidence	0.95	Prob < t	0.0517

Table 14: Area Under the Curve ISO Analysis

t Test

PCB-DHA

Assuming equal variances

Difference	-33.42	t Ratio	-0.74145
Std Err Dif	45.07	DF	39
Upper CL Dif	57.75	Prob > |t|	0.4629
Lower CL Dif	-124.59	Prob > t	0.7686
Confidence	0.95	Prob < t	0.2314

Table 15: Area Under the Curve DOMS Analysis

t Test

PCB-DHA

Assuming equal variances

Difference	65.86	t Ratio	2.01231
Std Err Dif	32.73	DF	39
Upper CL Dif	132.07	Prob > |t|	0.0511
Lower CL Dif	-0.34	Prob > t	0.0256*
Confidence	0.95	Prob < t	0.9744

Table 16: Area Under the Curve ROM Analysis

t Test

PCB-DHA

Assuming equal variances

Difference	38.56	t Ratio	0.440814
Std Err Dif	87.47	DF	39
Upper CL Dif	215.47	Prob > |t|	0.6618
Lower CL Dif	-138.36	Prob > t	0.3309
Confidence	0.95	Prob < t	0.6691

Table 17: Area Under the Curve Natural Log of CK Analysis

t Test

PCB-DHA

Assuming equal variances

Difference	2.1335	t Ratio	0.56893
Std Err Dif	3.7499	DF	39
Upper CL Dif	9.7184	Prob > |t|	0.5727
Lower CL Dif	-5.4515	Prob > t	0.2863
Confidence	0.95	Prob < t	0.7137

Table 18: Area Under the Curve CRP Analysis

t Test
PCB-DHA
Assuming equal variances

Difference	7.727	t Ratio	0.918871
Std Err Dif	8.409	DF	39
Upper CL Dif	24.736	Prob > |t|	0.3638
Lower CL Dif	-9.282	Prob > t	0.1819
Confidence	0.95	Prob < t	0.8181

Table 19: AUC of Markers Correlation Analysis

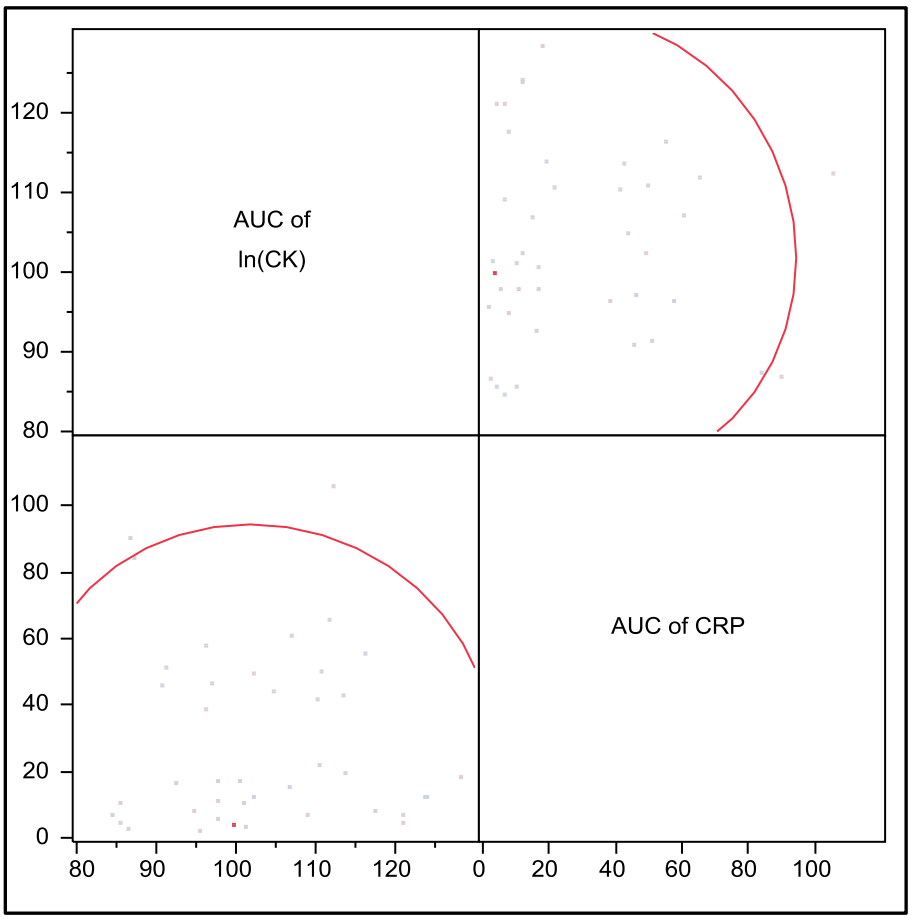
Correlations

	AUC of lnCK	AUC of CRP	AUC of ROM	AUC of DOMS	AUC of ISO
AUC of lnCK	1	-0.0498	-0.1176	-0.0201	0.0387
AUC of CRP	-0.0498	1	-0.0728	-0.022	0.1165
AUC of ROM	-0.1176	-0.0728	1	-0.0015	0.3629*
AUC of DOMS	-0.0201	-0.022	-0.0015	1	-0.0292
AUC of ISO	0.0387	0.1165	0.3629*	-0.0292	1

CI of Correlation

Variable	by Variable	Correlation	Lower 95%	Upper 95%
AUC of CRP	AUC of ln(CK)	-0.0498	-0.3521	0.2619
AUC of ROM	AUC of ln(CK)	-0.1176	-0.4104	0.1972
AUC of ROM	AUC of CRP	-0.0728	-0.3721	0.2403
AUC of DOMS	AUC of ln(CK)	-0.0201	-0.3257	0.2894
AUC of DOMS	AUC of CRP	-0.0220	-0.3275	0.2876
AUC of DOMS	AUC of ROM	-0.0015	-0.3090	0.3063
AUC of ISO	AUC of ln(CK)	0.0387	-0.2722	0.3423
AUC of ISO	AUC of CRP	0.1165	-0.1983	0.4095
AUC of ISO	AUC of ROM	0.3629*	0.0622	0.6032
AUC of ISO	AUC of DOMS	-0.0292	-0.3339	0.2809

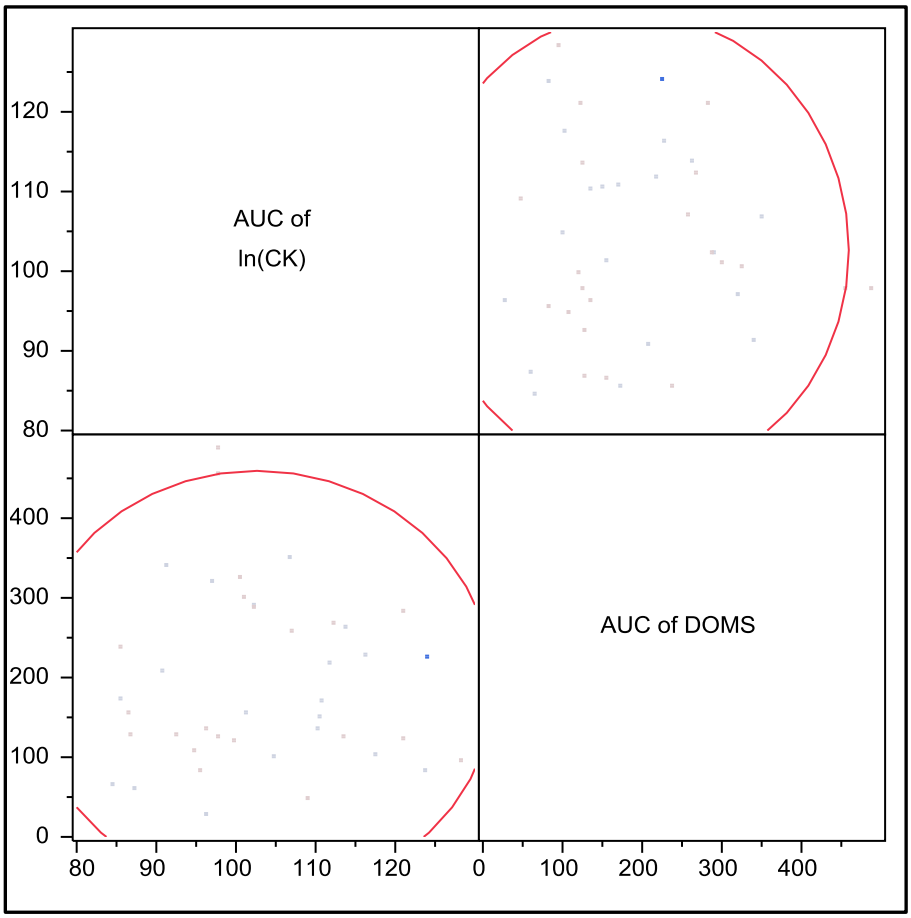
Table 20: Bivariate Fit of AUC of lnCK by AUC of CRP



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of lnCK	103.227	11.9002	-0.0498	0.7572	41
AUC of CRP	28.6644	26.8618			

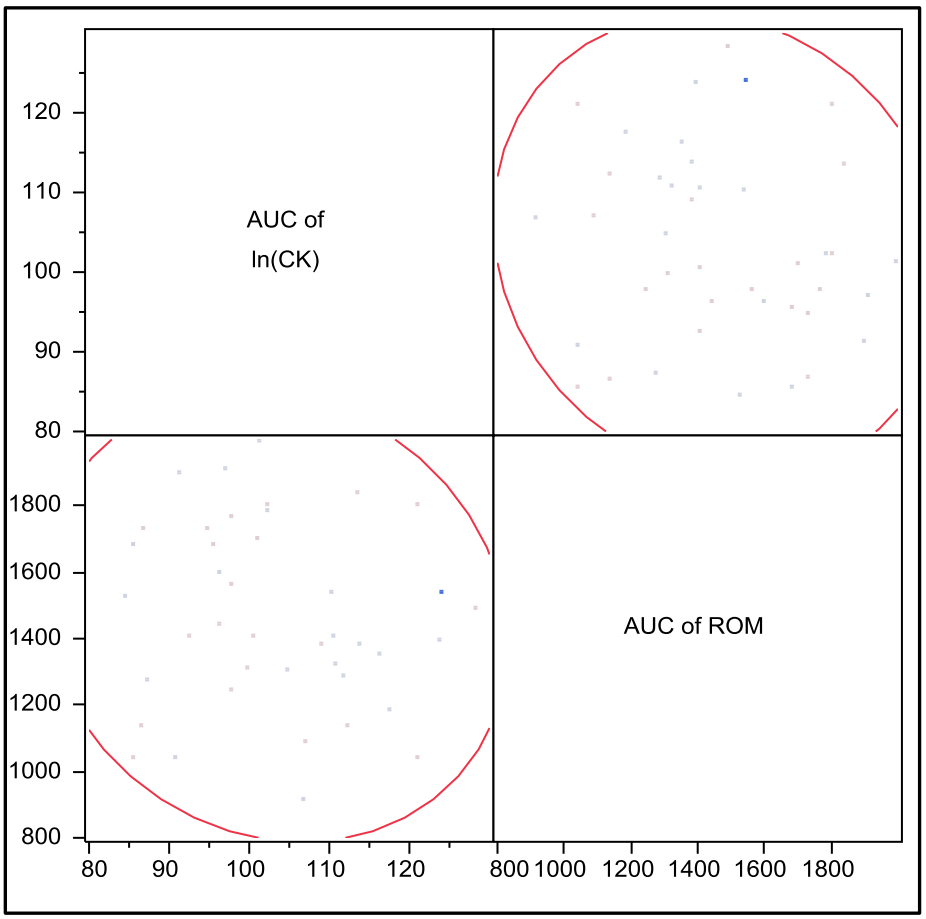
Table 21: Bivariate Fit of AUC of lnCK by AUC of DOMS



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of lnCK	103.227	11.9002	-0.0201	0.9008	41
AUC of DOMS	193.415	108.678			

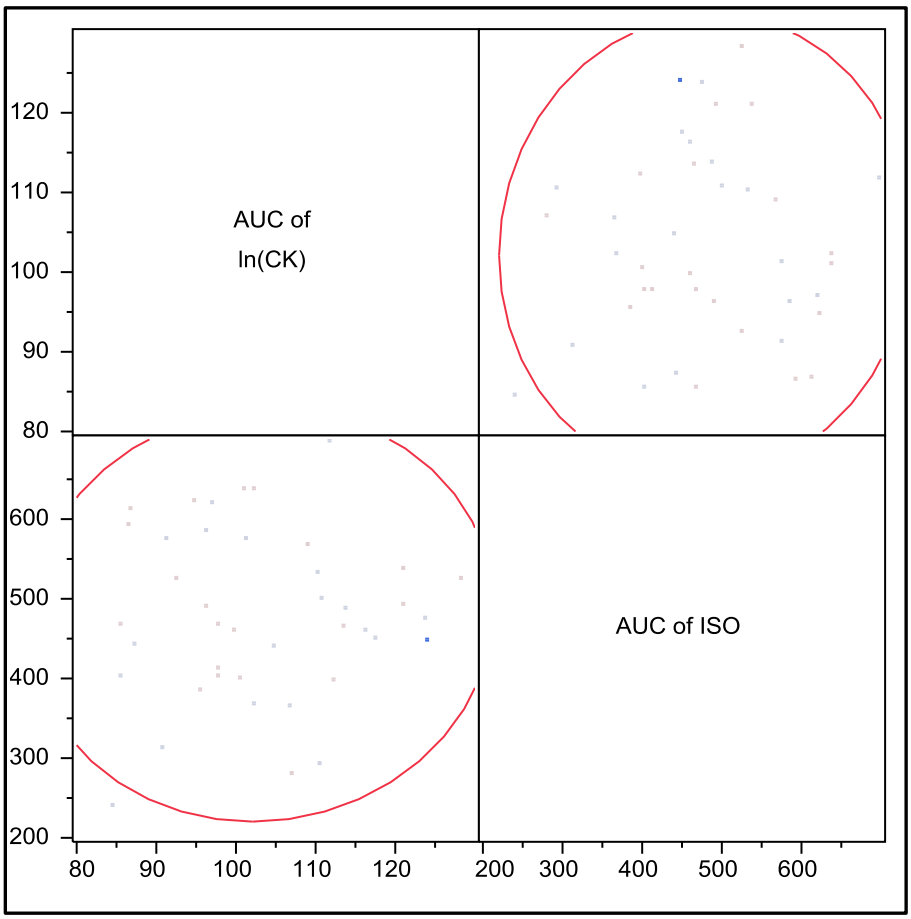
Table 22: Bivariate Fit of AUC of lnCK by AUC of ROM



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of lnCK	103.227	11.9002	-0.1176	0.4641	41
AUC of ROM	1464.93	277.109			

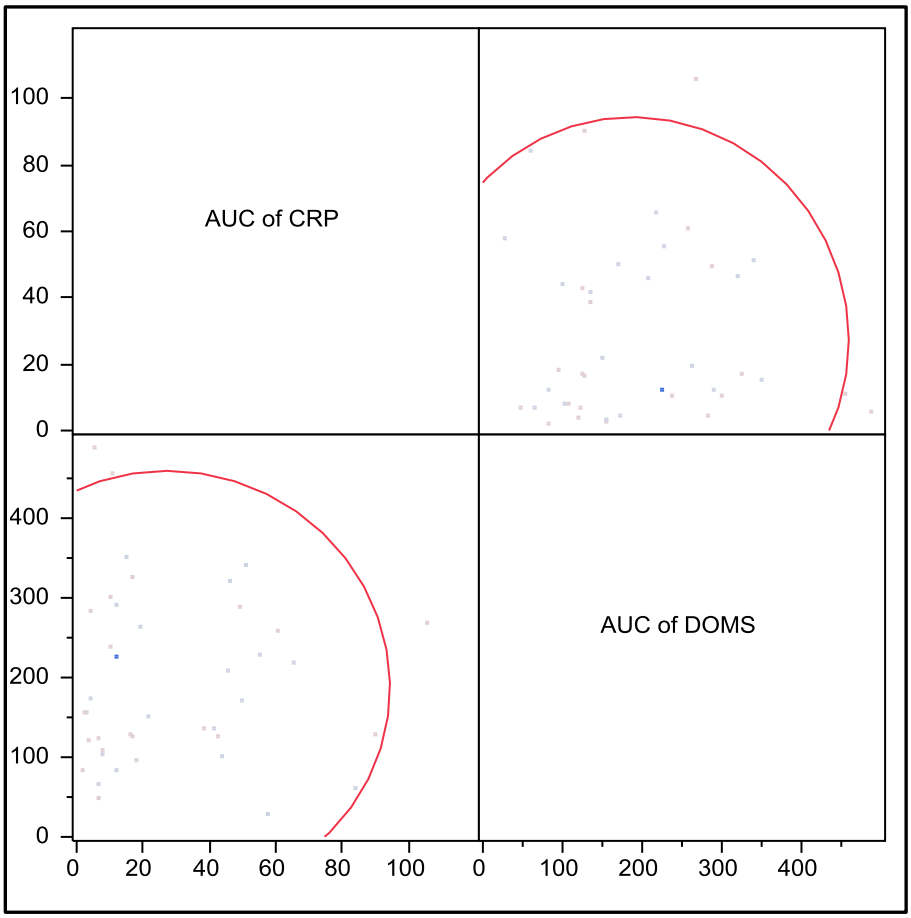
Table 23: Bivariate Fit of AUC of lnCK by AUC of ISO



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of lnCK	103.227	11.9002	0.0387	0.8099	41
AUC of ISO	479.288	105.803			

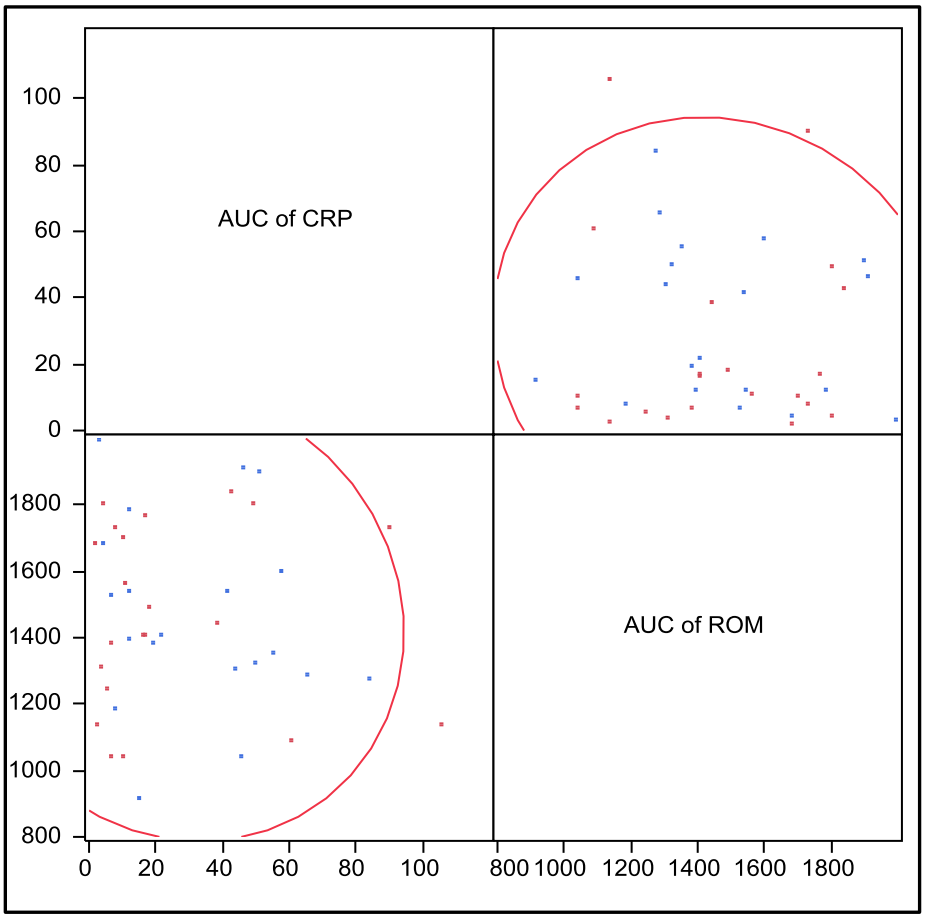
Table 24: Bivariate Fit of AUC of CRP by AUC of DOMS



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of CRP	28.6644	26.8618	-0.0220	0.8912	41
AUC of DOMS	193.415	108.678			

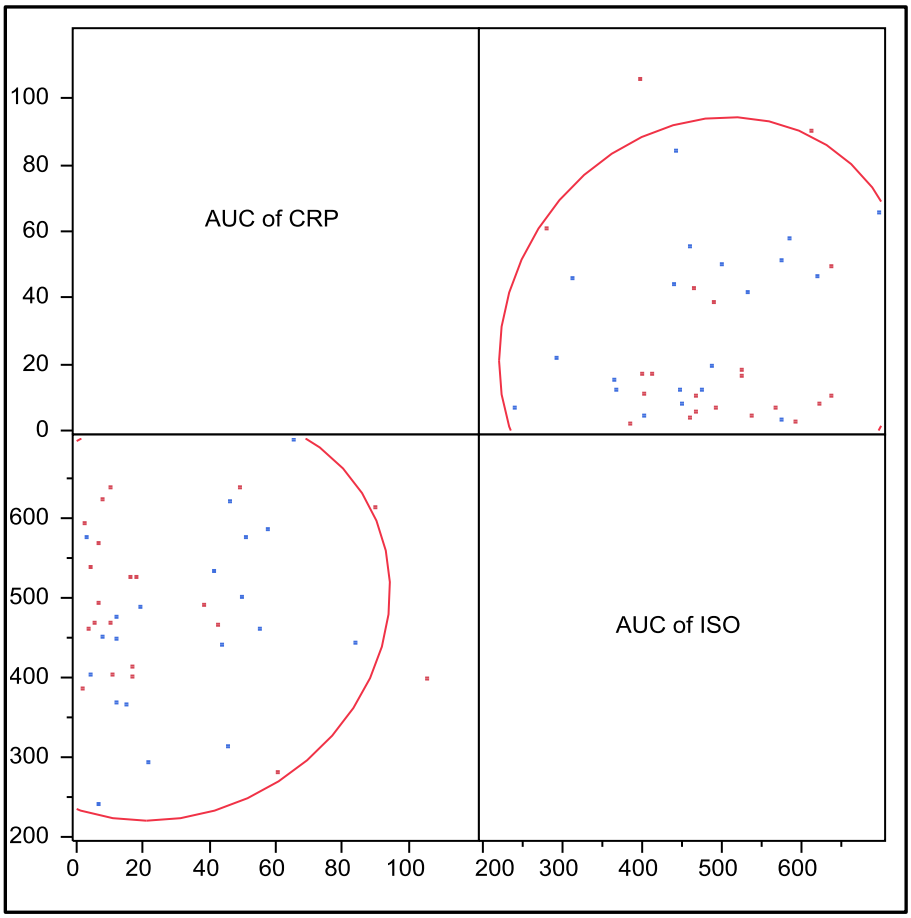
Table 25: Bivariate Fit of AUC of CRP by AUC of ROM



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of CRP	28.6644	26.8618	-0.0728	0.6512	41
AUC of ROM	1464.93	277.109			

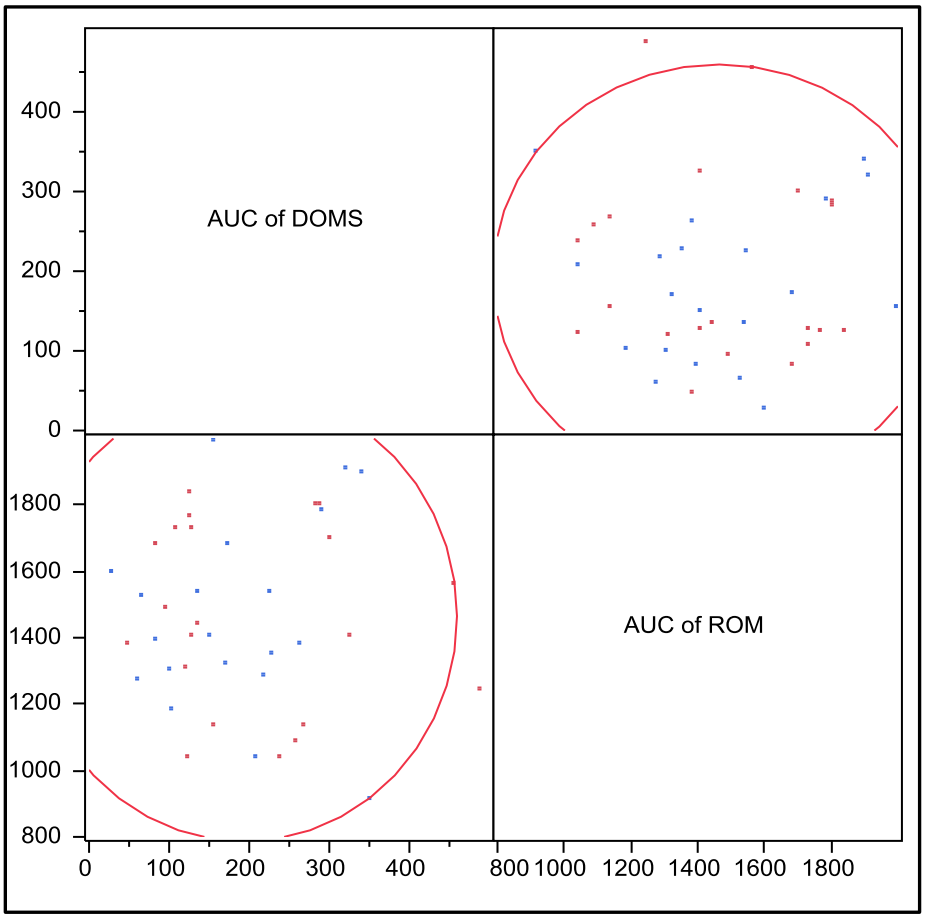
Table 26: Bivariate Fit of AUC of CRP by AUC of ISO



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of CRP	28.6644	26.8618	0.1165	0.4682	41
AUC of ISO	479.288	105.803			

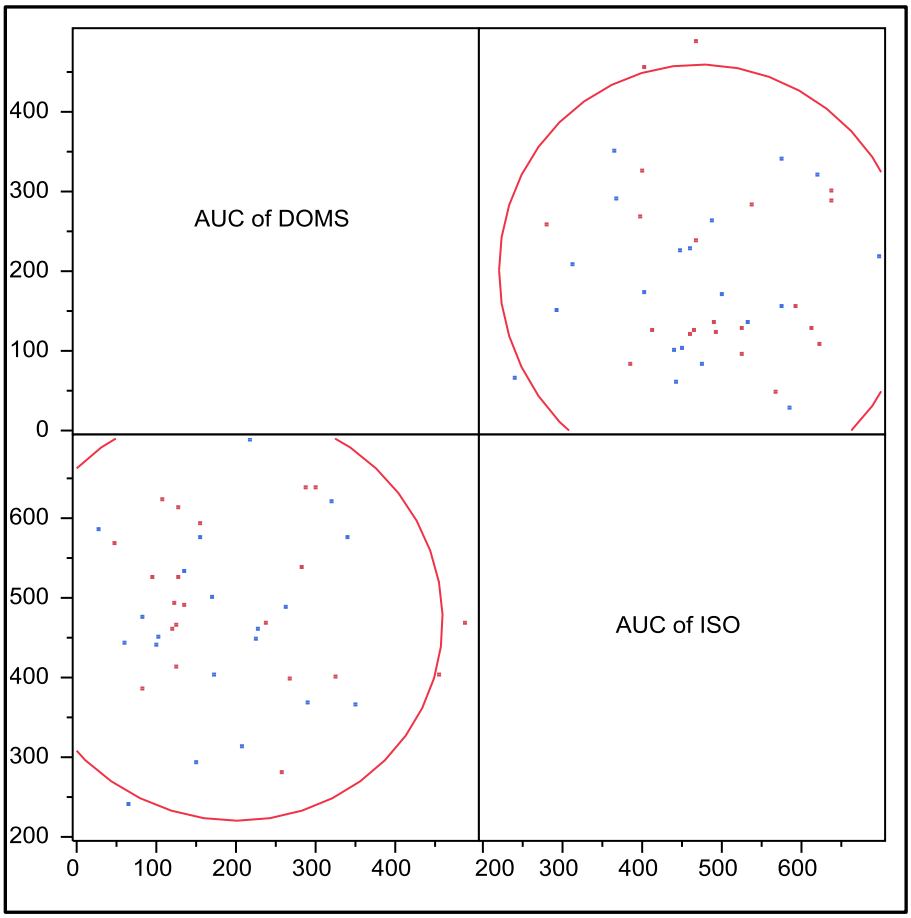
Table 27: Bivariate Fit of AUC of DOMS by AUC of ROM



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of DOMS	193.415	108.678	-0.0015	0.9927	41
AUC of ROM	1464.93	277.109			

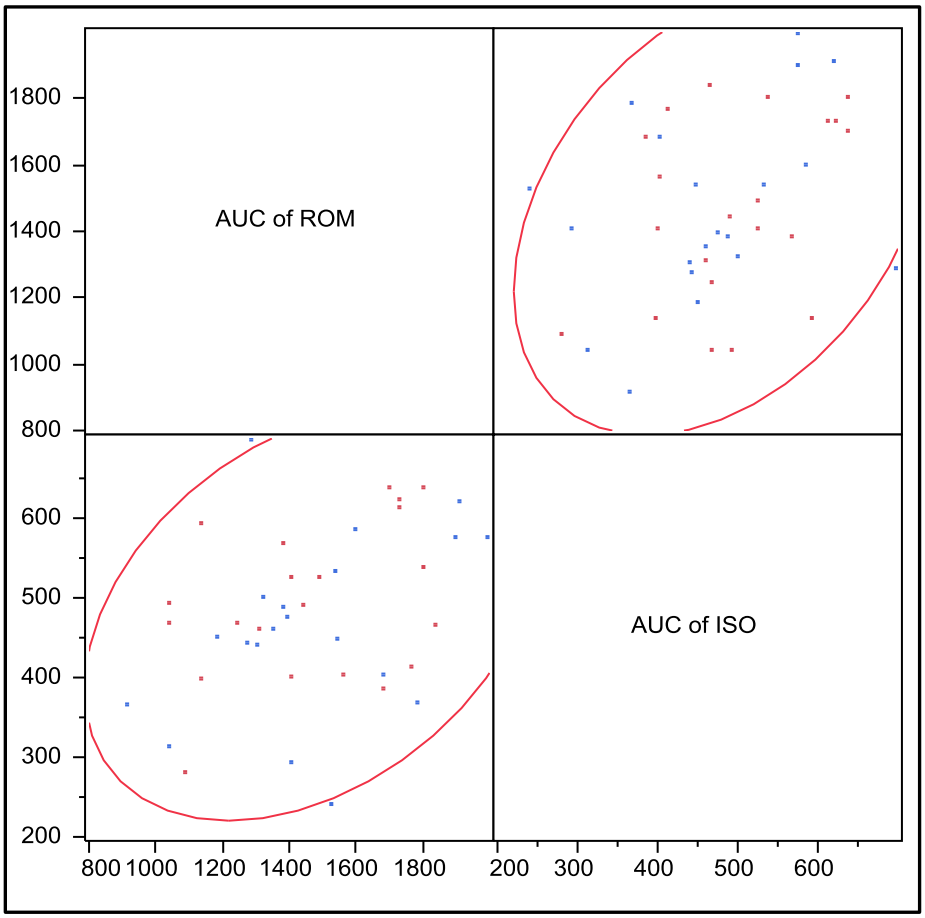
Table 28: Bivariate Fit of AUC of DOMS by AUC of ISO



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of DOMS	193.415	108.678	-0.0292	0.8560	41
AUC of ISO	479.288	105.803			

Table 29: Bivariate Fit of AUC of ROM by AUC of ISO



Correlation

Variable	Mean	Std Dev	Correlation	Signif Prob	Number
AUC of ROM	1464.93	277.109	0.3629	0.0197*	41
AUC of ISO	479.288	105.803			