The Effects of Acute Omega-3 Fatty Acid Supplementation on Delayed-Onset Muscle Soreness and Recovery

Colin A. Butler

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Delayed-onset muscle soreness (DOMS) is the feeling of discomfort that occurs after being exposed to unaccustomed eccentric resistance training often resulting in diminished athletic performance. Previous research has shown positive effects with omega-3 fatty acid (O3FA) supplementation to ameliorate DOMS. The purpose of this study was to investigate the effects of acute O3FA supplementation on perceived muscle soreness, ratings of exertion, and recovery after a lower body resistance training protocol in college-aged males. A double blind, repeated-measures design was utilized with 10 healthy, college-age males. Participants were placed in the placebo (olive leaf oil) or experimental (O3FA) group, consuming 3,000 mg O3FA for seven days. Exercise protocol consisted of two testing sessions, separated by 24 hours, of the leg press, leg extension, and lying leg curl exercises at 75% 1RM completing three sets until failure for each exercise. Following a seven-day washout period, participants repeated the protocol in the opposite supplement group. A paired sample t-test (α = 0.05) was conducted for 1) total number of repetitions; 2) difference in number of repetitions; 3) perceived ratings of exertion; 4) 24-hour perceived muscle soreness; 5) and recovery status. O3FA supplementation was not effective in improving any variable tested when compared to the placebo: total number of repetitions between conditions (O: 96 ± 17, C: 90 ± 24, p = 0.45); difference in repetitions between tests (O: 6 ± 12, C: 14 ± 11, p = 0.19); RPE (Test 1: Mdn = 5.5, C: Mdn = 6.0, Z = 13.0, p = 0.86; Test 2: Mdn = 6.0, C: Mdn = 7.0, Z = 7, p = 0.88); 24 hour perceived muscle soreness (O: 26.8 ± 21.0, C: 33.7 ± 17.6, p = 0.47); and recovery status (O: Mdn = 6.0, C: Mdn = 4.0, Z = 25.5, p = 0.38). The results suggest that acute O3FA supplementation does not attenuate perceived muscle soreness or improve rating of exertion or recovery 24 hours following induction of DOMS.

INDEX WORDS: Omega-3 fatty acids, Delayed-onset muscle soreness, Visual analog scale, Perceived recovery status, Rating of perceived exertion, College of Graduate Studies, Georgia Southern University
THE EFFECTS OF ACUTE OMEGA-3 FATTY ACID SUPPLEMENTATION ON DELAYED-ONSET MUSCLE SORENESS AND RECOVERY

by

COLIN A BUTLER

B.S., Georgia Southern University, 2014

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DEDICATION

I am dedicating this to my family and friends who have supported me through my eight years in college and supported my decision to leave my previous job and return to school. I would also like to dedicate this to Kelvin O’Neal because without his help and tutelage for the last eight years, I would not have graduated with a Bachelor of Science, nor would I have ever had the opportunity to come back to school and complete this achievement. Finally, I want to dedicate this thesis to anyone who believes that strong work ethic, not talent, is the key to success.
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CHAPTER 1
INTRODUCTION

Purpose of the Study

The purpose of this study is to investigate the effects of acute, lasting seven to ten days, omega-3 fatty acid supplementation on perceived muscle soreness, ratings of exertion, and recovery after a lower body resistance training protocol in college aged males. Past research has shown omega-3 fatty acid (O3FA) supplements positively influence the effects of delayed-onset muscle soreness (DOMS), but there is little consistency in the methods used to induce DOMS, type of O3FA used, and supplementation dose or supplementation period. The information from this study may be helpful in determining whether O3FA supplementation could be used to accelerate recovery to a person’s baseline of sports performance.

How This Study Is Original

The present study deals with a particular age level in the range of 18 to 24 and a supplement protocol not yet paired with a lower body exercise protocol to induce muscle soreness in known research. To the author’s knowledge, this is the first study observing the effects of omega-3 fatty acid supplementation on delayed-onset muscle soreness using perceived recovery status as an evaluation method during acute supplementation. To the author’s knowledge, this is the first study evaluating total repetitions completed and the difference in repetitions completed between exercise training sessions with O3FA supplements. The author knew that college age individuals are more susceptible to muscle soreness than older or younger individuals, and made the assumption that the majority of college age males do not regularly engage in lower body exercises or eat a diet high in fatty fish. Each of the participants in the study completed a health history questionnaire and a food allergy questionnaire before being admitted to the study. The participants were eligible for a raffle at the end of the study. All of the participants were enrolled as full-time students at a southeastern university.
Delayed-onset muscle soreness (DOMS) is the feeling of discomfort experienced by elite and novice athletes that occurs after being exposed to unaccustomed eccentric resistance training as well as high-intensity, or long-duration exercise (Serravite, Perry, Jacobs, Adams, Harriell, & Signorile, 2014). Symptoms of DOMS include soreness, loss of range of motion (ROM), and a decrease in strength production during training which can negatively affect athletic performance (Serravite et al, 2014). Performance-inhibiting effects attributed to DOMS reaches its peak 24 to 48 hours after exercise (Contro, Mancuso, Proia, 2016; Gulick, Kimura, Sitler, Paolone, Kelly IV, 1996). Research has shown no evidence that the effects of DOMS from eccentric exercise are permanent, also showing that it may serve as a precursor to muscle adaptation with increased use (Armstrong, Warren, & Warren, 2016).

Classified as a Type I muscle strain, DOMS is caused by unaccustomed stretching and/or microtrauma of the muscle tissue during eccentric exercise (Cheung, Hume, & Maxwell, 2003; Ryan, Long, Bishop, Herron, & Katica, 2013). Eccentric exercise is well documented in being more effective than concentric or isometric exercise in generating muscle damage leading to DOMS (McHugh, Connolly, Eston, & Gleim, 1999). White blood cell (WBC) concentrations increase in both eccentric and concentric exercise, and Bazgir, Salesi, Koushki, and Amirghofran (2015) showed there was not a significant difference in increased WBC concentration between the two exercise modalities when comparing athletes and non-athletes.

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the body’s ability to detoxify free radicals in the body (Leeuwenburgh & Heinecke, 2001). During exercise, ROS production is stimulated due to an increase in leukocyte production caused by cell damage during an inflammatory response in the body which act as mediators of inflammation (Leeuwenburgh & Heinecke, 2001; Sousa, Teixeira, & Soares, 2014). The influx of calcium ions during an inflammatory response disrupts the sensory regulators in the sarcomeres which can play a role in the formation of DOMS following eccentric exercise.

Polyunsaturated fatty acids (PUFA) are essential fatty acids (FAs) that are not synthesized in the body and must be obtained in the diet. There are two classes of PUFAs, omega-3 fatty acids (O3FA) and
omega-6 fatty acids (O6FA) (Allam-Ndoul, Guenard, Barbier, & Vohl, 2016). O6FA produce arachidonic acid (AA) which produces pro-inflammatory cytokines while O3FA produce alpha linolenic acid (ALA) which produces anti-inflammatory cytokines like eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. EPA and DHA produce anti-inflammatory eicosanoids such as prostaglandins. (Allam-Ndoul, Guenard, Barbier, & Vohl, 2016). Prostaglandins are hormones that reduce inflammation in the body, improve blood flow, decrease swelling, and inhibit inflammatory WBC recruitment (Jouris, McDaniel, & Weiss, 2011).

Exercise-induced muscle soreness (EIMS) results from exhaustive or intense exercise that the body is unaccustomed to performing (Ryan, Long, Bishop, Herron, & Katica, 2013). Common symptoms of EIMD include a loss in force production, range of motion (ROM) and DOMS. DOMS causes tenderness or stiffness in the affected limbs and loss of strength caused by a decrease in ROM. The inability to effectively perform through a full ROM reduces the amount of muscle fibers recruited during training and competition. Lamb (2009) states that an influx of calcium ions from the cytoplasm can result in soreness and disrupt the excitation-contraction coupling process decreasing muscle fiber recruitment. This results in decreased force production and, therefore, decreased athletic performance (Haff & Triplett, 2016).

O3FA can be found naturally in fatty fish, nuts, green, leafy vegetables, fortified foods, and in supplement form. Fish sources include salmon, tuna, halibut, herring, and oysters (Gligor & Gligor, 2016). Nuts and seeds including walnuts and flaxseed are natural sources of polyunsaturated FAs (Gligor & Gligor, 2016). Green, leafy vegetables, such as Brussel sprouts, kale, spinach, broccoli, and cauliflower are good sources of ALA (Williams, Rawson, & Branch, 2017). Lastly, fish oils supplements are rich in O3FA and are the commonly used to supplement O3FA in the diet (DiLorenzo, Drager, & Rankin, 2014).

The success of past research suggests that it is important to investigate the effects O3FA have on recovery time to determine if O3FA helps athletes not only not feel the effects of DOMS, but to also determine if it helps return their performance to baseline levels. Chapman, Newton, McGuigan, and
Nosaka (2008) and Marginson, Rowlands, Gleeson, and Eston (2005), determined that college-age males (25 ± 6 years) are more likely to experience DOMS than males nine to ten years of old and elderly males (64 ± 4 years). It was hypothesized that this was due to younger and older males having fewer type II muscle fibers compared to college-age individuals. The younger population has not gone through puberty and had less trained type II muscle fibers, and the elderly population may have lost type II muscle fibers due to sarcopenia.

**Hypothesis:** Omega-3 fatty acid supplementation will significantly reduce the effects of delayed-onset muscle soreness resulting in a faster recovery period.

**Rationale:** Until recently, scientific research investigating the efficacy of omega-3 fatty acid supplementation on the effects of DOMS has been scarce. There are mixed results on the benefits of O3FA supplementation and DOMS typically depending on the supplementation and exercise protocols. Therefore, the purpose of this study is to investigate the effects of acute O3FA supplementation on perceived muscle soreness, ratings of exertion, and recovery after a lower body resistance training protocol. The information from this study may be helpful in determining whether O3FA supplementation could be used to accelerate recovery to an athlete’s baseline of sports performance. It is hypothesized that acute supplementation of O3FA at 3,000 mg per day for seven days will alleviate the effects of delayed-onset muscle soreness.

**Limitations:** Participants between the ages of 18-24 years will be recruited from physical education classes at a southeastern university. In order to participate in this study, individuals must be untrained in resistance training exercise. Another limitation is the supplementation protocol as there is conflicting research on the efficiency of acute O3FA supplementation on attenuating DOMS using acute supplementation protocols.

**Delimitations:** Participants in the study will be college-aged (18-24 years old) students from a southeast university. Criteria to participate will include individuals that are untrained in resistance training, have not had any lower body injuries in the last six months, and are not currently taking nonsteroidal anti-inflammatory drugs or eating an anti-inflammatory diet.
**Assumptions:** It will be assumed that every participant gives maximal effort during all testing protocols and each participant will be able to perform leg extension and lying leg curl exercises. In addition, it will be assumed that participants will properly follow supplementation, exercise, and nutrition protocols throughout the duration of the study.
CHAPTER 2
REVIEW OF LITERATURE
EXERCISE-INDUCED MUSCLE DAMAGE

Exercise-Induced Muscle Damage (EIMD) results from exhaustive or intense eccentric exercise that the body is not accustomed to performing (Sousa, Teixeira, & Soares, 2014). Symptoms of EIMD include a reduction in force production and range of motion, delayed onset muscle soreness (DOMS), and inflammation, which all result in a decreased athletic performance (Sousa, Teixeira, & Soares, 2014). Proske and Allen (2005) stated that following eccentric exercise, force production is inhibited for up to a week though recovery is completed with two hours post-exercise. This is likely due to muscle damage that is suffered during eccentric exercise.

MECHANISMS OF EIMD

There are three primary forms of resistance exercises; isometric, concentric, and eccentric exercise. These forms of resistance training vary based on the muscle action required to perform the exercise. Isometric exercise is characterized by a muscle contraction without muscle shortening or lengthening (Haff & Triplett, 2016; Mike, Cole, Herrera, VanDusseldorp, Kravitz, & Kerksick, 2017). Concentric exercise is characterized by a shorting of the muscle tissue without the eccentric, or muscle lengthening, phase during the exercise (Haff & Triplett, 2016). Eccentric exercise involves a contraction, muscle shortening, and an eccentric phase and is the type of exercise most commonly associated with DOMS (Mike, Cole, Herrera, VanDusseldorp, Kravitz, & Kerksick, 2017).

According to Proske and Allen (2005), a primary cause of DOMS stems from instability in the sarcomere length-tension curve. It was stated that during the eccentric phase of resistance training, some overlapping sarcomeres in the muscle fiber resist the lengthening of the muscle to the detriment of weaker sarcomeres that become stretched more frequently than others. Over time, these weaker sarcomeres become overstretched causing stronger sarcomeres to do more work causing those to also become overstretched. This causes a disruption of one or more sarcomere resulting in the disruption of adjacent sarcomeres and myofibrils causing muscle damage. If this occurs, membrane damage of the sarcoplasmic
reticulum will result due to structural damage caused by the prevalence of overstretched sarcomeres. While structural distortions to the sarcomeres and myofibrils are occurring during eccentric training, intracellular calcium (Ca\(^{2+}\)) moves into the sarcoplasm which begins the next stage of the damage process.

Another theory suggested by Proske and Allen (2005) suggests that muscle damage is caused by changes in the excitation-contraction (E-C) uncoupling caused by intracellular Ca\(^{2+}\) movement into the sarcomere. However, this theory does not explain changes in the length-tension relation of the muscle, which can lead to increases in muscle length.

A popular theory regarding the secondary mechanisms of EIMD points to the role of Ca\(^{2+}\) influx into the muscle cell caused by muscle damage. An excessive influx of Ca\(^{2+}\), caused by an increased permeability of the sarcolemma, leading to an accumulation of Ca\(^{2+}\) in the muscle cell is involved in various processes that lead to muscle cell damage and DOMS (Gissel, 2005). An increase in intracellular Ca\(^{2+}\) activates PLA\(_2\) which attacks the mitochondria as well as other membrane phospholipids releasing free fatty acids, such as arachidonic acid (Gissel, 2005; Connolly, Sayers, & McHugh, 2003). Arachidonic acid may increase reactive oxygen species (ROS) production damaging cell structures, which will stimulate an inflammatory response in muscle cells leading to DOMS.

INFLAMMATORY MECHANISMS

Each of the theories regarding the primary and secondary mechanisms of EIMD ultimately leads to an inflammatory response in the muscle cell. Phospholipase A\(_2\) production results in arachidonic acid being separated from the cell membrane and signals the production of eicosanoids (prostaglandins, thromboxanes, and leukotrienes), which amplify the initial signs of inflammation (Serhan, Chiang & Van Dyke, 2008; Connolly, Sayers, & McHugh, 2003). Prostaglandin E\(_2\) is responsible for the sensation of pain during DOMS by stimulating type III and IV pain receptors while leukotrienes attract neutrophils to the site of damage (Connolly, Sayers, & McHugh, 2003). Neutrophils will enter damaged tissue causing further damage by stimulating the production of free radicals during phagocytosis (Connolly, Sayers, & McHugh, 2003). The production of eicosanoids is upregulated by the production of pro-inflammatory
cytokines [tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and interleukin-6 (IL-6)] after exercise (MacIntyre, Reid, & McKenzie, 1995). These cytokines play various roles in the body including facilitating the influx of lymphocytes, neutrophils, and monocytes into the cell to clear antigens and begin healing damaged tissue (Ostrowski, Rhode, Asp, Schjerling, Pedersen, 1999). TNF-alpha and IL-1 share similar roles in the body in that they are responsible for the production of acute-phase response and fever following exercise as well as and both can stimulate the release of IL-6. IL-6 is released when nerve injuries have occurred and has been shown to play a role in nerve regeneration by inducing apoptosis of neutrophils and macrophages (Zhang & An, 2007; MacIntyre, Reid, & McKenzie, 1995; Bucci, 2000).

**DELAYED-ONSET MUSCLE SORENESS**

DOMS, classified as a type I muscle strain, shows symptoms including tenderness or stiffness in the affected limb, loss of strength and range of motion, and soreness (Cheung, Hume, & Maxwell, 2003; Serravite, Perry, Jacobs, Adams, Harriell, & Signorile, 2014). Symptoms begin approximately 8 hours following exercise, reach their peak between 24 to 48 hours following unaccustomed exercise, and progressively decrease with time (Sayers, Dannecker, 2004; Cheung, Hume, & Maxwell, 2003).

In 2005, a study by Cleary and Kendrick (2005) investigated the effects of hydration status on DOMS and muscular strength in ten healthy males between the ages of 18 and 35 who regularly engaged in lower body exercise within the last six months. Participants performed a heat-stress test by walking in a controlled hot, humid environment for 60 minutes. The euhydrated group was permitted to drink water during the exercise protocol while the dehydrated group was fluid restricted to induce dehydration. Following the heat-stress trial, participants underwent a DOMS-inducing workout consisting of a 45-minute downhill run and were evaluated for DOMS within 60 minutes of the downhill run. In the study, muscular strength was significantly (p < 0.05) less in the dehydrated group by 10.5% 24 hours following exercise. There was also a significant increase in DOMS in the fluid restricted group (p < 0.001) at 24- and 48-hours following exercise protocol. Theories regarding the causes of EIMD and DOMS suggest an increase in inflammation of muscle tissue. Results of this study concluded that hydration status affects perceived soreness and muscle strength following eccentric exercise. It also showed that higher ratings of
perceived soreness accompanied a decrease in muscle strength 24 hours when compared to 72- and 96-hours following exercise.

**ELICITING DOMS**

To date, the majority of studies examining the effects or treatment of DOMS use eccentric exercise to induce soreness with most of the subjects participants being untrained or recreationally trained college-aged volunteers as it elicits a greater response to DOMS than professionally trained athletes (Ryan, Bishop, Herron, & Katica, 2013; Serravite, Perry, Jacobs, Adams, Harriell, & Signorile, 2014; Mike, Cole, Herrera, VanDusseldorp, Kravitz, & Kerksick, 2017; Houghton, & Onambele, 2012; Nosaka & Newton, 2002; Corder, Newsham, McDaniel, Ezekiel, & Weiss, 2016; Gray, Chappell, Jenkinson, Thies, & Gray, 2014). Though these studies used untrained or recreationally trained individuals, not all exercise protocols elicited an adequate DOMS response following exercise.

Age appears to be an important factor to consider when eliciting DOMS because college age individuals (18 to 24 years) are more likely to develop DOMS than older or younger individuals. Chapman, Newton, McGuigan, and Nosaka (2008) compared the reaction of DOMS between college-aged (25 ± 6 years; n = 10) and older (64 ± 4 years; n = 10) men following eccentric exercise. With the exception of age, no other demographic profiles were significantly (p < 0.05) different between groups. Participants were untrained individuals engaging in less than one structured resistance training session in the previous six months. Both groups completed five sets of six maximal lengthening bicep curl contractions with 90 seconds of rest between sets. Total work performed was not significantly (p > 0.05) different between groups though the old group had a greater (p > 0.05) decline in strength one hour following exercise. 24 to 72 hours following exercise, perceived muscle soreness was significantly lower (p > 0.05) in the old group than the young group. Based on the findings of this study, it was concluded that college-aged individuals are more likely to experience DOMS than older individuals.

In a similar study, Marginson, Rowlands, Gleeson, and Eston (2005) investigated the effects of DOMS on college-aged and younger individuals. 10 young boys (9.9 ± 0.3 years) and 10 men (22.2 ± 2.7 years) completed eight sets of ten plyometric jumps to induce DOMS two times, separated by two weeks.
Isometric strength of the quadriceps and perceived soreness were measured 30 minutes, 24 hours, 48 hours, and 72 hours following exercise. Perceived soreness was significantly (p < 0.05) higher in men at 30 minutes, 24, 48, and 72 hours following exercise in both bouts and a significant (p < 0.05) decrease in strength was observed in men at the same intervals. Perceived soreness for the younger group was significantly increased 30 minutes and 24 hours following exercise in the first bout and no significance (p < 0.05) was observed in the second bout for either soreness or isometric strength. It was concluded that college-aged men were more likely to experience the effects of DOMS, including perceived soreness and less of strength than younger individuals following damage inducing exercise.

In both studies, it was concluded that a difference in type of muscle fiber type and number of type II muscle fibers caused the difference in soreness as type II muscle fibers are more susceptible to soreness. Type II muscle fibers are less developed in prepubescent boys whereas older men may have fewer type II muscle fibers due to sarcopenia (Chapman, Newton, McGuigan, & Nosaka, 2008).

To further support the role of type II muscle fibers in eliciting DOMS, Macaluso, Isaacs, and Myburgh (2012) tested eight untrained individuals (22 ± 1 years) by performing ten sets of ten squat-jumps with one minute of rest in between sets. The purpose of this study was to determine the percent of muscle fibers utilized in an eccentric exercise. Perceived soreness of the knee extensor was measured using a VAS, and a muscle biopsy of the vastus lateralis was performed to measure what muscle fibers were damaged following eccentric exercise. All participants completed ten sets of ten squat jumps with one minute of rest between sets. Creatine kinase levels increased, but were not significant, and remained elevated for one day following exercise. Perceived soreness from squat jumps increased (p < 0.05) 6 hours following exercise and peaked at two days. Results found that 7.6% of type I fibers, 10.3% of type IIa fibers, and 14.3% type IIx muscle fibers were used by assessing muscle damage and measuring dystrophin staining. It was concluded that type II muscle fibers perform the majority of the work during eccentric exercise and, therefore, experience the most damage.

MEASURING THE EFFECTS OF DOMS
Using a visual analog scale (VAS) to determine changes in perceived pain has been shown to be an effective way in evaluating the success or failure of an exercise protocol (Jouris, McDaniel, & Weiss, 2011; Corder, Newsham, McDaniel, Ezekiel, & Weiss, 2016; Nosaka & Newton, 2002; Mike, Cole, Herrera, VanDusseldorp, Kravitz, & Kerksick, 2017; Gulick, Kimura, Sitler, Paolone, & Kelly IV, 1996). Using a VAS to measure perceived soreness has been validated as a reliable method in previous research (Gallagher, Bijur, Latimer, and Silver, 2002). The most common method of using a VAS involves using a ten-centimeter line on a piece of paper with zero (no pain) on the left and ten (very painful) on the right side of the line. The participant places a mark on the line where they perceive their pain to be. A ruler is then used to measure the distance in centimeters from the left side of the line to the mark placed by the participant.

**FATTY ACID SUPPLEMENTATION AND DOMS**

In recent years, more research has been conducted investigating the possible anti-inflammatory benefits of O3FA supplementation on athletic performance in untrained, recreationally trained, and collegiate athletes. The most common forms of O3FA are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which produces alpha linolenic acid (ALA) to produce anti-inflammatory cytokines. O3FA are found in fatty fishes (salmon and tuna), walnuts, and chia seeds (Pickova, 2009; Baran, Kelly, Kress, Landgraf, & Weiss, 2015).

There is some variation in the supplementation protocol in the research, ranging from acute supplementation (approximately one week) to more long-term chronic supplementation (approximately a month or longer). Studies following acute supplementation protocol prescribe 3,000 mg of EPA and DHA per day, which is the United States Food and Drug Administration’s safe limit for O3FA daily intake (Food and Drug Administration, 2004). The United States FDA does not specify values for EPA or DHA for acute or chronic supplementation. In research, dosages for chronic supplementation range from 1.8 grams to 2.4 grams per day (Tsuchiya, Yanagimoto, Nakazato, Hayamizu, & Ochi, 2016; Rajabi, Lofti, Abdolmaleki, & Rashid-Amiri, 2013; Gray, Chappell, Jenkinson, Thies, & Gray, 2014; Tartibian, Maleki, & Abbasi, 2009; Lenn et al., 2002; DiLorenzo, Drager, & Rankin, 2014).
In 2009, Tartibian, Maleki, and Abbasi (2009) evaluated the effects of O3FA supplementation in 27 untrained men (experimental group, n = 9; placebo, n = 9, control, n = 9; 33.4 ± 4.2 years) for perceived pain and ROM following 30 days of omega-3 supplementation. Participants consumed 1.8 grams of O3FA per day prior to the start of exercise and 48 hours following exercise. The O3FA group also took 100 IU of d-alpha-tocopherol/d-alpha-tocopherol acetate to minimize long chain fatty acid oxidation during the study. Participants performed 40 minutes of eccentric exercise consisting of bench stepping for five minutes with one minute of rest between stepping periods at a pace of 15 steps per minute. There was no significant difference (p > 0.05) for perceived soreness between the control and O3FA groups immediately after exercise or 24 hours later. 48 hours after exercise, perceived pain was significantly greater (p < 0.001) in the control group, experiencing more pain. The same result was seen in ROM 48 hours following exercise with the control group having a decreased ROM when compared to the O3FA group (p = 0.031). Results from this study concluded that ingestion of 1.8 grams of O3FA per day can alleviate the effects of DOMS induced by eccentric exercise.

Lenn et al. (2002) used a similar supplementation protocol as the previous mentioned study (Tartibian, Maleki, and Abbasi, 2009) but did not report any anti-inflammatory benefits from O3FA supplementation. Ten men (22.7 ± 3.92 years) and six women (24.5 ± 5.47 years) completed the study. Participants were divided into three groups: fish oil group (n=7) receiving 1.8 grams of O3FA and wheat flour, isoflavone group (n=8) receiving 120 mg of soy isolate and Western fat blend, and placebo group (n=7) receiving comparable amounts of Western fat blend or wheat flour compared to the other two groups. All groups followed supplementation protocol for 30 days prior to exercise and during the week of the exercise protocol. All groups received 100 IU of d-alpha-tocopherol/d-alpha-tocopherol acetate to minimize long chain fatty acid oxidation. Following supplementation protocol, participants performed 50 maximal effort eccentric bicep curl contractions using a KinCom Dynamometer. Participants returned on days 2, 4, and 7 following exercise to determine measures for soreness and ROM. Soreness and ROM were significantly changed (p < 0.05) from day zero to day two in all groups but there was no significance observed between groups in that time period. Unlike the findings of Tartibian, Maleki, and Abbasi
(2009), there was not an indication that O3FA supplementation could help reduce the symptoms of DOMS.

Gray, Chappell, Jenkinson, Thies, and Gray (2014) showed six weeks of 3,000 mg of fish oil supplementation reduced markers of oxidative stress but did not alleviate perceived muscle soreness resulting from eccentric exercise. 20 recreationally active males (23 ± 2.3 years) were divided into two groups: placebo (n=10) and fish oil (n=10). The placebo group took 3 grams of olive oil while the fish oil group took 3 grams of fish oil per day for six weeks prior to an exercise protocol consisting of 200 eccentric knee contractions. Perceived muscle soreness increased significantly (p < 0.05) in both groups 48 hours following exercise indicating that the exercise protocol effectively elicited a response, however, there was no difference between groups (p > 0.05). Creatine kinase levels significantly increased (p<0.05) in both groups 48 hours following exercise with no interaction seen observed between the groups (p > 0.05). Thiobarbituric acid reactive substances (TBARS) serum levels, a byproduct of lipid peroxidation, was significantly lower (p<0.05) in the fish oil group at 48 hours than the control group. A limitation of the study was although fish intake was monitored every week during the study, a full nutritional evaluation was not made. This could raise the possibility that caloric and other nutrient intake may play a role in the efficacy of O3FA supplementation to alleviate the effects of DOMS.

Santos et al. (2012) monitored seventeen male participants in the military (18.6 ± 0.5 years) who were placed into one of two groups: placebo (n=9) or supplement group (n=8) for four weeks. The supplement group received 1,000mg of O3FA and the placebo group received a 700 mg of a gel with no caloric value, three times per day. The placebo group received a similar capsule as the supplement group that contained 80 mg of maltodextrin three times per day. Creatine kinase and C-reactive protein were measured before supplementation, following three days of no physical activity, the night before the start of boot camp, and on the third night of boot camp. The boot camp regimen consisted of five days controlled, caloric restriction with each participant receiving 3,000 to 3,600 calories per day. On the fourth day of boot camp, participants began military survival training. Only on the third day were participants permitted to sleep for exactly two hours. Creatine kinase levels were lower in the supplement
group on the third day of boot camp, but it was not significant when compared to the placebo group. C-reactive protein serum levels were significantly lower (p < 0.05) in the supplement group on the third night of boot camp compared to the placebo group. It was concluded that despite caloric deprivation common with boot camp, O3FA supplementation for three weeks was still effective in attenuating inflammatory activity in these participants.

In 2016, Tsuchiya, Yanagimoto, Nakazato, Hayamizu, & Ochi evaluated the effects of eight weeks of O3FA supplementation on maximal voluntary contractions (MVC) and ROM. Twenty-four healthy men (19.5 ± 0.8 years) were placed into either a placebo group (n=12), which received 2,400 mg of corn oil per day or the supplement group (n=12), which received 2,400 mg of omega-3 supplement. Perceived muscle soreness was evaluated using a VAS, and ROM was measured using a goniometer to determine differences in elbow extension. Using a Biodex Multi-Joint System 3, participants completed five sets of six repetitions of elbow flexors. The participants were told to resist the movement of the isokinetic arm, moving at 30° per second, for three seconds per repetition. Participants were evaluated two, three, and five days following eccentric exercise. MVC was significantly higher (p < 0.05) in the supplement group than the control group on days two, three, and five. Elbow ROM was significantly higher (p < 0.05) in the supplement group when compared to the control group at days two, three, and five. Perceived muscle soreness was shown to be significantly higher (p < 0.05) in the control group three days following exercise, however there was no significant change in soreness from baseline measures to any re-testing point in either group. The study concluded that O3FA supplementation can attenuate the effects of DOMS when measuring strength and ROM.

Recent research has shown acute supplementation lasting approximately seven to ten days at 3,000 mg per day has been effective at reducing the effects of DOMS (Atashak, Sharafi, Azarbayjani, Stannard, Goli, & Haghighli, 2013; Corder, Newsham, McDaniel, Ezekiel, & Weiss, 2016; Jouris, McDaniel, & Weiss, 2011).

In a 2013 study, Atashak, Sharafi, Azarbayjani, Stannard, Goli, and Haghighli blood levels of oxidative stress, muscle damage, and markers of inflammation following seven days of 3,000 mg of
O3FA supplementation in twenty men were evaluated. Participants were placed in either an O3FA group (n=10; 20.24 ± 1.87 years) or a placebo group (n=10; 21.55 ± 2.34 years). The O3FA group received three pills of 1,000 mg of O3FA to be consumed at three meals throughout the day for seven days. The placebo group received a supplement visually similar to the O3FA group at the same times. Participants performed four sets of 10 repetitions with three minutes of rest for 120% of their 1RM for the leg press, leg extension, and leg curl exercises. Blood samples were collected one week prior to supplementation, immediately before exercise, and 24 hours following exercise protocol. 24 hours following exercise, C-reactive protein and creatine kinase serum levels were significantly lower (p < 0.001) in the placebo group but not the O3FA group. Based on this study, it can be concluded that 3,000 mg of O3FA supplementation for seven days can reduce markers of inflammation following a single session of strenuous resistance exercise.

In a study by Corder, Newsham, McDaniel, Ezekiel, and Weiss (2016), 27 women (33 ± 2 years) received 3,000 mg of DHA or a matching placebo pill containing corn or soy oil. The purpose of the study was to investigate the effects of DHA supplementation on markers of inflammation in healthy women following eccentric exercise. In order to elicit DOMS, participants completed 120% of their predetermined 1RM for 4 sets of eccentric bicep curls with their non-dominant arm until exhaustion with three minutes of rest between sets. Sets ended when the participant could no longer lower the weight over four seconds for two consecutive repetitions. Participants began taking supplements seven days prior to the exercise protocol and two days afterwards. Leading up to testing, perceived muscle soreness and stiffness, and serum C-reactive protein levels were measured following the exercise protocol. Palpated soreness, full extension soreness, and passive elbow soreness levels were all significantly increased (p < 0.05) from baseline testing to the 48-hour follow-up within groups. C-reactive protein serum levels were not significantly different in the DHA group (p=0.55) or the placebo group (p=0.46). Between groups, palpated soreness and ROM were significantly different 48 hours following exercise protocol with the placebo group being significantly higher (p < 0.05) compared to the O3FA group. It was suggested that a lack of an increase in C-reactive protein levels did not increase because single-arm eccentric bicep curls
do not produce an adequate inflammatory response. A limitation of the study is that it only looks at DHA supplementation and not a combination of EPA and DHA. It was concluded that acute supplementation of 3,000 mg of DHA may be beneficial for healthy women to minimize the effects of DOMS following eccentric exercise.

Using a similar supplementation and exercise protocol as the previous study, Jouris, McDaniel, and Weiss (2011) tested perceived muscle soreness in three men (37.0 ± 9.6 years) and eight women (34.1 ± 11.2 years) following 7 days of O3FA supplementation. All 11 participants completed both the control protocol and the O3FA protocol. Participants were asked to refrain from eating foods that contained O3FA for the duration of the study. During the O3FA protocol, participants were given five capsules totaling 3,000 mg per day of omega-3 supplement. 120% of their 1RM for eccentric bicep curls were performed for two sets to exhaustion. The set ended when the participant was unable to lower the weight for four seconds for two consecutive repetitions. Soreness significantly increased (p < 0.001) within all trials from the baseline measurement to 48 hours following the exercise protocol. Between the control and O3FA trials, weighted soreness (p=0.02) and fully extended soreness (p=0.004) were both significantly higher in the control trial than in the O3FA trial. Based on the findings of this study, it was concluded that the exercise protocol was adequate enough to elicit an inflammatory response and the supplementation protocol of 3,000 mg of O3FA for seven days is adequate in alleviating the effects of DOMS.

MECHANISMS OF O3FA

When the inflammatory response in the body is activated, inflammation must be resolved in order for the inflamed site to return to homeostasis and to prevent inflammation from spreading or becoming chronic (Serhan, Chiang, & Van Dyke, 2008). In order to return to homeostasis, leukocytes and debris must be removed from the affected area, which until recently, was considered to be a passive process but is now considered an active metabolic process (Serhan, Chiang, & Van Dyke, 2008). Passive processes in the body do not require energy; however, returning to homeostasis is an active process. Returning to
homeostasis following an inflammatory response involves rapidly initiating responses by cellular pathways to synthesize dual-acting anti-inflammatory and pro-resolution lipid mediators.

The first step to return to homeostasis in the body begins with the secretion of prostaglandins and leukotrienes which become active and begin to increase at the beginning signs of inflammation (Serhan, Chiang, & Van Dyke, 2008). Prostaglandin E$_2$ and prostaglandin D$_2$ activate enzymes to produce lipoxins, resolvins, and protectins. These pro-resolution molecules serve to promote homeostasis in the body by playing a role in stopping the entry of neutrophils into the inflamed areas, stimulate monocyte recruitment, and stimulate phagocytosis of microorganisms and apoptotic cells (Serhan, Chiang, & Van Dyke, 2008).

Lipid mediators, such as prostaglandins and leukotrienes, are secreted during an inflammatory response and compete with arachidonic acid (ADA) for eicosanoid generation. As inflammation continues, leukotriene B$_4$ production is ceased by neutrophils and instead begin to convert ADA to lipoxins. Lipoxins, specifically lipoxin A$_4$ and lipoxin B$_4$ serve as agonists to end inflammation and return the body to homeostasis by preventing neutrophils from entering inflamed sites (Serhan, Chiang, & Van Dyke, 2008). Lipoxin interaction with other cells does not stimulate further Ca$^{2+}$ influx which is a known reason for the start of an inflammatory response (Serhan, Chiang, & Van Dyke, 2008). Lipoxin A$_4$ also plays a role in reducing pain during inflammation by preventing organ fibrosis and acting on smooth muscle (Serhan, Chiang, & Van Dyke, 2008).

Aspirin inhibits prostaglandin biosynthesis and stimulates the creation of aspirin-triggered lipoxins (ATLs) (Serhan, Chiang, & Van Dyke, 2008). ATLs protect tissue during inflammation and play an important role in endogenous anti-inflammatory systems by stimulating haeme oxygenase (HO1). As seen in mice, aspirin stimulates lipoxins to produce nitric oxide preventing leukocytes from attaching themselves to endothelial cells. Furthermore, lipoxins and ATLs act on human T cells to block TNF-alpha, preventing the stimulation of an inflammatory response (Serhan, Chiang, & Van Dyke, 2008).

Resolvins are derived from EPA and DHA and are classified as E-series and D-series resolvins. Resolvin E1 prevents neutrophils from traveling across the endothelium to the site of inflammation
(Serhan, Chiang, & Van Dyke, 2008). Resolvin E2 also displays anti-inflammatory characteristics by preventing zymosan-initiated neutrophil infiltration (Serhan, Chiang, & Van Dyke, 2008). EPA-derived resolvins may be responsible for the anti-inflammatory characteristics attributed to O3FA demonstrating that 5-lipoxygenase in human leukocytes may signal the production of leukotrienes to inhibit an inflammatory response. DHA-derived resolvins, 17S and 17R D-series display anti-inflammatory characteristics particularly in the DHA-rich areas of the body like the brain, synapses, and retinas (Serhan, Chiang, & Van Dyke, 2008). Both 17S and 17D have been shown to block TNF-inducing transcripts for interleukin-1β which is expressed primarily during a neuronal injury (Serhan, Chiang, & Van Dyke, 2008).

DHA is converted from resolvins to protectins via lipoxygenase pathways to a 17S-hydroperoxide-containing intermediate (Serhan, Chiang, & Van Dyke, 2008). The 17S-hydroperoxide-containing intermediate is then converted to a 16(17)-epoxide by leukocytes which is then opened in these cells to protectin D1. Protecting D1 plays a role in inflammation by reducing TNF-alpha secretion and promoting T-cell apoptosis (Serhan, Chiang, & Van Dyke, 2008).
CHAPTER 3

METHODS

The purpose of this study was to investigate the effects of acute O3FA supplementation on perceived muscle soreness, ratings of exertion, and recovery after a lower body resistance training protocol in college-aged males.

PARTICIPANTS

Twenty-one college aged males from a southeast university that were untrained in resistance training were recruited for the study. Inclusion criteria consisted of being 18-24 years old and engaging in less than one organized resistance training session per week for the previous six months. Participants were excluded if they consumed O3FA supplements in the previous six months, had a lower limb injury in the previous six months, had a history of hypertension, malignancy, diabetes, clotting disorders, or were currently taking non-steroidal anti-inflammatory drugs (NSAIDs) or anti-coagulants. Subjects were asked to refrain from taking NSAIDs for the duration of the study. Participants were also excluded if they ate a diet high in O3FAs, defined as more than two times the adequate intake (1.6 grams per day) for adult males (Williams, Rawson, & Branch, 2017), one week prior to the start of the study or had a fish or seafood allergy. Participants were asked to refrain from participating in resistance training for the duration of the study. Participants were given a thorough explanation on the resistance training protocol and the nutrition supplementation protocol. The participants were informed of possible benefits and risks from the training protocol as well as the nutrition supplementation protocol for this study. In addition, all participants were asked to complete and sign a written informed consent, a written health history questionnaire, and a written food allergy questionnaire prior to beginning the study. This study was be approved by the Institutional Review Board (IRB) at Georgia Southern University prior to beginning recruitment.

INSTRUMENTATION

Participant’s height and weight were assessed in the Human Performance Laboratory at a southeast university during the first week of the study. Body weight (kilograms) was measured using a
Siltec® digital scale and height (centimeters) was measured using a stadiometer and recorded to the nearest 0.1 centimeter. Participants were instructed to stand with their back against the stadiometer, with the scapulae and buttocks in contact with the wall (if possible), and weight evenly distributed between both feet. The participants were instructed to stand erect and look straight ahead.

Participants were asked to use a visual analog scale (VAS) to determine a baseline measure for perceived muscle soreness at the start of pre-testing and at the end of post-testing. For VAS, participants placed a mark on a ten-centimeter (100 millimeter) line with the distance measured from the left end of the line (zero, no pain) to the right end of the line (100, in extreme pain) using a ruler to determine the score in millimeters (mm). The VAS scale has been validated as a subjective measure for soreness (Gallagher, Bijur, Latimer, and Silver, 2002).

Prior to beginning the exercise protocol, participants were asked to rate perceived recovery using a perceived recovery status (PRS) scale. The PRS scale represents varying levels of an individual’s perceived recovery using a zero (very poorly recovered) to ten (very well recovered) scale (Laurent et al., 2011).

Following each testing session, participants were asked to rate perceived exertion for that particular training session using an OMNI Session Rating of Perceived Exertion (RPE) scale. The Omni Session RPE is a scale representing varying levels of an individual’s perceived exertion using a zero (extremely easy) to ten (extremely difficult) scale (Robertson et al., 2003).

PROCEDURES

Participants completed the leg press exercise, followed by the leg extension exercise, and then the lying leg curl exercise. The leg press exercise was performed using a Cybex Leg press machine (Lumex Inc, Ronkonkoma, NY, model no. 5320). The seated leg extension and lying leg curl exercises were performed using an Optima Series Leg Extension/Curl machine (Life Fitness, Rosemont, IL, model no. OSLEC-0102-203). Exercise technique protocol has been outlined by Haff and Triplett (2016).

LEG PRESS

Starting Position:
Sit in the machine with the lower back, hips, and buttocks pressed against the seat. Place the feet on the platform shoulder-width apart with the toes slightly pointed out and legs parallel to each other. Grasping the handles on the side of the machine, move the hips and fully extend the knees without forcefully locking the knees. Keeping the hips on the seat and back firmly pressed against the back pad, remove the support mechanism from the foot platform and grasp the handles on the seat.

Downward Movement Phase:

Slowly flex the knees and hips to lower the platform towards the body. Keep the buttocks and back firmly pressed against the pad. Keeping the knees aligned over the feet as they flex, allow the hips and knees to flex until the thighs are parallel to the platform. Do not allow the buttocks or hips to lose contact with the pads or the heels to rise off of the platform.

Upward Movement Phase:

Extend the hips and knees pushing the platform away from the body without forcefully extending the knees. Keeping the back and buttocks in contact with the pads, do not allow the buttocks to rise and keep the knees over the feet as they extend. At the end of each set, return the support mechanism to the starting position, remove the feet, and exit the machine.

**LEG EXTENSION**

Starting Position:

Sit down on the machine with the back firmly pressed against the back pad. Place feet behind and in contact with the roller pad in contact with the ankles. Legs should be parallel to each other. Align the knees with the edge of the seat. Adjust the back pad or roller pad if necessary. Grasp the handles located on the sides of the seat.

Upward Movement Phase:

Raise the roller pad by fully extending the knees keeping the back erect and firmly in contact with the pad. Keep the thighs, lower legs, and feet parallel to each other. Maintain a tight grip on the handles. Do not forcefully lock out the knees.

Downward Movement Phase:
Allow the knees to slowly flex back to the starting position for a count of three seconds to ensure the weight is controlled during the eccentric phase in order to reduce the risk of injury. The technician will verbally count down for each repetition. Keep the torso erect and back firmly pressed against the back pad. Keep the thighs, lower legs, and feet parallel to each other. Do not allow the buttocks to lift off of the seat by maintaining a firm grip on the handles.

**LYING LEG CURL**

**Starting Position:**

Lie down on the machine with the front of the torso firmly pressed on the back pad. Place the feet underneath and in contact with the roller pad in contact with the ankles. Legs should be parallel to each other. Align the knees with the edge of the seat. Adjust the back pad or roller pad if necessary. Grasp the handles located on the front of the pad.

**Downward Movement Phase:**

Curl the roller pad towards the buttocks by fully flexing the knees. Keep the torso stationary with the hips and torso firmly pressed against the pads. Do not allow the hips or torso to lift off of the pads by maintaining a tight grip on the handles located on the front of the seat.

**Upward Movement Phase:**

Allow the knees to slowly extend back to the starting position for a count of three seconds to ensure the weight is controlled during the eccentric phase in order to reduce the risk of injury. The technician will verbally count down for each repetition. Keep the torso stationary and the hips and torso firmly pressed against the pads by maintaining a tight grip on the handles. Do not forcefully lock out the knees.

**Determination of 10-Repetition Maximum:** The ten-repetition maximum (10RM) protocol has been established as valid and reliable (McLester et al., 2003) and was used in this testing. On the same day as the informational meeting, participants reported to the weight room facility to determine a 10RM for the leg press, seated leg extension, and lying leg curl exercises. Using a protocol similar to Ryan, Long, Bishop, Herron, and Katica (2013), an estimate weight for the starting load of the 10RM testing was needed for all exercises. To determine this weight, participants performed a warm-up set at a weight
easily allowing for 15 repetitions, then were asked to estimate what weight that could be lifted for 10 repetitions to reach fatigue on the final repetition. The process was repeated, either raising or lowering the weight by five or ten pounds until the participant reached fatigue on their tenth repetition. 10RM was determined within three sets, and participants were given four minutes of rest between sets to ensure sufficient recovery before starting the next set. Once a 10RM weight was established for all exercises, it was recorded and used for all post-training sessions. To ensure proper technique, the same technician supervised all 10RM tests. Rest time will be kept using a DICK’S Sporting Goods Stopwatch (Dick’s Sporting Goods, Coraopolis, PA).

**Exercise Protocol:** Participants reported to the testing facility at the same time of day for induction of DOMS and 24 hours later for retest. The testing protocol for the current study is similar to the protocol in a study by Ryan, Long, Bishop, Herron, and Katica (2013). Prior to the exercise protocol, participants were asked to determine perceived muscle recovery using the PRS scale. A 100-millimeter visual analog scale was used to determine perceived muscle soreness. Using 60% of the participants pre-determined 10RM for the leg press, leg extension and lying leg curl exercises, one, ten repetition warm-up set were performed. All exercises were performed in a pattern of three sets of 75% of a one repetition maximum, until fatigue, with four minutes of rest between sets. Participants were instructed to continue each exercise until fatigue was achieved during each set. Verbal encouragement was not be given to participants. The technician verbally counted the overall repetitions for the set, and count to three during the eccentric phase of the lift. Following the completion of each set, the total number of repetitions completed were recorded for that set by the technician. This procedure was repeated for all sets, all three exercises, during all exercise protocols.

Fifteen minutes after the completion of both lifts during the exercise protocol, participants were asked to rate the overall workout difficulty using the OMNI Session RPE and complete a post-exercise VAS for perceived muscle soreness. Participants were sent home and instructed to return 24 hours after the first trial to complete the second exercise protocol. They were asked to estimate muscle recovery
using the PRS scale and complete a pre-exercise VAS for perceived soreness. After these are recorded, participants underwent the same exercise protocol as the first testing session.

Following the completion of the second exercise protocol, participants began a one-week (seven days) washout period. The exercise protocol, in its entirety, was repeated following the washout period and the second supplementation period.

Supplementation Protocol: On the same day as determining 10RM, participants received the first round of supplements by an independent party and began the first supplementation period the following day. Participants were randomly placed in either the experimental group or placebo group. Using a cross-over protocol, participants were given 35 O3FA capsules (Rx Omega-3 Factors, Natural Factors, Everett, Washington; 600 mg O3FA per soft gel capsule; 400 mg EPA, 200 mg DHA) or 35 olive leaf extract capsules (Olive Leaf Extract, Spring Valley Herbs and Natural Foods, Springfield, MO; 150mg). Participants were instructed to take five capsules over three meals throughout the day totaling 3,000 mg O3FA (2,000 mg EPA, 1,000 mg DHA) per day, taking no more than two capsules per meal. To monitor adherence to the protocol, participants were given a sheet to fill out the times they took their supplements every day throughout the supplementation periods and were asked to turn these sheets in at the end of each supplement period.

Nutrition Intervention: Participants were asked to limit the amount of OMFA in the diet during the study. To help ensure compliance, all participants were provided with a list of food sources that were moderate to high in O3FAs and were asked to refrain from eating these foods for the duration of the study. The purpose of asking participants to refrain from moderate-to high O3FA food sources was to control for any variance in O3FA intake that may come from their diet. Participants were asked to keep a three-day food record journal during the supplementation periods covering the day before the first exercise protocol, the day of the first exercise protocol, and the day of the second exercise protocol and replicate their diet on the day prior to the third exercise protocol, the day of the third exercise protocol, and the day of the fourth exercise protocol. This was to prevent large variations in caloric and macronutrient intake that may alter the outcome of the tests. Food journals were evaluated using the
United States Department of Agriculture SuperTracker (United States Department of Agriculture, Alexandra, VA).

STUDY TIMELINE

**Days 1:** Details regarding the procedures of this study were provided to all participants, including the exercise and supplement protocols and study timeline. Participants were asked to sign an informed consent form, and complete a health history questionnaire and a food allergy questionnaire. Anthropometric measures (height and weight) and demographic data (age) were collected from all participants. Next, participants were introduced to the OMNI Session RPE, Perceived Recovery Scale (PRS), and a 10 centimeter visual analog scale to determine perceived muscle soreness. They were informed of the proper technique for the three exercises: (1) Leg press (2) Seated knee extension (leg extension) (3) lying knee flexion (leg curl). After proper technique was discussed, each participant had their 10RM determined for the leg press, leg extension and lying leg curl exercises. Lastly, participants were randomly given either the experimental or placebo supplement and were asked to begin taking the assigned supplement the next day.

**Days 2-8:** Participants consumed no more than two experimental or placebo capsules at every meal totaling five capsules (3,000 mg O3FA or 150 mg olive oil extract) per day.

**Days 9-10:** Following seven days of the first supplementation period, participants reported to the weight room facility for the first exercise training session. Twenty-four hours following the first exercise training session, participants reported back to the weight room facility for the second exercise training session.

**Days 9-15:** Participants did not receive any supplements during the seven day “washout period”. Participants were asked to continue to follow the O3FA restrictive diet and refrain from resistance exercise during the 7-day washout period. On the last day of the washout period, participants received the other supplements for the group they were not in during the first supplementation period to start the next day.
**Days 16-22:** Participants consumed no more than two experimental or placebo capsules of the different supplement at every meal totaling five capsules (3,000 mg O3FA or 150 mg olive oil extract) per day. Groups receiving the placebo in the first supplementation period received the experimental supplement in the second supplementation period. Participants receiving the experimental supplement in the first supplementation period received the placebo supplement in the second supplementation period.

**Day 23-24:** Following the second supplementation period, participants reported back to the weight room facility for the third exercise session. Twenty-four hours following the third testing session, participants reported back to the weight room facility for the fourth exercise training session.

**STATISTICAL ANALYSIS**

Before any statistics are run, all data was tested for normal distribution. In the event of a violation in normative data, a nonparametric test of the null hypothesis, Wilcoxon Signed-rank Test, was conducted. Descriptive statistics (means, frequencies) on all demographic variables were calculated. A paired-sample t-test was conducted between the placebo and O3FA supplementation protocol for perceived muscle soreness, total number of combined repetitions completed, difference of number of repetitions from both test sessions, subjective ratings of exertion, and perceived recovery. Significance was set at $p \leq 0.05$. All statistical analyses will be calculated in SPSS v. 23 (IBM Inc, Armonk, NY).
CHAPTER 4
RESULTS

Participant Characteristics
14 enrolled in the study and four dropped out during the first supplementation period, one for personal reasons, two due to unforeseen scheduling conflicts, and one due to injury sustained outside of the study. A total of 10 of the 14 college-age males completed the study. The mean ± standard deviation age for all participants (n = 10) are shown in Table 1.

Muscle Soreness
No significant difference was observed in the change in perceived muscle soreness after 24 hours between conditions (Control: 16.6 ± 27.4; O3FA: 19.7 ± 17.4; p=0.72). Results for Change in 24 Hour Perceived Soreness between Conditions are shown in Table 2. There was no significant difference in perceived soreness 24 hours after induction of DOMS between conditions (Control: 33.7 ± 17.6; O3FA: 26.8 ± 21.0; p=0.47).

Difference in Number of Repetitions
There was a significant difference in total number of repetitions between the first and second trials in the placebo group (p = 0.01) resulting in a decrease of 15% in the total number of repetitions from testing session one and testing session two. In the O3FA group, there was no significant difference between trial one and trial two in total number or repetitions (p = 0.16). There was no significant difference in the change in total repetitions completed between trial one and trial two for the placebo and O3FA groups (p=0.19). Difference in number of repetitions is shown in Table 4, and difference in number of repetitions between conditions is shown in Table 5.

Rating of Perceived Exertion
No significant difference was observed in perceived exertion between placebo testing session 2, Mdn = 7, and O3FA testing session 2, Mdn = 6, Z = 7, p = 0.88. Perceived exertion between conditions is shown in Table 6.

Perceived Recovery
O3FA perceived recovery scores, Mdn = 4.0, were not statistically higher than the control median placebo scores, Mdn = 6.0, Z = 25.5, p = 0.38. Perceived recovery results are shown in Table 7.

**Nutrient Intake**

There was no significant difference in total energy intake (Placebo: 5,547.7 ± 1,705.2 kcal; O3FA: 4,972.1 ± 1,709.8 kcal; p = 0.25) between groups. There was no statistical difference in fat intake (Placebo: 235.5 ± 81.4; O3FA: 215 ± 87.7; p = 0.08) between groups. A Wilcoxon Signed-rank Test indicated that the median carbohydrate intake O3FA scores, Mdn = 518.0, were not significantly higher than the median placebo scores, Mdn = 587.0, Z = 17, p = 0.51. There was no observed difference in median protein intake O3FA scores, Mdn = 237.5, and the median placebo scores, Mdn = 221, Z = 25.5, p = 0.83. Nutrient analysis results are shown in Table 8.
CHAPTER 5

DISCUSSION

The purpose of this study was to investigate the effects of acute O3FA supplementation on perceived muscle soreness, ratings of exertion, and recovery after a lower body resistance training protocol in college-aged males. While there has been an increase in research investigating the effects of O3FA supplementation on DOMS in recent years, there is a large variation in supplementation protocol, the source of O3FA, and the method of inducing DOMS.

This study suggests that acute supplementation of O3FA at 3,000 mg per day for seven days may inhibit the negative effects caused by resistance training 24 hours after the induction of DOMS by accelerating recovery, but supplementation did not improve perceived muscle soreness. The primary findings of this study showed there was a significant decrease in the number of repetitions completed during the second exercise training session in the control group, but not the O3FA group. This means that the O3FA group was objectively better recovered from the first exercise training session than the control group providing reason to believe acute O3FA supplementation may be effective in accelerating recovery. There was no significant difference in changes in perceived muscle soreness 24 hours following the induction of DOMS between the control and O3FA groups. There was no significant difference between total repetitions completed during either exercise training session between conditions or in the difference in number of repetitions from training session one to training session two between the control and O3FA groups. There was no difference in perceived recovery status at the beginning of training session two between supplement groups. Perceived exertion was not significantly different between the control and O3FA groups.

The majority of O3FA supplementation studies have determined decreased perceived muscle soreness as a primary benefit of O3FA supplementation (Tartibian, Maleki, & Abbasi, 2008; Lenn et al., 2001; DiLorenzo, Drager, & Rankin, 2014; Gray, Chappell, Jenkinson, Thies, & Gray, 2014; Santos et al., 2012; Rajabi, Lotfi, Abdolmaleki, & Rashid-Amiri, 2013; Tsuchiya, Yanagimoto, Nakazato, Hayamizu, & Ochi, 2016; Atashak, Sharafi, Azarbeyjani, Stannard, Goli, & Haghighli, 2013; Corder,
Newsham, McDaniel, Ezekiel, & Weiss, 2016; Jouris, McDaniel, & Weiss, 2011; Houghton & Onambele, 2012; Baran, Kelly, Kress, Landgraf, & Weiss, 2015). In each of these studies, DOMS was measured at multiple points, ranging from immediately following the induction of DOMS (Gray, Chappell, Jenkinson, Thies & Stuart, 2014; Tartibian, Maleki, Abbasi, 2009; Rajabi, Lofti, Abdolmaleki, & Rashid-Amiri, 2013; Tsuchiya, Yanagimoto, Nakazato, Hayamizu, & Ochi, 2016) to five days later (Tsuchiya, Yanagimoto, Nakazato, Hayamizu, & Ochi, 2016). The current study observed significant increases in perceived muscle soreness from baseline on the first training session to the beginning of the second training session, 24 hours later, in both groups indicating the exercise protocol was effective in inducing DOMS. To date, most research involving O3FA and DOMS has evaluated the effects of DOMS 48 hours post-exercise, as this is when the effects of DOMS peaks in the body. Less research has been conducted evaluating DOMS 24 hours post-exercise.

To date, one study has evaluated the effects of O3FA supplementation on DOMS over 24 hours post-exercise and saw a significant increase in perceived muscle soreness 24 hours after baseline measures (Rajabi, Lotfi, Abdolmaleki, & Rashid-Amiri, 2013). The researchers observed a significant increase in perceived muscle soreness 24 hours following induction of DOMS as well as a significant difference between the experimental and control groups at this time point. Much like the study conducted by Rajabi, Lotfi, Abdolmaleki, and Rashid-Amiri, the current study also observed a significant increase in perceived muscle soreness 24 hours from baseline testing. This study also utilized an exercise protocol consisting of eccentric leg presses at a pre-determined %RM which is similar to the current study though only one exercise was used. There was a difference in the volume of repetitions between the studies with Rajabi, Lotfi, Abdolmaleki, and Rashid-Amiri completing a set volume of four sets of 20 repetitions while the current study completed three sets until failure for three different exercises. Nonetheless, both studies showed improvements in perceived muscle soreness following O3FA supplementation 24 hours following the induction of DOMS.

In contrast, a study conducted by Gray, Chappell, Jenkinson, Thies, and Gray (2014) did not observe a significant increase in DOMS from baseline measures or a significant difference between
groups in DOMS 24 hours after baseline measures. The researchers measured perceived muscle soreness at multiple points after the induction of DOMS and found no significant increase in DOMS at 24 hours post-exercise. They concluded that O3FA supplementation ameliorated markers of oxidative stress, but not DOMS, following the exercise protocol. It’s possible that the reason a significant increase in DOMS was not observed is due to the nature in which DOMS was induced in the study. Different from the current study, resistance training exercises were not used to induce DOMS. Though the volume of work was greater than the current study, because there was no resistance in the exercise protocol, there may have been insufficient muscle damage occurring to adequately induce DOMS. A study conducted by Tsuchiya, Yanagimoto, Nakazato, Hayamizu, and Ochi (2016), used five sets of six eccentric bicep curls using a dynamometer to induce DOMS. Participants were instructed to resist the dynamometer’s movement through the full range of motion for at least three seconds for each repetition. Unlike the current study, the researchers also saw no significant difference in DOMS 24 hours post-exercise or any time point when compared to pre-test values in either group. The O3FA group had significantly less perceived muscle soreness 72 hours following exercise than the placebo group, but there was no significant difference between groups until that time. Unlike Gray, Chappell, Jenkinson, Thies, and Gray (2014), this study used a form of resistance training to induce DOMS, however, both studies completed a specific number of sets and repetitions instead of performing until complete muscle fatigue. Both studies also utilized single-joint exercises while the current study which used both multi- and single-joint exercises which may have affected the effectiveness in inducing DOMS 24 hours after the first exercise training session. Though previous studies have used resistance training protocols to induce DOMS, the study conducted by Tartibian, Maleki, and Abbasi (2008) used a bodyweight stepping protocol of eight sets for five minutes of stepping at a rhythm of 15 steps per minute totaling 600 steps. The researchers measured DOMS before exercise, immediately after exercise, 24 hours, and 48 hours post-exercise. At 24 hours following exercise, DOMS was not significantly different between groups, but the experimental group’s perceived muscle soreness was significantly lower than the other groups at 48 hours. Much like Gray, Chappell, Jenkinson, Thies, and Gray, the work volume was greater than the current study, and
though the step protocol was a multi-joint exercise, the fact that it did not involve resistance exercises may have played a role in the effectiveness of inducing DOMS during the study. Differences in perceived muscle soreness from pre-exercise to 24 or 48 hours later was not evaluated to determine if the exercise protocol was effective in inducing DOMS. By not measuring the change in perceived muscle soreness from pre-exercise to 24 or 48 after resistance training, it is difficult to determine if the protocol in this study was effective in inducing DOMS at all. By comparing the methods used in the studies by Tsuchiya, Yanagimoto, Nakazato, Hayamizu, Ochi (2016) and Tartibian, Maleki, and Abbasi (2008), and Tartibian, Maleki, and Abbasi (2008) to the methods used in the studies described in the above paragraph, it can be suggested that multi-joint resistance exercises may be more effective at inducing DOMS 24 hours post-exercise.

Although the current study found no significant difference in perceived recovery between the two different supplements, the control group recorded a higher score on the PRS scale than the O3FA group prior to starting the second exercise training session. The median score for the control group was “adequately recovered”, noted as a six on the PRS scale, while the median O3FA group was reported to be “somewhat recovered”, noted as a four on the PRS scale. Though these results were not statistically significant in this sample size, it is possible that the O3FA group’s median score might be considered practically significant during training meaning that O3FA supplementation may not be effective in accelerating recovery 24 hours following the induction of DOMS. Korak, Green, and O’Neal (2015) observed the effects a high-volume resistance training protocol had on perceived recovery and concluded that a lower value on a PRS scale resulted in a greater change in performance. In contrast, the current study found an inverse relationship between the two. The control group, which felt more recovered than the O3FA group according to the PRS scale, completed significantly fewer repetitions during the second exercise training session when compared to the first exercise training session. The current study found no significant difference between the control and O3FA groups in difference in number of repetitions completed during the first and second exercise training sessions between supplementation periods. The O3FA group completed fewer repetitions during the second exercise training session when compared to
the first exercise training session, but not by a significant amount. Although the O3FA group reported being less recovered according to the PRS scale, they were more recovered than the control group based on the number of repetitions they were able to complete during the second exercise training session. This measure suggests that O3FA supplementation may be effective at inhibiting the negative effects of DOMS. To date, the current study was one of the few studies measuring the effects of O3FA supplementation on perceived recovery following a resistance training program, and was the first study investigating the effects O3FA supplementation on the total number of completed repetitions and the difference in completed repetitions between exercise training sessions. The mixed recovery results may be due to a lack of understanding on how the PRS scale worked. This could have led to participants in one group picking a number that was not accurately indicative of their perceived recovery.

No significant difference in RPE between the control and the O3FA group at any exercise training session was observed in the current study. Between exercise training sessions one and two, the control group reported slightly higher ratings of perceived exertion than the O3FA group, though neither were significant in our sample size. The results of the current study are similar with the results from Houghton and Onambele (2012), which concluded that O3FA supplementation had no effect on RPE following a resistance training protocol. Like the current study, a combination of multi- and single-joint exercises were used to induce DOMS, but there was no difference in RPE between groups in either study. Korak, Green, and O’Neal (2015) investigated the effects of resistance training recovery using an RPE scale, which showed the O3FA group reported a lower RPE score at the beginning of the second testing session. This study suggested that RPE was a useful predictor of performance and that a lower RPE rating was indicative of greater performance during testing. Similar findings were also seen in the current study; all training sessions with a lower RPE were associated with completing more repetitions. One of the inclusion criteria for the current study was that participants were to be untrained in resistance training, defined as averaging less than one organized resistance training session per week for the last six months. However, all of the participants were recruited from physical education classes from a southeastern university, so it is possible that some of the participants had higher fitness levels than the average
individual. A study by Travlos and Maris (1996) evaluated the perceived exertion between individuals with high and low fitness levels and concluded that the perceived exertion was significantly greater in those with lower fitness levels when exercising at similar workloads. Because there was not a significant difference in the number of repetitions completed at either testing day between both conditions in the current study, it can be suggested that these participants were engaging in similar relative workloads during the exercise training sessions.

No significant differences in total energy, carbohydrate, fat, and protein intake were found between groups throughout the current study. The control group consumed more total calories, carbohydrates, and fat than the O3FA group, though not significantly more. Despite none of these measures being significantly different than the O3FA group, there is no denying a higher carbohydrate intake may have influenced muscle glycogen synthesis and storage, potentially influencing the number of repetitions completed during testing sessions. As exercise intensity increases, so does the utilization of carbohydrates as the primary source of fuel during anaerobic exercise, such as resistance training, while fat is the primary source of fuel during long-duration, aerobic exercise. For this reason, it can be assumed that the difference in fat intake, which was approaching statistical significance, is less important than carbohydrate intake which was not significant. The overall caloric intake of the control group was approximately 10% higher than the O3FA group though the bulk of these calories likely came from a difference in fat intake. Of the studies compared to the current study, none utilized a food diary to monitor and compare macronutrient intake between groups to determine if a significant difference in may have affected performance during the exercise training sessions.

Limitations

Because of the time of year that recruitment began and the nature of the study involving participants experiencing muscle soreness multiple times throughout the study, a small sample size completed the study though the sample size was enough to provide adequate statistical power. While most studies involving DOMS measure 48 hours following the induction of DOMS, this study measured at 24 hours potentially leading to a lack of significance between groups. Water intake and hydration
status was not accurately monitored though participants were encouraged to drink at least two liters of water per day during testing. It is difficult to ensure that participants kept honest food records during the study as a level of trust is assumed between the study conductor and participants. It is also difficult to ensure participants measured accurate portion sizes during this period as most individuals are not well-educated on how to estimate portion sizes as they received no training on how to estimate food portions or maintain a food journal at the beginning of the study. However, based on the findings that there were no significant differences in nutrient intake, it appears the participant’s diets were consistent throughout the study. Activity was not monitored or restricted during testing days. Participants were recruited from physical education classes potentially causing a discrepancy in the amount of physical activity they were engaged in outside of the study. The washout period length, though used in other studies involving O3FA supplementation and DOMS, may have been inadequate as some research has suggested washout periods ranging eight to sixteen weeks (Cao, Schwichtenberg, Hanson, & Tsai, 2006) may be more appropriate to allow erythrocyte membrane EPA and DHA levels to return to baseline measures following O3FA supplementation. Due to the rate at which EPA and DHA are cleared from the erythrocyte membrane, DHA, which is cleared at a slower rate, was theorized by Cao, Schwichtenberg, Hanson, and Tsai to be the storage form of O3FA. Despite remaining in the body longer than EPA, DiLorenzo, Drager, and Rankin, (2014) saw that DHA did not play a role in attenuating DOMS, but was helpful in attenuating other markers of inflammation. However, Corder, Newsham, McDaniel, Ezekiel, and Weiss (2016) saw a significant decrease in DOMS in their study. The difference between these two studies in the supplementation protocol may be the deciding difference as both received varying amount for different time periods.

**Conclusions**

To date, this is the first study to measure the total number of repetitions completed, the difference in repetitions completed between exercise training sessions, and perceived recovery status when supplementing with O3FA. Although there were improvements in recovery between training sessions in the O3FA group, there was no difference in RPE, PRS, or perceived muscle soreness 24 hours after
exercise training sessions between groups, so it cannot be concluded that supplementing with O3FA will improve performance or ratings of recovery or ratings of exertion. The study showed no significant difference in perceived muscle soreness, perceived exertion, or perceived recovery. However, the control group completed significantly fewer repetitions on the second exercise training session while the O3FA group was closer to baseline numbers indicating they may have been more recovered during the second exercise training session. The lack of significance in this study may be attributed to low sample size.

Further studies should be conducted in which participants are asked to re-test 48 hours following the first training session, as some studies have shown a greater increase in perceived muscle soreness 48 hours after induction of DOMS compared to 24 hours. Studies should also utilize a washout period that allows for O3FAs to be adequately released from fat cells to allow O3FA levels to return to baseline measures before re-testing the sample size. Participants should also be given adequate training on how to estimate portion sizes and record a food journal to help remove bias from the study. Lastly, based on the results of this study and previous studies, future studies may be better served by focusing on large muscle, multi-joint, resistance exercises when inducing DOMS, particularly if the intention is to measure DOMS 24 hours after exercise.
REFERENCES


Pickova, J. (2009). Importance of knowledge on lipid consumption of foods to support development towards consumption of higher levels of n-3 fatty acids via freshwater fish. *Physiological Research, 58*(Suppl. 1), S39-S45.


APPENDIX A

Research Questions

1. Will 3,000 mg of O3FA per day for seven days result in lower levels of perceived soreness and faster perceived recovery?

Assumptions

1. That all participants comply with the supplementation protocol.
2. That all participants give maximal effort during training sessions.
3. That all participants comply with the nutrition intervention.
4. That all participants comply with the exercise protocol.
5. That all participants will accurately document their food diaries.

Limitations

1. Only using untrained, college-aged (18-24) males.
2. Time of school year limited recruitment possibilities.
3. Cannot control diet or exercise outside of the lab setting.
4. Compliance with placebo and O3FA supplementation
5. Current research is conflicting on the efficacy of O3FA on attenuating DOMS.
6. Time point at which DOMS was measured.
7. Water intake and hydration status was not accurately monitored.
8. Activity was not monitored during testing days.
9. Participants had different levels of physical fitness at the start of the study.
10. Washout period may not have been adequately long enough.
11. Participants were not educated on how to properly measure accurate portion sizes for the food journal.
APPENDIX B

GEORGIA SOUTHERN UNIVERSITY

WATERS COLLEGE OF HEALTH PROFESSIONS

DEPARTMENT OF HEALTH SCIENCES AND KINESIOLOGY

INFORMED CONSENT

1. Principal Investigators:
   Colin A Butler, Graduate student, Department of Health and Kinesiology, 678-362-3968
   Amy Jo Riggs, Ph.D., RD, LD Associate Professor, Department of Health and Kinesiology, 478-7753

2. Purpose of the Study: The purpose of this study is to investigate the effects of acute omega-3 fatty acid supplementation on perceived muscle soreness, ratings of exertion, and recovery after a prescribed lower body resistance training protocol in college aged males.

3. Procedures to be followed: You will be provided a list of foods moderate to high in omega-3 fatty acid (O3FA) and be asked to refrain from consuming these foods for the duration of the study. You will be provided a health history questionnaire (2017 PAR-Q) and an allergy questionnaire to sign and complete. You will be asked to report to the Hanner Fieldhouse Human Performance Lab where your height and weight will be taken and recorded. You will then report to the Hanner Fieldhouse weight facility to determine a ten-repetition maximum (10RM) for the leg press, leg extension, and lying leg curl exercises. Determining a 10RM consists of a warm up of each exercise at a weight that easily allows for 15 repetitions. You will then estimate a weight you think you could complete to reach fatigue on the tenth repetition. Weight will be raised or lowered by five to ten pounds reaching your 10RM by the third set. Four minutes of rest between sets will be given to ensure recovery. Once this is completed, you will be randomly assigned to either the placebo or O3FA supplements the same day as 10RM testing to begin the following day. You will take five capsules per day, no more than two per meal, for seven days. You will then report after the supplementation period to the Hanner Fieldhouse weight facility to induce delayed-onset muscle soreness (DOMS). Prior to testing sessions, you will be asked to estimate your perceived recovery using a perceived recovery scale and perceived muscle soreness using a visual analog scale. You will complete three sets to fatigue using your 10RM for each exercise with four minutes of rest between sets. Fifteen minutes after testing is completed, you will estimate your perceived exertion using an Omni Session rating of perceived exertion scale and perceived soreness. Twenty-four hours after induction of DOMS, you will report back to the Hanner Fieldhouse weight facility to repeat the testing process in its entirety. You will then have a seven-day washout period. During the washout period, you will be asked to continue to follow nutrition intervention protocol and refrain from resistance training. At the end of the washout period, you will receive the opposite supplement, following the same supplementation procedure as the first supplementation period completing the cross-over protocol. At the end of the second supplementation period, you will report to the Hanner Fieldhouse weight facility to induce DOMS a second time. Twenty-four hours after induction of DOMS, you will report back to the Hanner Fieldhouse weight facility to repeat the testing process in its entirety. Prior to the first
testing session, you will be asked to keep a three-day food journal for the day before induction of DOMS as well as both testing days. You will be asked to replicate your diet from the first testing session for the second testing session. All data will be archived for three years before being destroyed.

4. **Discomforts and Risks:** Risks associated with resistance training are minimal in healthy populations, but include muscle soreness, loss of range of motion, tenderness or stiffness in the affected limb, increase in limb circumference, and a decrease in muscle force production peaking 48 hours following exercise, and typically disappears within 72 hours after appearing. No research has shown the effects of DOMS to be permanent. In extreme cases, injury such as muscle strains, or rhabdomyolysis (RML) are possible. Primary factors resulting in RML include engagement in eccentric exercise by those with low exercise experience. Secondary factors including electrolyte imbalance, low protein intake, excessive carbohydrate intake, and excessive exercise in high humidity and temperature have also been reported to increase the risk of RML. Extreme soreness and loss of range of motion in the affected limb may occur with this type of exercise, and it is possible that you may experience brown-colored urine. You will be required to engage in a warm-up before exercise to reduce the chance of muscle damage and follow scheduled rest periods between exercises in accordance with the testing protocol. The investigator will encourage you to stay properly hydrated by drinking at least 2 liters of water throughout the day, and that you bring water to each testing session as well as eat a recovery meal with 0.5g/kg of protein and 1.5g/kg carbohydrates following exercise to aid in recovery. If you experience symptoms such as brown-colored urine, extreme muscle pain, or extreme loss in range of motion in the affected limbs, you will be encouraged to report to health services immediately and inform the investigator. At that time, you will be removed from further participation. The investigator will then inform the Georgia Southern University Institutional Review Board of the issue within 24 hours of its occurrence. You understand that medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. O3FA supplementation risks also include the possibility of belching, halitosis, heartburn, nausea, loose stools, rash, and nosebleeds. Olive oil supplementation up to 28 grams per day is well tolerated, but may lower blood sugar in diabetics and those who have recently had surgery. Risks will be minimized through examination of medical health history forms to determine any health risks for fish allergies or resistance training testing. Although an allergic reaction to the fish oil supplements is not likely, you will be monitored closely to ensure you don’t have any adverse reaction from them. If you do begin to feel nauseous or lightheaded, or develop a rash, please communicate this immediately to one of the researchers.

5. **Benefits:** O3FA supplementation may be effective in alleviating the effects of DOMS, which is beneficial for athletes. Reducing the effects of DOMS and decreasing recovery time can help athletes train at a high level faster and improve competitive performance. O3FA supplementation research suggests that O3FA can reduce triglyceride levels and be effective in reducing the risk of heart disease.

6. **Duration/Time:** Each session at the weight facility will take approximately 1 hour. This includes the warm up and the actual experimental trial. This leads to a total time commitment of five hours at the weight facility; one hour for determining a ten-repetition maximum, and one hour each for the following four testing periods.

7. **Statement of Confidentiality:** All scientific and personal data collected on subjects for presentation purposes will be kept confidential and stored in a locked file drawer in Hollis 2121-A. This information will be available only to the principal investigators. Your identity will not be revealed in publications or presentations that result from this study so as to protect your privacy
and confidentiality. All data will be reported as means and standard errors. Data will be kept for 3 years and then destroyed. Electronic data will be kept on a private hard drive and will be destroyed as soon as the data analysis has been run and the manuscript is complete.

8. **Right to Ask Questions:** You have the right to ask questions and have those questions answered. If you have questions about this study, please contact Colin A Butler, graduate student, Department of Health and Kinesiology, 678-362-3968, cb05120@georgiasouthern.edu or Dr. Amy Jo Riggs, Ph.D., RD, LD Associate Professor, Department of Health and Kinesiology, 478-7753, ajriggs@georgiasouthern.edu. For questions concerning your rights as a research participant, contact Office of Research Compliance IRB@georgiasouthern.edu or call (912) 478-5465.

9. **Compensation:** All participants will be eligible for a one-time drawing to win a one-hundred-dollar gift card at the end of the study. Names will be placed into a bowl and an independent third party will draw one name from the bowl. The name drawn will be contacted to retrieve their gift card.

10. **Voluntary Participation:** Your participation in this study is entirely voluntary. If you decide to participate, you are free to withdraw your consent and to stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

11. **Penalty:** If you decide not to participate, you will not be penalized, and you will not lose any benefits or services to which you are otherwise entitled.

12. **You must be 18 years of age or older to consent to participate in this research study.** If you consent to participate in this research study and to the terms above, please sign your name and indicate the date below.

You will be given a copy of this consent form to keep for your records. This project has been reviewed and approved by the GSU Institutional Review Board under **tracking number H18212.**

**Title of Project:** The effects of acute omega-3 fatty acid supplementation on delayed-onset muscle soreness and recovery

**Principal Investigators:**

Colin A Butler, Graduate student, Department of Health and Kinesiology, 678-362-3968

Amy Jo Riggs, Ph.D., RD, LD Associate Professor, Department of Health and Kinesiology, 478-7753

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Participant Signature  Date

I, the undersigned, verify that the above informed consent procedure has been followed.

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Investigator Signature  Date
2017 PAR-Q+
The Physical Activity Readiness Questionnaire for Everyone
The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including vigorous exercise).</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITION(S) HERE:</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past, but it does not limit your current ability to be physically active. PLEASE LIST CONDITION(S) HERE:</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>7) Has your doctor ever said that you should only do medically supervised physical activity?</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

☑ If you answered NO to all of the questions above, you are cleared for physical activity. Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age ([www.who.int/dietphysicalactivity/en/](http://www.who.int/dietphysicalactivity/en/)).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

⚠️ Delay becoming active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at [www.eparmedx.com](http://www.eparmedx.com) before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.
2017 PAR-Q+
FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?
   If the above condition(s) is/are present, answer questions 1a-1c
   If NO go to question 2
   1a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       (Answer NO if you are not currently taking medications or other treatments)
       YES NO

   1b. Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylothesis), and/or spondylolisthesis/pars defect (a crack in the bony ring on the back of the spinal column)?
       YES NO

   1c. Have you had steroid injections or taken steroid tablets regularly for more than 3 months?
       YES NO

2. Do you currently have Cancer of any kind?
   If the above condition(s) is/are present, answer questions 2a-2b
   If NO go to question 3
   2a. Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?
       YES NO

   2b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?
       YES NO

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm
   If the above condition(s) is/are present, answer questions 3a-3d
   If NO go to question 4
   3a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       (Answer NO if you are not currently taking medications or other treatments)
       YES NO

   3b. Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)
       YES NO

   3c. Do you have chronic heart failure?
       YES NO

   3d. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?
       YES NO

4. Do you have High Blood Pressure?
   If the above condition(s) is/are present, answer questions 4a-4b
   If NO go to question 5
   4a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       (Answer NO if you are not currently taking medications or other treatments)
       YES NO

   4b. Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication?
       (Answer YES if you do not know your resting blood pressure)
       YES NO

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes
   If the above condition(s) is/are present, answer questions 5a-5e
   If NO go to question 6
   5a. Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?
       YES NO

   5b. Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.
       YES NO

   5c. Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, or the sensation in your toes and feet?
       YES NO

   5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?
       YES NO

   5e. Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?
       YES NO
6. Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer’s, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome
   If the above condition(s) is/are present, answer questions 6a-6b if NO go to question 7
6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? YES NO
6b. Do you have Down Syndrome AND back problems affecting nerves or muscles? YES NO
7. Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure
   If the above condition(s) is/are present, answer questions 7a-7d if NO go to question 8
7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? YES NO
7b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy? YES NO
7c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week? YES NO
7d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs? YES NO
8. Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia
   If the above condition(s) is/are present, answer questions 8a-8c if NO go to question 9
8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? YES NO
8b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/fainting? YES NO
8c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)? YES NO
9. Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event
   If the above condition(s) is/are present, answer questions 9a-9c if NO go to question 10
9a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? YES NO
9b. Do you have any impairment in walking or mobility? YES NO
9c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months? YES NO
10. Do you have any other medical condition not listed above or do you have two or more medical conditions?
    If you have other medical conditions, answer questions 10a-10c if NO read the Page 4 recommendations
10a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months? YES NO
10b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)? YES NO
10c. Do you currently live with two or more medical conditions? YES NO

PLEASE LIST YOUR MEDICAL CONDITION(S)
AND ANY RELATED MEDICATIONS HERE:

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.
2017 PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered YES to one or more of the follow-up questions about your medical condition: You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the ePARmed-X+ at www.eparmedx.com and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

All persons who have completed the PAR-Q+ please read and sign the declaration below.

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME ___________________________ DATE ___________________________

SIGNATURE ___________________________ WITNESS ___________________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ___________________________

For more information, please contact www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+

Key References

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Food Allergy Questionnaire

First Name: ___________________________  Last Name: ___________________________

Do you have a food allergy?
☐ Yes (fill out form below, sign and return to study administrator)  ☐ No (initial bottom of page, return to study administrator)

Please describe your food allergy or food intolerance.

Was your food allergy diagnosed by a medical professional?

Do you have medical documentation, such as a Medical Alert bracelet or necklace?

Please describe what happens when you have a reaction.

Is the information you provided accurate to the best of your ability? Please sign below and return the form to the study administrator.

Signature: ___________________________  Date: ___________________________

# CAPSULE DIARY

<table>
<thead>
<tr>
<th>Day no. - Date</th>
<th>Meal</th>
<th>No. of Capsules</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 3</td>
<td></td>
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<td></td>
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<td>Day 4</td>
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<td>Day 5</td>
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<td></td>
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<tr>
<td>Day 6</td>
<td></td>
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<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meal Number (Time)</td>
<td>Food/Beverage</td>
<td>Amount</td>
<td>Notes/Food Description</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------</td>
<td>--------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Day 7</td>
<td>Testing Day 1</td>
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<td></td>
</tr>
</tbody>
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## FOODS HIGH IN OMEGA-3 FATTY ACIDS

<table>
<thead>
<tr>
<th>Source</th>
<th>ALA (g)</th>
<th>EPA + DHA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flaxseed oil, 1 tbsp.</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Walnuts, 1 ounce</td>
<td>2.57</td>
<td></td>
</tr>
<tr>
<td>Soybean oil, 1 tbsp.</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Beechnuts, 100g</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Butternuts, 100g</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td>Chia seeds, 1 ounce</td>
<td>5.06</td>
<td></td>
</tr>
<tr>
<td>Anchovy, 100g</td>
<td></td>
<td>1.449</td>
</tr>
<tr>
<td>Herring, 3 ounces</td>
<td></td>
<td>1.71</td>
</tr>
<tr>
<td>Mackerel, 3 ounces</td>
<td></td>
<td>1.02</td>
</tr>
<tr>
<td>Salmon, 3 ounces</td>
<td></td>
<td>1.83</td>
</tr>
<tr>
<td>Sardine, 3 ounces</td>
<td></td>
<td>1.19</td>
</tr>
<tr>
<td>Trout, 3 ounces</td>
<td></td>
<td>0.884</td>
</tr>
<tr>
<td>Tuna, canned, 3 ounces</td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>Tuna, steak, 3 ounces</td>
<td></td>
<td>0.10</td>
</tr>
</tbody>
</table>

**OMEGA-3 FATTY ACID SIDE EFFECTS AND USES**

**O3FA Side effects**

Fish oil is LIKELY SAFE for most people when taken by mouth in low doses (3 grams or less per day). There are some safety concerns when fish oil is taken in high doses. Taking more than 3 grams per day might keep blood from clotting and can increase the chance of bleeding.

High doses of fish oil might also reduce the immune system's activity, reducing the body's ability to fight infection. This is a special concern for people taking medications to reduce their immune system's activity (organ transplant patients, for example) and the elderly.

Fish oil can cause side effects including belching, bad breath, heartburn, nausea, loose stools, rash, and nosebleeds. Taking fish oil supplements with meals or freezing them can often decrease these side effects.

**Bipolar disorder:** Taking fish oil might increase some of the symptoms of this condition.

**Liver disease:** Fish oil might increase the risk of bleeding in people with liver scarring due to liver disease.

**Depression:** Taking fish oil might increase some of the symptoms of this condition.

**Diabetes:** There is some concern that taking high doses of fish oil might make the control of blood sugar more difficult.

**High blood pressure:** Fish oil can lower blood pressure and might cause blood pressure to drop too low in people who are being treated with blood pressure-lowering medications.

**Fish or seafood allergy:** Some people who are allergic to seafood such as fish might also be allergic to fish oil supplements. There is no reliable information showing how likely people with seafood allergy are to have an allergic reaction to fish oil. Until more is known, advise patients allergic to seafood to avoid or use fish oil supplements cautiously.

**O3FA Uses**

**Effective for:** High triglycerides. Research suggests that fish oil from supplements and food sources can reduce triglyceride levels.

**Likely Effective for:** Heart disease. Research suggests that eating fish can be effective for keeping people with healthy hearts free of heart disease. People who already have heart disease might also be able to lower their risk of dying from heart disease by eating fish or taking a fish oil supplement.
APPENDIX C
TABLES & FIGURES

Table 1
Summary of Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.0 ± 1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>89.5 ± 16.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.3 ± 2.9</td>
</tr>
</tbody>
</table>

SD indicated Standard Deviation

Table 2
Change in 24 Hour Perceived Soreness between Conditions

<table>
<thead>
<tr>
<th>Perceived Soreness</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.6 ± 27.4</td>
</tr>
<tr>
<td>O3FA</td>
<td>19.7 ± 17.4</td>
</tr>
</tbody>
</table>

Note: Values shown represent mean ± standard deviation

Table 3
24 Hour Perceived Soreness between Conditions

<table>
<thead>
<tr>
<th>Perceived Soreness</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.7 ± 17.6</td>
</tr>
<tr>
<td>O3FA</td>
<td>26.8 ± 21.0</td>
</tr>
</tbody>
</table>

Note: Values shown represent mean ± standard deviation

Table 4
Difference in Number of Repetitions

<table>
<thead>
<tr>
<th>Test 1</th>
<th>Test 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>105 ± 20</td>
<td>90 ± 24</td>
</tr>
<tr>
<td>O3FA</td>
<td>101 ± 17</td>
<td>96 ± 17</td>
</tr>
</tbody>
</table>

Note: Values shown represent mean ± standard deviation

Table 5
Difference in Number of Repetitions between Conditions

<table>
<thead>
<tr>
<th>Number of Repetitions</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14 ± 11</td>
</tr>
<tr>
<td>O3FA</td>
<td>6 ± 12</td>
</tr>
</tbody>
</table>

Note: Values shown represent mean ± standard deviation
Table 6
Rating of Perceived Exertion between Conditions

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>IQR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>O3FA</td>
<td>6.0</td>
<td>4.0</td>
<td>0.88</td>
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</tbody>
</table>

Note: p-values for differences are from Wilcoxon signed-rank test (N=10)

Table 7
Perceived Recovery Status

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>IQR</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.0</td>
<td>4</td>
<td>0.38</td>
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<tr>
<td>O3FA</td>
<td>4.0</td>
<td>4.5</td>
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</table>

Note: p-values for differences are from Wilcoxon signed-rank test (N=10)

Table 8
Nutrient Intake

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>O3FA</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>5,547.7 ± 1,705.2</td>
<td>4,972.1 ± 1,709.8</td>
<td>0.25</td>
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<tr>
<td>Fat (g)</td>
<td>253.5 ± 81.4</td>
<td>215 ± 87.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>587.0</td>
<td>518.0</td>
<td>0.51</td>
</tr>
<tr>
<td>IQR</td>
<td>179.0</td>
<td>306.8</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>221.0</td>
<td>237.5</td>
<td>0.83</td>
</tr>
<tr>
<td>IQR</td>
<td>172.3</td>
<td>180.8</td>
<td></td>
</tr>
</tbody>
</table>

Note: p-values for carbohydrates and protein intake are from Wilcoxon signed-rank test (n=10)
FIGURES

Figure 1
Total Repetitions

Figure 2
Difference in Total Number of Repetitions
Figure 3
*Ratings of Perceived Exertion*

![Ratings of Perceived Exertion](image)

Figure 4
*Perceived Recovery*

![Perceived Recovery Status](image)