ABSTRACT

The Anabolic and Anti-Catabolic Effects of Long-Chain Omega-3 Polyunsaturated Fatty Acid Supplementation on Functional Muscular Outcomes

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Long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) are associated with multiple benefits, primarily related to cognitive and cardiovascular health. Recently, LC n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have received considerable attention from the athletic and military communities for their potential application to physical performance and recovery. While multiple lines of evidence have provided valuable insight into the plausible mechanisms, the influence of LC n-3 PUFAs on functional skeletal muscle outcomes remain elusive. As such, this dissertation will investigate the potential anabolic influence of LC n-3 PUFA supplementation on body composition and strength. Additionally, the differential effects of two bioactive LC n-3 PUFAs, EPA and DHA, on muscle recovery following exercise-induced muscle damage will be explored.

The Anabolic and Anti-Catabolic Effects of Long-Chain Omega-3 Polyunsaturated Fatty Acid Supplementation on Functional Muscular Outcomes

by

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The last three years have been a whirlwind, not just from the rigors of a demanding doctorate program, but also the difficulties of being a husband and father of three in a world consumed by the uncertainties surrounding a global pandemic. As my personal and professional lives collided with a rapidly changing and unpredictable community and research environment, I was blessed to have the support, mentorship, and understanding of a thoughtful advisor, Dr. LesLee Funderburk. It was truly an honor to work alongside a professional of her caliber. I would also like to acknowledge all the researchers that have assisted me with the various aspects of my research. Lastly, I'd like to acknowledge my funding sources for making my projects possible: Dr. Funderburk's faculty discretionary funding, the Mead Johnson Student Pilot Study Grant from the Research Dietetic Practice Group, the Student Development Research Award from the Texas Chapter of the American College of Sports Medicine, and the Doctoral Student Research Grant from the Department of Health, Human Performance, and Recreation at Baylor University.

DEDICATION

To God, who is the genesis of all that is good and beautiful in *the* world and inspires me to be an example and model of the ideal leader, personally and professionally.
To my wife, Caroline, who is everything that is good and beautiful in *my* world and encourages me to dream big and is the reason I work hard and aim for excellence.
To our three amazing boys who keep me grounded and youthful.

CHAPTER ONE

Introduction

Omega-3 polyunsaturated fatty acids have received considerable research interest from the athletic and military communities.[1,2] Omega-3 fatty acids belong to the family of polyunsaturated fats and their name related to the presence of a double bond at the third carbon from the methyl terminus of the fatty acid chain. Metabolism of α-linoleic acids (ALA, 18:n-3) by desaturase and elongase enzymes generates eicosapentaenoic acid (EPA, 20:5n-3) and, eventually, docosahexaenoic acid (DHA, 22:6n-3). Humans are unable to pre-form ALA and thus it is termed an essential dietary fatty acid. Humans also have a limited capacity to convert ALA to EPA and DHA.[3] Thus, the main dietary sources of EPA and DHA are fish, especially fish such as mackerel, salmon, sardines, and herring. However, EPA and DHA intake tends to be low globally and, especially low in military personnel and athletes based on food frequency questionnaires and biomarker data.[4–6] Thus, EPA and DHA supplementation from fish oil may be viable strategy to promote military and athlete health and, potential, performance.

Fish oil-derived omega-3 fatty acid (FO n-3) supplementation has steadily grown in popularity since the late 1990's[7] and is associated with health benefits important to athletic populations. Multiple studies have demonstrated FO n-3's ability to augment muscle protein synthesis (MPS).[8–11] FO n-3 PUFA supplementation has also been shown to increase lean body mass,[12–14] strength,[13,15] enhance recovery from muscle damaging exercise,[16,17] and preserve muscle mass during physiological stress.[18–21] Additionally, FO

n-3 are incorporated into skeletal muscle altering cell membrane fluidity[22] and play a role in anti-inflammation.[23] It is proposed that in an athletic or military populations FO n-3 supplementation can (1) enhance training adaptations from a regimented resistance exercise program (such as lean body mass accretion and strength) and (2) improve recovery from rigorous training or exercise.

Smith et al. demonstrated that eight weeks of FO n-3 supplementation increased the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content in skeletal muscle phospholipids and enhanced muscle protein synthesis (MPS) during the infusion of amino acids and insulin, mimicking a fed state, in both older[8] and younger[11] adults. An intervention conducted under more physiologically relevant conditions, found that sixteen weeks of FO n-3 supplementation enhanced the anabolic effect of nutrition and physical activity in older adults.[24] In combination with exercise, Rodacki et al.[25] also found an increase in strength after three months of FO n-3 supplementation and resistance training in older women. Another group of investigators evaluated both older men and women over an 18 week intervention of FO n-3 plus resistance training, and found significant improvements in strength in the female subjects but not in male subjects.[26] Possible physiological mechanisms for this phenomena are, the posited increased rate of MPS may be partially due to an upregulation in the mTOR-p70s6k signally pathway, known to be a crucial for cell growth.[8,11] Also FO n-3 may improve muscle function by changing the fluidity of the membrane and acetylcholine sensitivity.[27] Acetylcholine, a neurotransmitter, triggers the muscle contraction process, therefore it may enhance the contraction via a faster synaptic transmission at the neuromuscular junction and elicit faster muscle contraction.[25] Currently, there are no reported studies that have directly

measured muscle growth or strength responses to n-3 supplementation in athletic populations. However, some studies have indicated beneficial effects on anaerobic endurance capacity[28] and explosive power[29,30] in athletes.

Due to FO n-3's anabolic capabilities,[8,11,31] it is plausible that the effect identified in these studies is related to an increase in lean body mass (LBM), and significant improvements of LBM with FO n-3 supplementation have been reported in recent studies.[13,14,32,33] However, a majority of the studies were conducted in an older population that included participants with comorbities,[34] or in the presence of a weight loss diet.[35–37]

Physical training can cause exercise-induced muscle damage (EIMD). The symptoms of EIMD include soreness, decreased range of motion, decreased muscular strength,[38,39] and delayed-onset muscle soreness (DOMS).[40] Any of these symptoms can inhibit muscle activity and physical performance.[40] Effective therapies to manage or attenuate symptoms are important to allow athletes to continue to train and perform at desired performance levels. In a FO n-3 intervention with professional rugby players, Black et al.[30] found that there was maintenance of explosive power during training and reduced muscle soreness in those supplemented with FO n-3. Several other investigators have found significant decreases in DOMS at 48, 72 and 96 hours post exercise,[41–43] as well as a tendency to attenuate a decrease in performance following additional bouts of exercise or sporting events.[28,44–46]

A slew of recent systematic and narrative reviews have concluded that FO n-3 supplementation in athletic populations is a safe and effective method that may benefit certain aspects of performance and recovery.[1,2,47–49] However, multiple questions remain unanswered. While plausible mechanism exists, it is unclear if FO n-3 supplementation

can affect skeletal muscle functional outcomes in a meaningful way. By investigating anabolic and anti-catabolic functional skeletal muscle outcomes, this dissertation seeks to better understand the effectiveness of FO n-3 supplementation for athletic populations. The purpose of this dissertation is to determine if an appropriate dose of FO n-3 supplementation increases LBM and strength during a resistance training program in young men and women and to determine if differing types of FO n-3's, EPA and DHA, elicit differential effects on recovery from EIMD.

CHAPTER TWO

Literature Review

The Anabolic Effects of Fish Oil-Derived Omega-3 Polyunsaturated Fatty Acids

Lean Body Mass

Hypertrophy is mediated by the positive balance of muscle protein synthesis (MPS) and breakdown (MPB).[50] Resistance training independently increases MPS above MPB, thus creating a hypertrophic environment.[51,52] To date, five trials have investigated the hypertrophic influence of omega-3 supplementation combined with resistance training.[53–57]

Hayward et al.[53] randomized 28 healthy untrained young females into one of three groups, all of whom performed a supervised resistance exercise protocol: 1) resistance training only (RT); 2) higher-protein diet + omega-3 (HP+n-3); and 3) higher-protein diet + omega-3 + creatine monohydrate (HP+n-3C). The study protocol lasted 8 weeks, with 4 weeks devoted to pre-training and 4-weeks of resistance training plus the dietary intervention. Although the groups containing n-3 experienced the greatest increase in LBM (HP+n-3 1.35kg and HP+n-3C 0.96kg vs RT 0.38kg), this result was not statistically significant.

However, conclusions must be interpreted with caution as the study only provided n-3 supplementation for 4 weeks. Although research shows that n-3 fatty acids are incorporated into the muscle cell in as little as two weeks, McGlory et al.[58] found that n-3 muscle content continues to increase after 4 weeks, without a known plateau. Although

speculative, this suggests that greater than 4 weeks of n-3 supplementation may be needed to maximize its assimilation into the muscle cell. McGlory et al.[58] also supplemented subjects with 5g/d of n-3 PUFAs, so it is plausible that the low n-3 supplementation dose provided (0.9g n-3/d) would not elicit an appropriate anabolic response to resistance training. Although the results were not statistically significant, combining a high protein supplement and n-3 PUFAs for both intervention groups can make interpretation of the results difficult as well. While the LBM differences between the HP+n-3 and the HP+n-3C groups could be attributed to creatine, a single variable change, it is difficult to interpret the difference between the control group and HP+n-3, since the change may be related to protein alone, omega-3 alone, or a combination. It would have been more informative if the study included a group that received n-3 supplementation with the resistance training protocol, since it would have unambiguously demonstrated that the observed effects were solely attributed to the treatment.

In a cohort of 18 healthy trained young males, Georges et al.[54] found that 8 weeks of periodized RT with concomitant intake of 3g/d of a krill oil supplement improved LBM (1.4kg or 2.1%); however, this finding was not statistically significant compared to the control (0.3kg or 0.5%) despite a 1.1kg difference. The results of this trial are remarkably similar to Hayward et al. in that the intervention group experienced a greater increase in LBM but the results were not statistically significant, which, again, may be related to an inadequate n-3 PUFA dose. When compared to a typical fish oil supplement, krill oil at 3g/d only contains 0.96g of omega-3 fatty acids.[54] The authors also noted that post hoc analysis determined that the study was underpowered to detect a statistical significance.

Cornish and Chilibeck[55] randomized 51 older men and women to receive a supplement consisting of either 30 mL of corn oil (placebo) or 30 mL (~14 g) of alpha-linolenic acid (ALA) for 12 weeks. All subjects performed a periodized RT program 3 times per week. Significant increases in LBM were found in both groups. The change in LBM was greater in the male ALA (1.2kg) and female ALA (0.7kg) cohorts when compared to the placebo groups (0.4kg); however, the between group differences failed to reach statistical significance. Interestingly, knee flexor muscle thickness significantly increased in the male ALA group and the female placebo group. The difference may be related to the reduction of IL-6, an inflammatory cytokine, in the male cohort that did not occur in the female ALA group. Sex differences in markers of inflammation and ALA conversion may have been influenced by the decreased estrogen in the postmenopausal female participants, especially since estrogen has been shown to enhance the conversion of ALA to DHA.[59] Additionally, EPA seems to be the main driver of musculoskeletal adaptations[18], so it should be noted that the complete conversion of ALA to EPA is approximately only 8% in males[60] and 21% in females.[61] The poor conversion rate may also make it necessary to supplement ALA for a greater period to ensure appropriate incorporation of EPA into the muscle.

Similarly, Da Boit et al.[57] allocated 50 older men and women to either 3g/d of omega-3 supplementation or 3g/d of placebo (safflower oil). Both groups participated in a resistance training program twice weekly for 18 weeks. The study failed to demonstrate a beneficial effect of omega-3 supplementation on LBM compared to placebo. Despite a lengthy study period, it is surprising that none of the groups significantly increased muscle anatomical cross-sectional area (ACSA). Evidence clearly indicates that older

individuals achieve robust increases in LBM from structured RT.[62] Although the exercise intensity was set at 70% of subjects' 1RM and reassessed periodically, this raises questions as to whether the program was challenging enough to achieve hypertrophic adaptations.

Cornish et al.[56] conducted a double-blind study in which 23 untrained older men were randomly assigned to consume either 3g of an omega-3 supplement (1.98 g of EPA and 0.99 g of DHA) or 3g of an omega fatty acid blend (45% ALA, 26.5% oleic acid, and 17.5% of short-chain fatty acids, saturated fats, and phospholipids) per day. Both groups completed a 12-week total body resistance training program carried out three times weekly (nonconsecutive days). Results showed that both groups similarly increased LBM (RT: 0.7kg vs RT+n-3: 0.5kg) as measured by dual energy x-ray absorptiometry. It should be noted that the placebo contained a relatively high ALA content (45%) that may have contributed to the results. Although plausible, this is unlikely since it would only equate to ~0.11g of EPA per day.[60]

Overall, the studies presented here may not have provided an adequate dose of n-3 PUFAs to elicit a hypertrophic effect on muscle mass. Based on previous trials without a structured resistance training component, FO n-3 supplementation at a dose below 2 g/d (0.51-1.8g EPA and DHA) does not affect LBM[14,63,64]; however, doses greater than 2g/d (2.4-4.0g EPA and DHA) elicited an increase in LBM.[12,13,32] Of the 5 studies included, three found that RT with n-3 supplementation increased LBM when compared to placebo but failed to reached statistical significance.[53–55] All 3 trials used different sources of omega-3 supplementation: fish oil[53], krill oil[54], and ALA[55]; and all used different doses. Two of the trials were conducted in young to middle-aged participants.[53,54] Both trials

found an increase in LBM by approximately 1 kg; however, the findings were not statistically significant. Both studies provided less than 2g of omega-3s, while one trial was underpowered[54] and the other was most likely too short.[53]

Strength

In cross-sectional studies, omega-3 supplementation has been linked to improved measures of upper- and lower-body strength, such as handgrip strength[65], knee extension strength[66], and 1RM leg press[67]. Presently, seven trials have investigated the impact of omega-3 supplementation with a RT program on strength[53–57,68,69].

Cornish and Chilibeck's[55] RT protocol was 3 days per week and included 13 exercises per session (full body) with a progressive increase in exercise intensity based on subject's 1RM throughout four phases. The phases were 1) familiarization and adaptation – 6 sessions at 2-3 sets of 10-12 repetitions at 60-65%, 2) hypertrophy – 6 sessions at 4 sets of 10-12 repetitions at 65-70%, 3) strength development - 12 sessions at 3-4 sets of 6-10 repetitions at 70-85%, and 4) maintenance – 12 sessions at 3 sets of 10-12 repetitions at 65-80%. Both groups significantly increased 1RM bench press and leg press strength, with no additional effects in the ALA group. The lack of an effect may be related to the inefficient conversion of ALA to EPA and DHA.[60,61]

In the Hayward et al.[53] trial, strength, as measured by 1RMs in bench press, deadlift, squat, and hip-thruster, increased across all groups. The HP+n-3 group outperformed the RT (control) group in all lower-body exercises (squat, deadlift, hip-thruster). However, the dietary intervention did not lead to statistically significant improvements in strength versus RT alone. As described by the study authors, the 4-week supplementation period may have not been adequate to elicit a benefit from omega-3

supplementation. Data from previous trials suggests that omega-3 administration can increase strength; however, the process of omega-3 fatty acid incorporation into the myocyte may take a minimum of 4-weeks[58], then another 3-6 months until improvements in strength plateau.[13,68]

Cornish et al.[56] conducted another 12-week trial in 23 elderly men in which all participants completed a RT program that was progressive and divided into four 3-week phases: 1) exercise familiarization and muscular adaptation, 2) hypertrophy, 3) strength, and 4) maintenance. Exercises included bench press, seated row, shoulder press, dumbbell biceps curl, dumbbell triceps overhead extension, leg press, knee extension, dumbbell split squats, squats, and plantar flexion. The intervention group received 3g/d n-3 (RT+n-3), while the control group (RT) received an omega 3-6-9 containing placebo. Bench press and leg press 1RMs improved to the greatest degree in the RT+n-3 group; however, the results were not statistically different between groups. The study may not have been sufficiently powered to demonstrate a difference and the placebo contained omega-3 fatty acids as well (45% ALA).

In another study[54], the RT program consisted of 4 sessions per week for 8 weeks. Eighteen young men attended two strength-based sessions weekly, while the remaining two sessions per week focused on endurance during odd weeks (1, 3, 5, etc.) and hypertrophy during even weeks (2, 4, 6, etc). The results for this trial remarkably mirror the results of another trial, although the population was elderly men.[56] The RT+n-3 group experienced the greatest increase in bench press and leg press performance; however, the difference between the groups failed to be statistically significant.

Da Boit et al.[57] only had participants conduct lower-body RT. The exercises included were leg press, leg extension, leg curl, and calf press. The RT and RT+n-3 groups trained twice weekly for 18 weeks and based on 70% of subject's 1RM. The load was reassessed every 6 weeks and adjusted as needed. Maximal isometric torque increased in both groups and in men and women. However, only women in the RT+n-3 group significantly improved strength. Based on the lack of change in muscle ACSA, the RT regime may not have been challenging enough to elicit strength adaptations in men and may be related to only training the lower body. It is unclear why only the female cohort improved in strength. The authors speculated that the women had a greater capacity for strength improvement, so the skeletal muscle was in a more conducive environment to create positive changes after n-3 supplementation.

In agreement, Rodacki et al.[68] randomly assigned 45 older women to one of three groups: RT alone, RT+n-3 for 90 days, or RT+n-3 for 150 days. The RT+n-3 for 150 days group began supplementation 60 days prior to initiating the RT protocol. The RT+n-3 groups received 2g of n-3 PUFA (0.8 g EPA and 0.6 g DHA) per day. Training began with a 2-week (6 sessions) familiarization phase comprised of 3 sets of 8 repetitions at 50% of participants 1RM. For the remaining 12 weeks, participants exercised 3 days per week at 70% of their 1RM during week 1, 80% during week 2, and adjusted weekly thereafter. Participants only conducted lower body exercises. In addition to RT, participants were instructed to maintain their usual diet and physical activity levels and had their eating habits evaluated by food frequency questionnaire. At study's end, all groups increased their muscle function; however, both RT+n-3 groups exhibited greater improvements in strength (approximately 50%). The increase in all but one measure of

strength (chair-rising test) did not occur prior to resistance training in the RT+n-3 for 150 days group. Hence, the anabolic response may not have been due to n-3 supplementation alone but augmented the anabolic response toRT. Unfortunately, the trial did not include a n-3 supplementation only group, so this conclusion is speculative.

More recently, Lee et al.[69] conducted a 12-week double-blind trial that randomized 28 participants (10 male, 18 female) to one of three groups: 1) control (CON), 2) resistance training (RT), 3) RT with 3g fish oil/d (RT+n-3). The RT program was twice per week and progressive. At baseline, subjects tested their 1RMs, then used a percentage of this total to gage subsequent training intensity. At week 1, subjects exercised at 50% of their 1RM; 70% at week 2; then, increased by 5% every week, if they were able to complete the workload. Handgrip strength significantly improved in the RT (+5.3%, $p = 0.007$) and RT+n-3 (+9.4%, $p < 0.001$) groups when compared to the CON group (-3.9%, $p = 0.003$). Despite an increase of 4.1% in the RTFO group compared to the RT group, the authors did not report this difference as statistically significant. It is plausible that this finding was clinically significant, especially in an elderly population. This study uniquely had a control group and included both elderly men and women, although the differences between men and women were not reported.

Although 6 of the 7 studies found that omega-3 supplementation increased some or all variables of strength compared to RT or placebo,[53,54,56,57,68,69] only 2 of the studies were statistically significant[68,69] and one was only significant in women.[57] Cornish and Chilibeck[55] represented the data graphically, so it is unclear what the exact differences were between the groups and this was the only trial to use a plant-based omega-3 supplementation protocol. The two trials with young participants supplied less than 1g

per day of n-3 PUFA and also had the shortest intervention periods.[53,54] Future trials should provide at least 3g/d of omega-3 fatty acids and last at least 10-12 weeks. Of the 5 trials conducted with an older cohort, 3 found that RT+n-3 led to significant increases in strength as measured by handgrip strength[69] and maximal isometric torque[57,68] Since one trial only included elderly women[68] and Da Boit et al. only demonstrated significant findings in the female cohort[57], Lee et al.[69] was the first trial to report statistically significant findings within a cohort that included a male population. Unfortunately, sex specific data was not presented in the analysis, so it is unclear if the results were shared equally or driven by the female cohort.

It should be noted that trials reporting significant findings exhibited specific characteristics in study design, population, and type and amount of omega-3 supplementation. The three trials used fish oil supplementation that contained at least 2g/d of omega-3's, were longer in duration, and conducted in an elderly population. It is unclear if RT+n-3 will increase strength compared to RT only in young adults.

The Anti-Catabolic Effects of Fish Oil-Derived Omega-3 Polyunsaturated Fatty Acids

One of the clearest benefits associated with FO n-3 supplementation is recovery from EIMD. A recent systematic review determined that FO n-3 supplementation "very likely" improves recovery as measured by subjective perception of soreness, range of motion, and/or swelling.[48] However, there is a dearth of information regarding the potential differential effects of EPA and DHA supplementation alone.

DHA Studies

Four studies were identified as DHA trials.[70–73] The studies tended to have small sample sizes, ranging from 15 to 41 participants, and totaled 123 participants (81.1% men; 21.9% women). Recovery from muscle-damaging exercise was assessed in several ways, including subjective perception of pain or muscle soreness, strength, ROM, and limb circumference. Length of intervention ranged from 9 days to 10 weeks and the daily DHA dose ranged from 0.8 g to 3 g. The earliest trial to assess recovery from muscle-damaging exercise (3 x 10 reps at 80% 1RM of eccentric arm curl) with DHA supplementation found that 0.8g/d for 2-weeks failed to decrease muscle soreness or improve ROM compared to placebo (sunflower oil and rice powder).[71] However, this study used the lowest DHA dose compared to other trials. In a study by DiLorenzo et al.,[72] 41 untrained volunteers received 28 days (2 g/day) of DHA or a placebo (corn oil), before a 17-day resistance exercise program. There were no differences in muscle strength and soreness between groups. In 27 healthy women, Corder et al.[73] examined the effect of 3 g DHA supplementation seven days before and two days after exercise on muscle soreness after an eccentric elbow flexion exercise. Results showed DHA supplementation reduced muscle soreness and stiffness after 48 hours but reported no effects on swelling compared to a placebo (corn and soy oil). Recently, Ramos-Campo et al.[70] reported that the ingestion of 2.1 g of DHA and 0.24 g of EPA every day for 10 weeks promoted lower muscle soreness after a damaging eccentric exercise in endurance athletes, when compared to placebo (olive oil). However, DHA supplementation did not preserve strength or modify rating of perceived exertion.

Overall, DHA supplementation may reduce subjective soreness in studies supplying doses greater than 2g/d. DHA supplementation most likely does not improve strength deficits, ROM, or muscle swelling after muscle-damaging exercise. Due to the lack of studies investigating DHA supplementation and muscle-damaging or eccentric exercise, it is difficult to make any definitive conclusions.

EPA Studies

Fifteen studies were identified as EPA trials.[16,17,45,74–84] Tartibian et al.[80] showed a beneficial treatment effect of 32 days of 1.8 g of EPA and DHA per day versus a placebo on perceived pain and a minor decrement of range of motion 48 h after damaging exercise (40 min of bench stepping exercise) in 27 untrained males. A recent double-blind, placebo-controlled, parallel design study showed that daily supplementation for 8 weeks of fish oil (EPA, 0.6 g and DHA, 0.26 g) has a positive role in inhibiting muscle stiffness after eccentric contractions and was able to inhibit the loss of muscle strength, reduce ROM, development of muscle soreness and to reduce muscle swelling in comparison to a placebo.[83] In 11 healthy men and women, Jouris et al.[16] tested the effects of 7 days of EPA and DHA supplementation, in a ratio 2:1 (2 g of EPA and 1 g of DHA) on inflammation induced by two sets of eccentric elbow flexion exercise (120% 1RM) and showed a reduction of post-exercise soreness at 48 h. In another study,[82] 63 healthy male and female college students were supplemented for 30 days with omega-3 (2.7 g per day) or placebo (sunflower oil) prior to forearm extension eccentric exercise. Results showed a decrease in muscle soreness at 72 h and 96 h after damaging exercise in the supplemented group compared to placebo. A more recent double-blind, placebo-controlled, parallel-group trial,[45] conducted on 24 healthy Japanese men, concluded that

EPA and DHA supplementation (0.6 g EPA and 0.26 g DHA per day) for 8 weeks prior to, and 5 days after maximal unilateral isokinetic elbow flexor exercise was more beneficial than a placebo to attenuate strength loss and limit reductions in ROM during the recovery period. Jakeman et al.[77] first examined the effect of an acute intake of two fish oil supplements, with different concentrations of EPA and DHA in a double-blind, placebo-controlled study conducted in 27 physically active males. Immediately following a damaging bout of exercise (100 drop jumps), volunteers ingested either a low-EPA fish oil (0.15 g EPA, 0.1 g DHA), high-EPA fish oil (0.75 g EPA, 0.05 g DHA) or a placebo. Results showed that acute ingestion of high-EPA fish oil was beneficial on some markers of performance compared to low-EPA fish oil and placebo. Tinsley et al.[17] supplemented both EPA and DHA in 17 non-resistance trained females. Participants were randomized into fish oil (6 g/day; 5:1 EPA:DHA) or placebo (corn/soybean oil mixture) groups and took the supplement for one week prior to, and one week after a damaging elbow flexion and extension exercise. Results showed that at 48 h post-exercise, the supplemented group reported less muscle soreness in the lower body than the placebo group.

In contrast, Houghton and Onambele[76] investigated the effects of a dose of 0.36 g of EPA supplementation per day for 3 weeks on muscle soreness induced by acute and chronic resistance exercise in a double-blind placebo-controlled study in 20 female participants. Data showed no effect in reducing muscle soreness in comparison to a placebo (lecithin). Also, in contrast to the previous studies, this trial used a very low dose of EPA, so this amount may not have been enough to effect muscle physiology. More recently, Vandusseldorp and colleagues[74] randomized 32 participants into one of four groups comprised of varying doses of fish oil supplementation (2 g, 4 g, and 6 g) and one

placebo (safflower oil) group for 7 weeks. After a bout of muscle-damaging exercise, the fish oil supplementation (6 g, EPA: 2.4 g; DHA: 1.8 g) group's vertical jump height returned to baseline after 1-hour, while the other groups jump performance remained suppressed until 48 hours. Regardless of dose, all fish oil groups reported significantly reduced muscle soreness compared to placebo at 48 hours post-exercise. For strength, the highest dose (6g/d) did not significantly attenuate the decline in strength after muscle-damaging exercise. Although not statistically significant, strength returned to baseline after 72 hours in the 6g/d group, while the placebo group was still at an 8.5% (19.5 Nm) strength deficit.

CHAPTER THREE

Manuscript One: The Effect of Fish Oil Supplementation on Resistance Training-Induced Adaptations

Introduction

Tactical athletes are subjected to physically demanding environments and situations such as arduous training courses, combat scenarios that require daily operations, and occupations that have varied physical demands (military, firefighting, law enforcement, etc) [85–87]. Skeletal muscle mass and strength are often core components of physical performance. For example, tasks associated with occupational performance such as load carriage, heavy lifting, power production, and anaerobic capacity are correlated with high lean body mass, low body fat, and strength [85,88,89]. Resistance exercise training (RET) may be one of the best- and well-established strategies to influence these parameters.

Muscle protein synthesis (MPS), an important determinant of muscle mass and commensurate strength enhancements, is stimulated by RET [50]. The most common and widely recognized nutritional strategy to augment RET-induced adaptations, including MPS, is the intake of dietary protein (1.2-1.6g·kg^{-1}), especially the provision of the essential amino acids [90,91]. Recently, long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA), primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been investigated for their roles in MPS and skeletal muscle health [92].

The incorporation of EPA and DHA into skeletal muscle phospholipid has been shown to enhance both nutrient and mechanically sensitive anabolic signaling proteins

known to regulate MPS [58]. Moreover, fish oil supplementation (FOS), a concentrated source of EPA and DHA, augments the anabolic response to nutrient stimuli in healthy young and middle-aged men and women through the activation of the mTOR-p70s6k signaling pathway leading to a ~50% increase in MPS [93]. A study in resistance-trained young men demonstrated that FOS augmented the anabolic response elicited by protein feeding alone and with the addition of RET relative to placebo (PL), as indicated by a ~30% and 35% increase in MPS, respectively, in the absence of a concomitant increase in kinase signaling activity [94]. Moreover, these findings have corroborated similar alterations in protein signaling following skeletal muscle EPA and/or DHA incorporation [95,96]. As such, it appears that FOS may attenuate signaling cascades without compromising functional outcomes such as muscular hypertrophy and strength. In fact, a recent preclinical study reported skeletal muscle hypertrophy following EPA supplementation without alterations in the anabolic signaling pathways [97]. Unlike targeted pharmaceutical interventions, LC n-3 PUFAs may act via several other mechanisms that may influence RET-induced adaptations such as muscle quality associated factors such as muscle fiber type transitions [98] or enhanced neuromuscular recruitment [68], muscle protein breakdown [18], improved insulin signaling [99], enhanced cell membrane fluidity [100], and modulation of inflammatory cytokines [101].

While plausible mechanisms exist, it is unclear if FOS influences functional skeletal muscle outcomes such as the promotion of hypertrophy, strength and fat mass reduction in young adults following a RET program [102,103]. As noted by Anthony et al. [104] and James et al. [105], n-3 PUFA research is plagued with methodological flaws that may render some interpretations on primary outcomes unreliable. Since the influence of EPA

and DHA on physiology are mediated by incorporation into tissue phospholipid membranes, the aforementioned authors proposed the use of a membrane-centric hypothesis to resolve these issues in n-3 PUFA research [104]. For example, two studies investigating the effects of RET and n-3 PUFA supplementation on skeletal muscle functional outcomes in young adults did not measure membrane nor blood LC n-3 PUFA status, likely employing both suboptimal EPA and DHA dosing (< 1 $g \cdot d^{-1}$) and duration (4-8 weeks) protocols to meaningfully influence skeletal muscle incorporation [53,54]. Moreover, Georges et al. [54] reported that 8 weeks of RET and krill oil supplementation increased LBM by 1.4 kg, reduced FM by 0.6 kg, and increased upper- and lower-body strength; nevertheless, the effects where similar to PL. Since the krill oil supplement ($3g \cdot d^{-1}$) only contained 0.96 $g \cdot d^{-1}$ LC n-3 PUFAs, the dose was inadequate to maximize EPA and DHA skeletal muscle incorporation. Additionally, krill oil contains a higher proportion of various phospholipids that have been shown to independently influence MPS pathways [106]. Similarly, Hayward et al. [53] provided a multi-ingredient supplement containing protein and LC n-3 PUFAs (0.90 $g \cdot d^{-1}$) in combination with a 4 week RET program and found that young females had a greater, albeit non-significant, increase in LBM (1.35 kg vs. 0.38 kg) compared to the control group. Once again, the n-3 fatty acid dose provided most likely did not lead to maximal incorporation of EPA and DHA into the muscle phospholipid. Additionally, the control group was not protein-matched, hence, it is unclear if the results were influenced by protein, n-3 fatty acids, or a combination of factors. As stated by Rossato et al. [103] and others [102], it is apparent from the lack of available data that concurrent RET and FOS should be undertaken to determine if LC n-3 PUFAs augment skeletal muscle functional outcomes in young adults.

Therefore, the aim of this study was to determine if there is an augmented response of FOS (3.85 $g \cdot d^{-1}$ EPA+DHA), compared to PL, on body composition (lean body mass [LBM], fat mass [FM], percent body fat [%BF]) and 1-repetition maximum (1RM) strength during a 10 week RET program in young adults. Based on the current literature, we hypothesized that an adequate dose of FOS alongside RET, would facilitate increased LBM and strength, as well as a reduction in FM to a greater magnitude than RET plus PL. If so, targeted RET and FOS interventions can be developed for tactical athletes and personnel with similar characteristics.

Methods

Experimental Approach to the Problem

A randomized, single-blind, parallel-group design was used to examine the effects of FOS compared to placebo (PL) on body composition and strength during a 10-week RET program in young adults. Regarding blinding, one of the outcome assessors was aware of the allocations; however, all outcomes were conducted with other investigators present (see the *Strength Testing* section). A schematic overview of the study design is depicted in Figure 3.1.

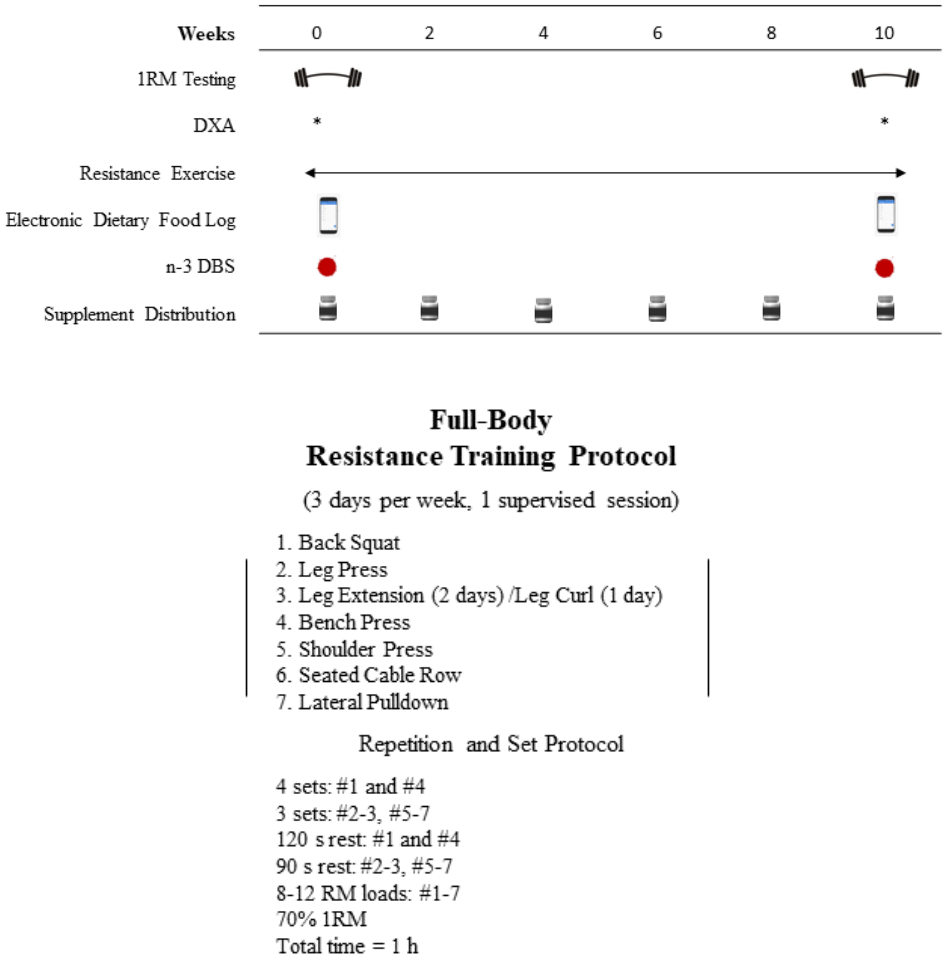


Figure 3.1. Schematic Overview of the Study Design

Subjects

Twenty-eight young male (n = 12) and female (n = 16) adults were recruited from the local central Texas area and university population for this study. All participants met the following criteria: 1) between 18 and 40 years, 2) free from neuromuscular/ musculoskeletal disorders and known chronic diseases (heart disease, type-2 diabetes mellitus, etc), 3) did not regularly consume ergogenic supplements within 6 months of starting the study, 4) does not take anabolic steroids or selective androgen receptor modulators, 5) reported both being recreationally-trained (defined as RET twice per week

for at least 6 months) and familiar with the barbell back squat and barbell bench press, and 7) have a body fat percentage (%BF) ≤ 24 in males and ≤ 34 in females in an effort to align with typical military retention standards. While we did not recruit tactical athletes directly *per se*, our aim was to include a variety of participants with similar characteristics of conventional forces personnel within the military. Of the initial 28 participants recruited, 7 dropped out during the course of the study. Figure 3.2 outlines subject recruitment, randomization, and reasons for drop out.

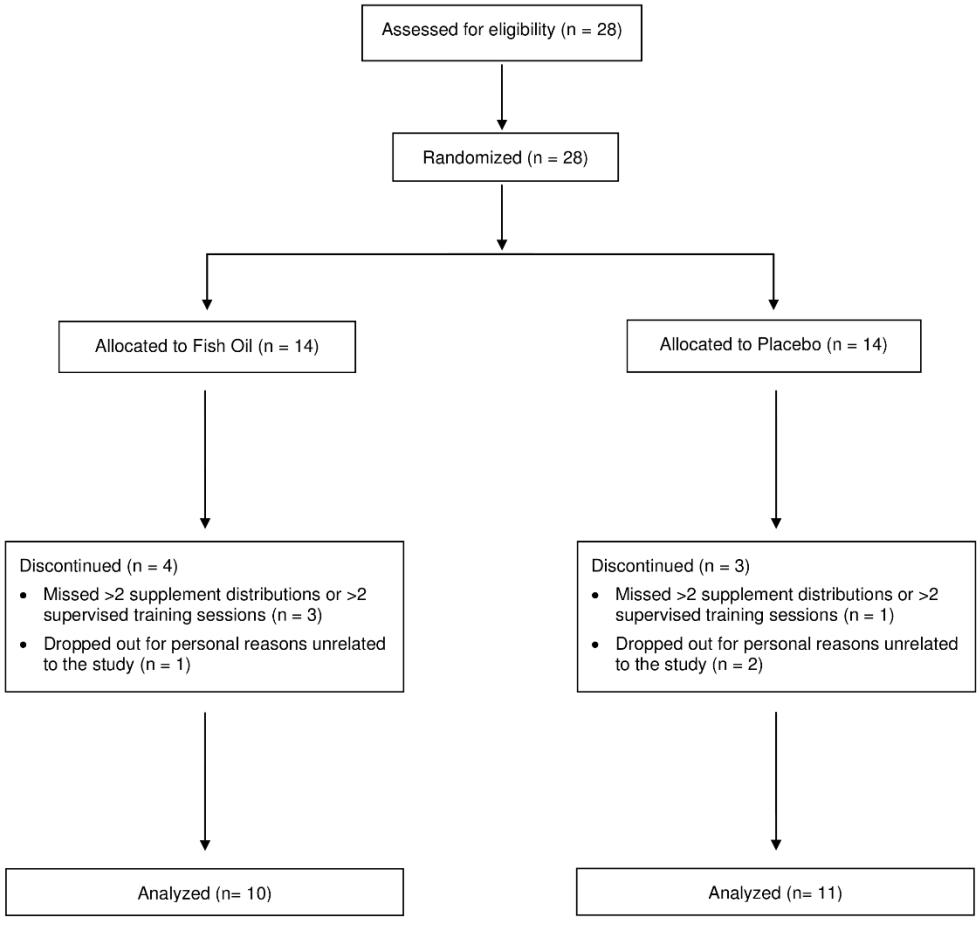


Figure 3.2. Participant Flow Diagram

Since there is little evidence to suggest sex differences in the anabolic response from RET [107], we opted to include male and female subjects. While hormonal variation does appear to uniquely impact women physiologically, there is no clear evidence that the menstrual cycle or oral contraceptive use significantly influences physical performance [108,109]. Furthermore, recent data suggests that RET-induced changes in hypertrophy and strength are minimally influenced by sex [110]. While some evidence suggests a differential response after FOS based on sex in older adults [57], this finding was not replicated in a recent trial [98] and has not been observed in young adults.

Supplementation Protocol

Groups were supplemented with FO (4.5 $g \cdot d^{-1}$ [2.275 $g \cdot d^{-1}$ EPA + 1.575 $g \cdot d^{-1}$ DHA], 7 capsules; Nordic Naturals, ProOmega, Watsonville, CA, USA) or PL (safflower oil, 4.5 $g \cdot d^{-1}$, 5 capsules; NOW, Bloomingdale, IL, USA) for 10 weeks. Based on recent data on skeletal muscle phospholipid incorporation of EPA+DHA following supplementation [58,111], our FOS dose needed to be > 3 $g \cdot d^{-1}$. To account for possible missed doses, we opted to provide a slightly higher dose. Supplements were distributed every two weeks to encourage compliance. Adherence was confirmed by verbal confirmation and upon visual inspection by the same laboratory technician. Additionally, blood fatty acid status was determined via fatty acid dried blood spot (DBS) as muscle and blood EPA+DHA content are highly correlated [58]. The supplements were packaged in similar bottles and the capsules were similar in size and shape in an attempt to blind the subject to the allocation. To assess blinding, at the end of the study, subjects were asked to guess which group they were in.

Resistance Training Procedures

As illustrated in Figure 3.1, subjects conducted a partially supervised (1 session per week) 10-week full-body RET protocol (3 non-consecutive days per week) consisting of seven exercises per session of 3-4 sets of 8-12 repetitions with 90-120 s rest intervals. Briefly, the following exercises were performed in order: barbell back squat, leg press, leg extension/leg curl, barbell bench press, shoulder press, seated cable row, and wide-grip lat pulldown. Similar exercise regimes have been used previously to study various hypertrophy and strength outcomes [112,113]. One RT session per week was supervised by trained research personnel, while the other two RT sessions was completed by the participant for a total of 10 supervised sessions and 20 unsupervised sessions over 10-weeks. Subjects were prohibited from performing additional RT or high-intensity anaerobic training until the completion of the study.

Initial training loads were selected based on 70% of the subject's baseline 1RMs. The load was adjusted for all exercises based on the subject's ability to reach momentary concentric failure between 8-12 repetitions. The load was decreased at the next training session if the subject completed less than eight repetitions on the final set or increased if the subject was able to complete all 12 repetitions on the final set. Load adjustments were approximately 5-10% for each exercise.

Measurements

Body composition. Total body mass (kg) and height (cm) were determined using a standard scale with a stadiometer (Seca 703, Hamburg, Germany). At PRE and POST, body composition (percent body fat [%BF], fat mass [FM], and lean body mass [LBM])

was obtained under laboratory conditions using dual-energy x-ray absorptiometry (DXA, Discovery DXA™, Hologic®, Bedford, MA). Based on previous studies in our lab, the accuracy of the DXA is ± 2 to 3.7% as compared to hydrodensitometry [114,115].

Strength testing. Lower- and upper-body strength were assessed by 1RM testing in the back squat ($1RM_{SQT}$) and bench press ($1RM_{BP}$) exercises, respectively. Subjects conducted a 5-minute bike warm-up. Prior to both 1RM tests, subjects conducted a self-directed dynamic warm up for an additional 5-minutes that included one set of 10 repetitions with an unloaded 20.4 kg bar. Subjects then completed a standardized warm-up protocol used previously in our lab [116] consisting of 10 repetitions at approximately 50% 1RM, 5 repetitions at 70% 1RM, 3 repetitions at 80% 1RM, and 1 repetition at 90% 1RM. All warm-ups were followed by 2-minute rest intervals. Subjects then performed sets of 1 repetition with increasing weight for 1RM determination. During the testing phases, 3-minute rest intervals were employed and all 1RM determinations were made within 5 attempts. For the $1RM_{SQT}$, subjects were required to reach parallel, in which the top of the thigh is discernably parallel to the floor, for a repetition to be considered successful. For the $1RM_{BP}$, the lift was deemed successful if the subject kept five-points of contact (bench: head, upper back, buttocks; ground: both feet), touched the barbell to their chest (no pause), and executed a full lock-out. While we do not have lab-specific reliability measures for the $1RM_{BP}$ and $1RM_{SQT}$, at least 2 study personnel were available for spotting and standards verification. All strength testing sessions were supervised and standards additionally verified by the lead technician and one additional technician - all National Strength and Conditioning Association Certified Strength and Conditioning

Specialist. The average PRE and POST $1RM_{SQT}$ and $1RM_{BP}$ will be reported as absolute and relative to body weight (1RM [kg]·body mass $[kg]^{-1}$) values.

Volume load. Volume load data, calculated as sets x reps x load, were obtained from bench press and back squat for every session. Total volume load and volume load data for back squat and bench press from each week were averaged across 3-RET sessions and used for data analysis.

Dietary Records

Subjects were required to submit three-day food logs (two weekdays, one weekend day) before and after the RET intervention using the MyFitnessPal mobile or desktop application (MyFitnessPal; San Francisco, CA, USA). Additionally, LC n-3 PUFA intake was assessed using a food frequency questionnaire (FFQ) that has been validated against whole blood EPA and DHA [5,117]. While the subject diets were not standardized, they were asked to keep their dietary habits as consistent as possible. Additionally, subjects were asked to consume at least 1.0 $g \cdot kg^{-1}$ per day of protein and given gram-specific targets per day by a registered dietitian. Macronutrients (kcals, protein, carbohydrate, fat) and n-3 fatty acids intake (EPA and DHA) were averaged both over the three-day tracking period and per group for analysis by the same aforementioned registered dietitian. Lastly, macronutrient data were normalized to body mass (kg) for further analysis.

Fatty Acid Dried Blood Spot

Fatty acid dried blood spot (DBS) was obtained to track supplementation compliance and ensure adequate LC n-3 PUFA membrane incorporation. A drop of blood

was collected from each participant via finger stick on filter paper pre-treated with a cocktail solution (Fatty Acid Preservative Solution, FAPS™) and allowed to dry at room temperature for approximately 15 minutes. The DBS were shipped overnight on dry ice to OmegaQuant for fatty acid analysis. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of RBC (GLC OQ-A, NuCheck Prep, Elysian, MN, USA) which was also used to construct individual fatty acid calibration curves. Fatty acid composition was expressed as a percent of total identified fatty acids. PRE and POST values of EPA, DHA, and the omega-3 index (O3i) were reported. The O3i is defined as the sum of EPA and DHA adjusted by a regression equation (r = 0.96) to predict the O3i in the RBC.

Statistical Analyses

All statistical analyses were performed using IBM SPSS version 28 (Armonk, NY, USA). Data were tested for normality and homogeneity using the Shapiro-Wilks and Levene's tests, respectively. Baseline characteristics were analyzed using an independent samples *t*-test. The sample size for this project was 26. This sample size was justified by *a priori* power analysis in G*power using a target effect size (ES) of f = 0.35, alpha of 0.05 and power of 0.80, which determined that 20 subjects were required for participation with an additional number of participants recruited to account for potential drop-outs. Of note, similar RET investigations with a nutritional intervention used identical per group sample sizes (n = 8-11) [53,54], even with cohorts including males and females [118]. The primary outcome data (body composition [LBM, FM, %BF], strength [$1RM_{SQT}$ and $1RM_{BP}$]), and strength relative to body weight (kg) were analyzed using an ANCOVA on the change scores with baseline values as the covariate. All other data with timepoints

(PRE/POST: Fatty acids [O3i, EPA, DHA], dietary variables [kcals, protein, carbohydrate, fats, EPA, and DHA]; weeks 1-10: volume load) were analyzed using a two-way repeated measures ANOVA (group x time). If the assumption of sphericity (Mauchly's test) was violated, the Greenhouse-Geisser correction was used. If significant interaction effects were present, pairwise comparison analyses were used with a Bonferroni adjustment for alpha inflation. Significance was set a priori at $p < .05$. ES values are reported as Cohen's *d* to infer the between-group magnitude of differences in change scores. ES values were classified according to Cohen [119] as trivial, < 0.2; small, 0.2 – 0.49; moderate, 0.5 – 0.79; and large, ≥ 0.8. All data presented as mean ± SD, unless otherwise stated.

Results

Seven subjects dropped out during the study, resulting in a total of 21 subjects (FOS group, n = 10; PL group, n = 11). Reasons for dropouts are noted in the participant flow diagram (Figure 3.2). The FO and PL groups had similar ($p > .05$) baseline characteristics (Table 3.1).

Table 3.1. Baseline participant characteristics

Variable	Fish Oil (n = 10; 5 men, 5 women)	Placebo (n = 11; 5 men, 6 women)	*p* value
Age (*y*)	28.0 (7.4)	30.5 (5.7)	.403
Height (*cm*)	169.7 (9.6)	171.8 (8.9)	.679
Weight (*kg*)	75.1 (16.0)	79.0 (16.0)	.906
BMI ($kg \cdot m^{-2}$)	25.8 (3.5)	26.6 (4.3)	.496
Body Fat (%)	23.9 (6.9)	24.9 (8.0)	.766
Training Age (*y*)	1.8 (1.1)	2.0 (1.0)	.652
Bench Press (kg)	62.7 (37.0)	66.3 (37.0)	.826
Back Squat (kg)	82.9 (35.0)	90.9 (43.1)	.650

Compliance

Supplement. Self-reported supplement compliance was 95.2% for all participants. There was no difference in supplement compliance between groups (FOS: 94.6%, PL: 95.8%, F (1,19) = 0.331, p = .572). Fifty-seven percent of subjects (12 of 21; FOS: 6 of 10, PL: 6 of 11) were unable to ascertain their allocated group. Only 2 subjects in the FOS group reported experiencing "fishy burps". No other symptoms or adverse effects were reported.

RET protocol. Overall attendance for those who completed the study was similar between groups (supervised: F (1,19) = 0.022, p = .883; unsupervised; F (1,19) = 0.1118, p = .734). Participants in the PL and FOS groups had an 94.6% and 95.0% attendance for the RET supervised sessions, respectively, and a self-reported unsupervised session attendance of 95.0% and 94.0%, respectively.

Fatty acid dried blood spot. The baseline average O3i for all subjects was 4.58% ± 1.12 (FOS: 4.9% ± 1.3, PL: 4.3% ± 0.9). There were no baseline group differences in the O3i (F (1,19) = 1.688, p = .209) or whole blood values of EPA (F (1,19) = 0.309, p = .585) and DHA (F (1,19) = 1.829, p = .192). As noted in Figure 3.3, the O3i did not change in the PL group (1.3%, p = .938) and significantly increased from PRE to POST in the FOS group (109.7%, p < .001). Similarly, whole blood EPA and DHA did not significantly change in the PL group (14.7%, p = .869; -0.8%, p = .952, respectively) and significantly increased in the FOS group (613.0%, p < .001; 69.9%, p < .001, respectively). At the individual level, all subjects in the FOS group increased their O3i.

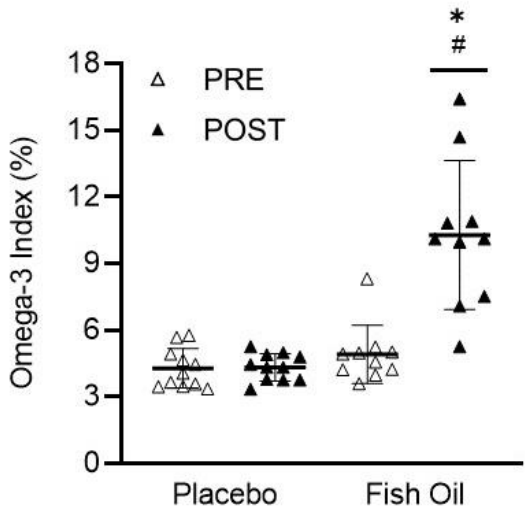


Figure 3.3. Participant Omega-3 Index Before (PRE) and After (POST) 10 Weeks of Supplementation. Black line with whiskers indicates mean ± SD.
*significantly different than PRE; #significant difference between groups

Dietary Intake

All dietary data, normalized by body mass (kg) for the macronutrients, are reported in Table 3.2. In brief, there were no significant differences between groups nor over time in self-reported calorie and macronutrient intake. Dietary intake of EPA and DHA was similar between groups and did not change from PRE to POST.

Table 3.2. Nutritional analysis

Variable	Group	Pre, Mean ± SD	Post, Mean ± SD	*p* (Time)	*p* (GxT)
Total energy (kcal)	FO	1889.2 ± 343.0	1905.1 ± 462.7	.676	.994
	PL	1901.1 ± 520.6	1916.5 ± 414.7		
Carbohydrate ($g \cdot kg^{-1}$)	FO	2.6 ± 1.0	2.6 ± 1.0	.985	.868
	PL	2.7 ± 1.4	2.7 ± 0.9		
Protein ($g \cdot kg^{-1}$)	FO	1.5 ± 0.3	1.5 ± 0.2	.889	.690
	PL	1.2 ± 0.3	1.3 ± 0.2		
Fat ($g \cdot kg^{-1}$)	FO	1.0 ± 0.2	1.0 ± 0.3	.529	.938
	PL	1.0 ± 0.4	0.9 ± 0.2		
EPA (mg)	FO	29.6 ± 28.8	17.6 ± 18.6	.306	.114
	PL	13.9 ± 22.4	16.6 ± 17.1		
DHA (mg)	FO	63.8 ± 59.1	39.6 ± 41.0	.345	.104
	PL	33.3 ± 53.0	40.0 ± 37.8		

Data are mean ± SD. Amount of EPA and DHA does not include supplementation
Abbreviations: FO, fish oil; PL, placebo; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

Volume Load

Total volume load over the 10 weeks was similar between conditions (FOS: 42,670 ± 18,925 kg, PL: 43,879 ± 22,765 kg, p = .897). There were no between-group differences in total volume load for the back squat (FOS: 24,888 ± 9,985 kg, PL: 26,197 ± 12,914 kg, F (1,19) = 0.017, p = .897) or bench press (FOS: 17,782 ± 9,173 kg, PL: 17,683 ± 10,033 kg, p = .897). Back squat and bench press volume load significantly increased over 10-weeks (p < .001). Compared to baseline, back squat volume load was significantly higher at week 3 (p < .001), week 8 (p = .016), week 9 (p = .006), and week 10 (p < .001). For bench press, volume load was significantly higher at week 3 (p = .025), week 8 (p = .049), and week 10 (p < .001) compared to week 1. Volume load data for the back squat and bench press over 10-weeks and between groups are noted on Figure 3.4.

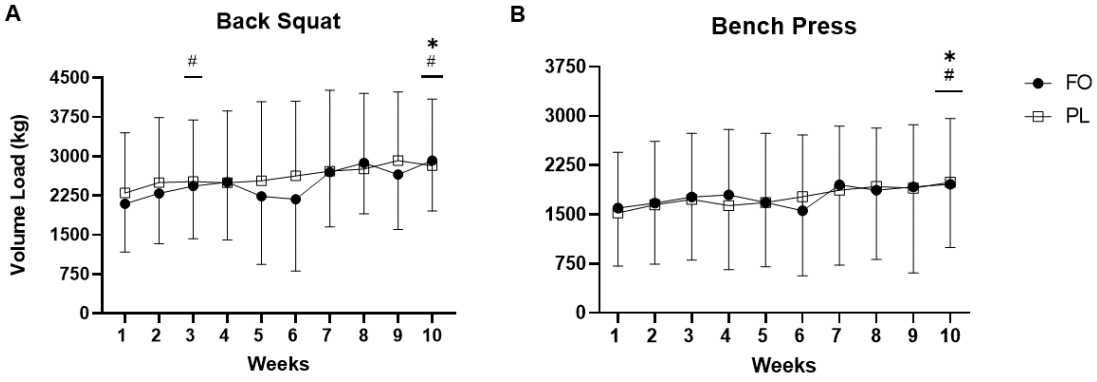


Figure 3.4. Weekly Volume Load for the A) Back Squat and B) Bench Press. Data are mean ± SD. *significantly different than week 1 for PL (Back Squat: p = .016; Bench Press: p = .001); #significantly different than week 1 for FO (Back Squat: week 3, p = .003; week 10, p < .001; Bench Press: p = .031). There were no group by time interactions (p > .05)

Body Composition

Total LBM increased and FM/%BF decreased in both groups. As indicted in Table 3.3, there was no significant between-group differences in LBM (FOS: +3.1%, PL:

+2.5%), FM (FOS: -4.3%, PL: +0.6%), nor %BF (FOS: -5.4%, PL: -1.3%). Notably, the between group magnitude was considered moderate and large for %BF (d = 0.74) and FM (d = 0.84), respectively, favoring the FOS group. The 0.6 kg difference in LBM, favoring the FOS group, was conversely considered small (d = 0.36).

Strength Testing

As noted in Table 3.3, absolute $1RM_{BP}$ significantly improved in the FOS group (+23.6%) compared to PL (+11.0%), whereas absolute $1RM_{SQT}$ was similar between groups (FOS: +32.6%, PL: +23.0%). FOS improved relative $1RM_{BP}$ (+22.9%) and tended to improve relative $1RM_{SQT}$ (+31.7%) compared to PL ($1RM_{BP}$: +8.7%, $1RM_{SQT}$: +20.5%).

Table 3.3 Data of study outcomes

Outcomes	Group	Pre, Mean ± SD	Post, Mean ± SD	Unadjusted Δ ± SD	Baseline Adjusted Δ (CI)	*P* (Group)	ES
Squat 1RM (kg)	FO	82.9 ± 35.0	106.8 ± 38.4	23.9 ± 8.1	24.2 (17.5, 30.9)	.191	0.64
	PL	90.9 ± 43.1	109.5 ± 48.6	18.6 ± 12.1	18.2 (11.8, 24.6)		
Relative Squat 1RM ($kg \cdot kg^{-1}$)	FO	1.1 ± 0.3	1.4 ± 0.3	0.3 ± 0.1	0.3 (0.2, 0.4)	.054	**0.89**
	PL	1.1 ± 0.4	1.3 ± 0.4	0.2 ± 0.1	0.2 (0.1, 0.3)		
Bench 1RM (kg)	FO	62.7 ± 37.0	73.9 ± 40.7	11.1 ± 6.8	11.3 (2.9, 9.7)	**.047**	**1.00**
	PL	66.3 ± 37.0	72.7 ± 39.6	6.4 ± 5.0	6.3 (7.7, 14.8)		
Relative Bench 1RM ($kg \cdot kg^{-1}$)	FO	0.78 ± 0.4	0.92 ± 0.4	0.14 ± 0.1	0.14 (0.1, 0.2)	**.013**	**1.20**
	PL	0.81 ± 0.4	0.87 ± 0.4	0.06 ± 0.1	0.06 (0.02, 0.1)		
LBM (kg)	FO	55.9 ± 15.6	57.9 ± 16.7	1.9 ± 1.9	2.0 (0.8, 3.1)	.457	0.36
	PL	58.2 ± 14.9	59.6 ± 15.4	1.4 ± 1.7	1.4 (0.3, 2.5)		
FM (kg)	FO	17.4 ± 3.8	16.5 ± 3.1	-0.91 ± 1.2	-1.0 (-1.9, -0.9)	.092	**0.84**
	PL	33.3 ± 53.0	19.3 ± 5.6	-0.02 ± 1.6	0.1 (-0.7, 0.9)		
BF (%)	FO	23.9 ± 6.9	22.4 ± 6.2	-1.4 ± 1.5	-1.5 (-2.4, -0.6)	.136	0.74
	PL	24.9 ± 8.0	24.2 ± 7.3	-0.63 ± 1.6	-0.6 (-1.4, 0.3)		

Bold indicates a significant p-value ($p < .05$) or large effect size (≥ 0.8)
Abbreviations: Δ, change; ES, effect size (Cohen's *d*); 1RM, 1-repetition maximum; LBM, lean body mass; FM, fat mass; BF, body fat

Discussion

To our knowledge, this is the first study to investigate the effects of FOS compared to PL on muscle adaptations following a 10-week RET program in young

adults. We demonstrated that 3.85g combined daily EPA and DHA resulted in significantly greater RET-induced gains in absolute and relative $1RM_{BP}$, as well as moderate-to-large between-group differences based on ES for absolute and relative $1RM_{SQT}$, %BF, and FM. Contrary to our hypothesis, FOS failed to differentially influence LBM compared to PL.

In young adults, changes in RET-induced muscular strength are often associated with changes in muscle mass [120]. While LBM improved in both experimental groups within the present investigation, 1RM strength - especially relative strength ($kg \cdot kg^{-1}$ body mass) - improved to a greater extent in the FOS group relative to PL. Given that MPS is the primary contributor to hypertrophy in young adults [121], we hypothesized that strength improvements with FOS would be mediated by concomitant LBM enhancements. However, despite demonstrating similar between-group LBM changes, absolute and relative $1RM_{BP}$ was increased by 12.6% and 14.2%, respectively, and relative $1RM_{SQT}$ was increased by 11.2% more than PL. While the relationship between muscle mass and increased strength is well-established, muscle quality, or strength per unit of muscle mass, can improve through several factors both independently or commensurate to LBM [122,123]. Thus, beyond hypertrophy, other factors such as fiber type distribution, reductions in intramuscular fat, and enhanced neuromuscular activation may uniquely improve measures of muscular strength [124].

Although speculative, the between-group LBM difference of 0.6 kg (+1.2%), may be partially explained by enhanced MPS. FOS in the presence of nutrient stimuli has been shown to improve MPS by 50% in young adults [93]. However, this effect appears to be modestly attenuated following an acute bout of RET in similar demographics [94].

Notwithstanding that trained-individuals experience a somewhat blunted RET-mediated MPS response [125], we expected a more pronounced MPS-associated LBM accrual amongst our recreationally trained subjects. Nevertheless, MPS changes following an acute RET bout may not correlate with chronic RET-induced skeletal muscle hypertrophy due to the initial muscle damage experienced at the early stages of unaccustomed exercise [51,126]. The between-group differences observed in the present investigation were nonetheless statistically equivocal; regardless, it remains plausible that FOS and its subsequent skeletal muscle phospholipid incorporation upregulated muscle protein synthetic machinery, albeit to a much smaller degree than expected.

Other alternate explanations for the FOS-mediated greater magnitudes of 1RM strength improvement despite similar LBM augmentations may be related to muscle quality and its associated factors such as muscle fiber type transition and neuromuscular function. Since relative strength in the bench press (d = 1.20) and back squat (d = 0.89) changed to a greater degree than absolute strength compared to PL, it appears that FOS influenced mechanisms related to muscle quality. Although performed in elderly populations, many fish oil supplementation studies with concurrent RET have noted similar strength improvements without significant LBM changes [57,98]. Furthermore. a study in resistance-trained young men found that 4 $g \cdot d^{-1}$ FOS improved 1RM leg extension, notably despite a loss in muscle mass during a 40% calorie restricted diet [127]. Recent evidence suggests that this may be influenced by muscle fiber type transitions [128]. In older adults, 6 weeks of 3.68 $g \cdot d^{-1}$ LC n-3 PUFA (1.86 g EPA, 1.54 g DHA) administration alongside RET significantly increased type II fCSA in the absence of whole body LBM alterations [128], corroborating similar investigations' fiber type-specific

hypertrophy following LC n-3 PUFA supplementation [129,130]. While LBM increased to a similar degree between groups, it is plausible, based on our differential 1RM strength outcomes, that type II fCSA increased to a greater extent in the FOS group. Our FOS protocol was intentionally EPA-biased (1.4:1, EPA:DHA) since it has been shown to uniquely influence MPS [18]; however, DHA is the prominent fatty acid involved in neuromuscular control [131]. As previously hypothesized by Philpott et al. [127], the DHA component of our supplement significantly increased blood DHA (~70%), thus, it is reasonable to assume that neural DHA concomitantly increased. Consequently, Rodacki et al. [68] demonstrated greater neural activation (i.e., faster muscular response after a stimulus) following 90 days of combined FOS and RET. Previous authors have reported that neuromuscular adaptations may occur as early as 21-days following FOS, although the effect may be attenuated compared to studies using higher doses or longer durations [44]. In agreement with previous investigations [127], these data may therein support an FOS-mediated neuromuscular enhancement that ultimately influenced the greater observed strength relative to PL.

Much of the evidence to date on RET and FOS is in older adults largely [57,68,98,128] [56] reports favorable results for LC n-3 PUFA supplementation. To our knowledge, there are only two studies that used a RET protocol combined with n-3 PUFA supplementation either isolated or as part of a protein-based supplement in young adults [53,54]. Specifically, Georges et al. [54] found that 8-weeks of RET with 3 $g \cdot d^{-1}$ krill oil administration significantly improved both LBM as well as 1RM bench and leg press from PRE to POST in young trained men; however, the results were nonetheless similar to placebo. Hayward et al. [53] presented similar findings in untrained females amidst a combined 4-week RET

program and protein-based supplement containing n-3 PUFAs. Although LBM and strength were improved from baseline, no significant differences were noted compared to controls. The present study also reported similar changes in LBM compared to PL; however, our investigation was the first to report greater improvements in strength outcomes. Notably, our observed FOS-mediated strength increases compared to other studies are ostensibly due to the skeletal muscle LC n-3 PUFA incorporation, likely facilitated by our more optimal dosing regimen.

Although there are significant strengths to the present investigation, such as the inclusion of young females to further our understanding of the effect of FOS and RET across the general young adult population, blood LC n-3 PUFA status measurement, equivalent macronutrient intake, as well as similar volume loads and RET-induced strength changes compared to resistance trained subjects [112,113,132], this study was not without limitations. While the number of subjects enrolled in our study was similar to other investigations, it's possible that our study may have been underpowered to detect significant between group changes in certain outcomes (e.g., LBM). The DXA is widely considered as one of the better methods to determine changes in body composition when using a standardized protocol; however, our findings could have been strengthened with the use of total body water. Lastly, subject diets were not controlled and, although unlikely, it remains possible that dietary fluctuations may have unintentionally influenced the participants' PRE-to-POST body composition.

In summary, FOS improves both absolute and relative $1RM_{BP}$, commensurate with moderate to large between-group differences for absolute and relative $1RM_{SQT}$, %BF, and FM in resistance trained young men and women. To facilitate the most

superior RET-induced adaptations, tactical athletes may need to opt for FOS, especially if consuming less than 2 servings of fish per week. Unlike targeted pharmaceutical interventions, the complex and often unspecified action of LC n-3 PUFAs - especially the notable divergent actions of EPA and DHA - on human physiology can make identification of an underlying mechanism challenging. Nevertheless, the convergence of multiple known contributors, including MPS, muscle quality characteristics, and neuromuscular control, likely contributed to our findings. As Anthony and colleagues [104] antecedently suggests, future research in tactical and non-tactical personnel is warranted to examine the influence of FOS on RET-induced adaptations using more precise tracer, muscle biopsy, and neuromuscular activation-associated assessments to ascertain the aforementioned underlying mechanisms. Additionally, these subsequent investigations should explore the differential effects of EPA and DHA on skeletal muscle functional outcomes, potentially benefiting from longer duration (> 10 weeks) supplementation protocols and doses commiserate with 3 servings of fatty fish per week.

Practical Applications

Our data reinforces the preceding literature, indicating that RET foundationally mediates beneficial skeletal muscle adaptations and body recomposition amongst young healthy adults. The addition of fish oil supplementation (4 $g \cdot d^{-1}$ [3.85 g EPA+DHA]) to a 10-week RET program may further augment absolute $1RM_{BP}$ and relative $1RM_{BP}$ and $1RM_{SQT}$, as well as a greater FM reduction. Nevertheless, it is unclear if FOS increases LBM to a greater extent than isolated RET. In light of our results and previous findings regarding the efficacy of FOS in athletic populations [47], sports medicine and dietetic staff should make a concerted effort to educate their tactical/non-tactical athletes on the

usefulness of FOS, primarily for those with low blood or suboptimal dietary intake of LC n-3 PUFAs.

CHAPTER FOUR

Manuscript Two: Dual- Versus Single-Model Exercise-Induced Muscle Damage Protocols on Indirect Markers of Muscle Damage and Cardiac Autonomic Control: A Randomized, Crossover Pilot Study

Introduction

Sporting and military environments expose individuals to intermittent periods of high-intensity movement such as sprinting, jumping, and tackling combined with the necessity for rapid deceleration and change of direction [86,133–135]. These physiological stressors, especially after extended breaks (i.e., summer break or off-season) or repeated exposure with minimal recovery (tournaments, daily operational missions, etc), may cause debilitating muscle damage that can contribute to sub-optimal physical performance and increase the susceptibility to injuries. Exercise-induced muscle damage (EIMD) results in skeletal muscle damage and the loss of myocyte integrity. As such, EIMD is characterized and quantified by significant elevations in perceived muscle soreness and serum creatine kinase. Much more recently, heart rate (HR) and cardiac autonomic activity inferred from heart rate variability (HRV) measures have been used to provide insight into the physical stress from EIMD. Systematic reviews have recognized the utility of HRV indices as an effective approach to measure an individual's adaptability to environmental demands (i.e., training load) and monitor physical stress [136,137]. Recent investigations have also shown that serum CK and training load are associated with heart rate (HR) and HR-indices of autonomic control [138–140].

While exercise stress can stimulate muscular adaptations, interventions should be in place to avoid inadequate recovery or maladaptation, especially in situations with

multiple sporting events or missions that occur within a short timeframe (e.g., tournaments, multi-day combat operations). Hence, the ability to accurately quantify the physiological impact of an EIMD protocol on the human body is crucial for understanding the recovery and rehabilitation needs of athletes and military personnel subjected to harsh training regimes or environments.

While there are numerous EIMD models, downhill running (DR) is a common method to investigate the physiological consequences and recovery kinetics of skeletal muscle. Unfortunately, DR models are oftentimes time-consuming and provide research outcomes that may have limited generalizability to athletic populations. However, in recent studies, short DR sessions (≤ 15 mins) have emerged as a new training modality to improve change of direction speed and increase knee extensor strength without substantially affecting HR, other HR-derived metrics [141] or the energy cost of running [142]. Similarly, plyometric training has become a widely accepted and effective way to improve power and sprint performance in athletes [143–145]. Additionally, acute plyometric exercise has been shown to elicit substantial muscle fiber damage with concomitant increases in serum CK activity and perceived muscle soreness [146]. Despite these recent developments and the need for protocols that can be readily translated from research to practice, an EIMD model has not been developed to include DR and plyometric movements. Due to the advent of DR as a novel training modality and the common use of plyometric exercise to augment training, a dual-model EIMD protocol combining short-duration DR with a plyometric exercise should be tested in athletic populations.

Therefore, the aim of this study was to determine the viability of a novel dual-EIMD model (DR plus plyometric) compared to two single-model protocols, DR only

and plyometric only, as measured by indirect markers of muscle damage and modulation of cardiac autonomic control. We hypothesized that a dual-model EIMD protocol would lead to a greater CK and muscle soreness response with similar modulation of HR and HR-derived indices of autonomic control compared to the single-model EIMD protocols.

Methods

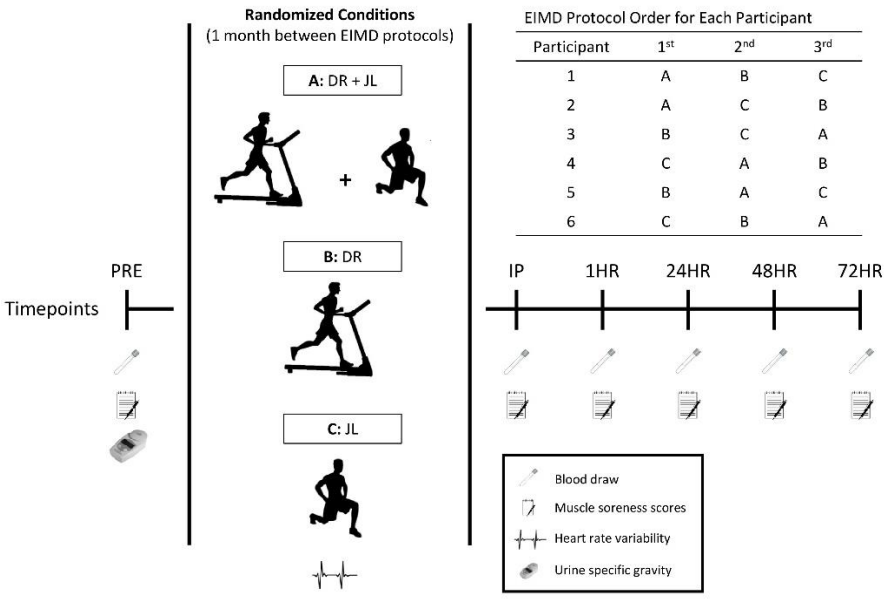
Participants

Six recreationally trained men were volunteered to participate and completed three EIMD protocols of the study as part of a randomized, crossover design. *A priori* power analysis for a repeated-measures design indicated that an estimated sample size of 6 would be sufficient to demonstrate a significant result with an α level of $p < .05$, power of 0.8, and partial eta squared (η_p^2) of ≥ 0.3. All participants were non-smokers, did not report any chronic diseases or comorbities, and exercised at least 3 days per week for the past 6 months. This study was approved by Baylor University's Institutional Review Board before any recruitment or testing occurred and was carried out in accordance with the guidelines of the declaration of Helsinki. After discussion of the study details, each participant provided written informed consent. Subjects were asked to avoid using any other therapeutic modality (massage, ice compression, or non-steroidal anti-inflammatory drugs) and lower-body exercise 2-days prior and throughout the experimental protocol days.

Experimental Design

This was a randomized, crossover study involved a screening visit and three experimental sessions separated by one month. Figure 4.1 outlines the experimental

sessions. Briefly, each participant was randomly assigned to one of the three EIMD protocols that consisted of A. downhill running plus jumping lunges (DR + JL), B. DR only, and C. JL only. All experimental sessions occurred at approximately the same time of day. Visit 1 included height and weight and an aerobic capacity treadmill test assessment. Blood draws and perceived muscle soreness assessments were obtained before the EIMD protocol and then, immediately (IP), 1-hr, 24-hr, 48-hr, and 72-hr post at visits 2, 3, and 4. The one-month time interval between conditions was to protect against the repeated bout effect, which can attenuate the extent of muscle damage during subsequent EIMD bouts. As noted by Bontemps et al. [147] endurance trained individuals experience an attenuated response to DR protocols. As such, the visit 1 aerobic capacity testing was used as a screening tool to identify those that are trained ($VO_2max > 54$ $ml \cdot kg^{-1} \cdot min^{-1}$). Additionally, participants were unable to wear compression garments during the protocols and were asked to refrain from using ice baths or anti-inflammatory medication.



EIMD Protocol Order for Each Participant

Participant	1st	2nd	3rd
1	A	B	C
2	A	C	B
3	B	C	A
4	C	A	B
5	B	A	C
6	C	B	A

Figure 4.1. Schematic overview of the experimental sessions. The study involved three experimental sessions in a randomized, crossover manner. At each visit, participants (n = 6) performed one of three exercise-induced muscle damage (EIMD) protocols: A) downhill running at 70% VO_2max for 20 minutes (DR) followed by 5 sets of 20 repetitions of jumping lunges (JL), B) DR only, or C) JL only. The participants conducted sessions at least one month apart.
Abbreviations: PRE, before EIMD; IP, immediately post.

Hydration Status

Urine sample was collected pre-EIMD to assess participants' hydration status. Hydration was verified by checking that urine specific gravity (USG) via handheld digital refractometer (MISCO Palm Abbe^{TM} PA201X-093, MISCO, Solon, OH) upon arrival was ≤ 1.02 prior to each EIMD condition [148]. The refractometer was calibrated with distilled water before each sample. A previous validation study indicated high precision (%CV < 0.01) for the MISCO Palm Abbe^{TM} handheld digital refractometer [149].

Aerobic Capacity Testing

To determine maximal aerobic capacity (VO_2max), participants performed a volitional maximal cardiopulmonary exercise (VO_2max) test on a laboratory treadmill using the Bruce protocol. Briefly, the protocol began at a grade of 10% and speed of 1.7

mi·hr^{-1}. Every 3 minutes, the incline increased by 2% and the speed increased by 0.8 or 0.9 mi·hr^{-1}. Participants were instructed to perform the test for as long as possible to ensure a true maximal attempt. Standard ACSM test termination criteria were monitored and followed throughout each test. Metabolic gases were obtained with the Parvo Medics 2400 TrueMax metabolic measurement system (Parvo Medics, Sandy, Utah, USA) to determine maximal aerobic capacity. Based on a previous investigation, the mean coefficient of variation for this protocol is 6.5% [150].

Exercise-Induced Muscle Damage Protocols

Each participant performed three EIMD protocols in random order (Figure 4.1). The conditions were separated by one month. Before each EIMD condition, participants completed a brief warm-up which consisted of 5 minutes on the stationary bike at 60 RPMs, followed by 5 minutes of self-directed dynamic stretching.

Downhill running. The DR condition was adopted from a protocol used previously [78]. Briefly, on a DR treadmill (MT200 Gait Trainer Treadmill, Spirit Medical, Jonesboro, AR, USA) at a 16% decline, participants ran for 20 minutes at 70% of their previously determined VO_2max. Oxygen consumption and heart rate were monitored continuously, and the treadmill speed was adjusted accordingly to maintain an intensity at 70% of VO_2max. Average speed during this condition was 10.7 km·h^{-1} ± 1.2.

Jumping lunges. For the plyometric component, we opted to use jumping lunges as this movement has been used previously as a secondary movement in a dual-model EIMD protocol [74]. Briefly, participants performed 5 sets of 20 repetitions of JLs, separated by 2 minutes of recovery time between sets. No side-to-side movement was

allowed and would result in a no-count and the knee had to touch the ground for the repetition to be counted.

Downhill running and jumping lunges. For the combined condition, participants conducted the DR protocol, then rested without sitting for 2 minutes, then began the JL protocol. The average speed during the DR portion of this condition was 10.6 $km \cdot h^{-1}$ ± 1.3.

Indirect Markers of Muscle Damage

Perceived muscle soreness. Muscle soreness was assessed at baseline, immediately post EIMD, 1-hour post, then at 24-, 48-, and 72-hours post EIMD using a 10-cm visual analog scale (VAS) with an anchor of "no soreness" (0 cm) to "worst soreness ever" (10-cm). The participant was asked to draw a vertical line corresponding to the level of soreness in the lower body. Muscle soreness was assessed in three conditions: rested (VAS-R), extended (VAS-E), and active (VAS-A). In VAS-R, the participant was in the seated position and for VAS-E, the participant assessed perceived muscle soreness after extending at the knee and ankle joints (full extension from the seated position). For VAS-A, the participant assessed perceived muscle soreness after performing a body weight squat parallel to the ground (90°).

Serum creatine kinase. Venous blood sample (~10 mL) was collected by a standard 46nalysed46ure technique from the antecubital space. All blood samples were allowed to clot, centrifuged for 10 mins to obtain serum, and immediately sent to Clinical Pathology Laboratories (Waco, TX) for CK analysis. Serum CK activity was measured

prior to EIMD (PRE) and immediately- (IP), 1 hr-, 24 hr-, 48 hr-, and 72 hr-post. The percent change (%Δ) in serum CK from baseline was used for analysis.

Heart Rate and HR-Derived Indices of Autonomic Control

All HR and HRV testing was conducted during the bout of EIMD. Participants wore a HR monitor using an elastic electrode Polar belt (H7™, Polar Wearlink®, Lake Success, NY). HRV indices obtained from HR were measured and processed by the CardioMood® smartphone application. The HRV score and stress index (SI [(amplitude of mode %)/(2*mode*(RR_{max} – RR_{min})]) were used to quantify the internal workload associated with each EIMD condition [151]. Other HRV analysis included the square root of the mean sum of the squared differences between N-N intervals (RMSSD) and the low- to high-frequency ratio (LF:HF). RMSSD reflects vagal activity [152] and has greater reliability compared to other spectral indices [153], especially in ambulatory conditions [154]. The LF:HF ratio can be used to identify whether sympathetic or parasympathetic activity predominates [155]. The HRV score, SI, RMSSD, and LF:HF are reported as the average over each EIMD condition.

Statistical Analyses

Data are reported as means ± SD, unless otherwise indicated. Normality and homogeneity were tested on all dependent variables using Shapiro-Wilk's and Levene's tests, respectively. The assumption of homogeneity was violated for VAS-R, VAS-E, VAS-A, and %Δ serum CK at one timepoint (IP). Since all other timepoints were considered homogenous and we had equal observations (n = 6 per condition), we opted to continue data analysis using parametric statistics. As such, data were 47nalysed using a

repeated measures ANOVA with condition (DR+JL vs DR vs JL) and time (PRE, IP, 1HR, 24HR, 48HR, 72HR). Violation of sphericity was corrected using the Greenhouse-Geisser method. When significant differences were confirmed with ANOVA, multiple comparison testing was performed using the Bonferroni post hoc correction to identify differences between conditions. Partial eta squared (η_p^2) was used to estimate the proportion of variance in the dependent variables explained by the independent variable for overall condition, time, and condition*time effects. Partial eta-squared effect sizes (ES) were determined to be: small ≤ 0.12, medium = 0.13 – 0.25, large ≥ 0.26 [156]. Cohen's *d* ES were calculated to quantify the magnitude of the between conditions differences at each timepoint for %Δ serum CK and cardiac autonomic activity. As recommended by Cohen [119], ES were interpreted as small = 0.20 – 0.49, medium = 0.50 – 0.79, and large ≥ 0.80. One-way ANOVA or the nonparametric Kruskal-Wallis ANOVA (stress index and LF:HF ratio) were used to identify differences between conditions for USG, HR, and other HR-derived variables. All statistical analyses were completed using SPSS (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY, USA). Significance was set *a priori* at $p < .05$.

Results

Descriptive characteristics of the participants are in Table 4.1. Every participant completed all assessments for each condition.

Table 4.1. Participant characteristics (n = 6)

Variable	Mean ± SD	Range
Age (y)	28.3 ± 3.8	24-35
Height (cm)	175.7 ± 6.0	168.0-185.0
Body Mass (kg)	90.3 ± 13.0	73.1-111.8
VO_2max		
$L \cdot min^{-1}$	3.3 ± 0.2	3.1-3.6
$ml \cdot kg^{-1} \cdot min^{-1}$	36.6 ± 4.0	32.2-42.8

Hydration Status

Participant's hydration status was similar between conditions (p = .659). The average USG for DR+JL, DR, and JL was 1.013 ± 0.007, 1.016 ± 0.005, and 1.018 ± 0.003, respectively.

Indirect Markers of Muscle Damage

Perceived muscle soreness. Muscle soreness scores in the rested condition (VAS-R) significantly increased over time (p < .001, η_p^2 = 0.273); however, no main effects for condition (p = .165, η_p^2 = 0.213) or interaction effects for condition*time (p = .701, η_p^2 = 0.088) were noted. In the extended condition (VAS-E), there were significant main effects for time (p < .001, η_p^2 = 0.355) and condition (p = .005, η_p^2 = 0.505). Perceived muscle soreness during extension was significantly higher in the DR+JL (p = .004) compared to JL. DR was similar to JL (p = .180) and DR+JL (p = .240). No condition*time effects were observed (p = .101, η_p^2 = 0.183). For VAS-A, there were significant main effects for time (p < .001, η_p^2 = 0.385) and condition (p < .001, η_p^2 = 0.627); however, there were no significant interactions between experimental factors (condition*time, p = .10, η_p^2 = 0.183). Muscle soreness in the active condition was significantly higher after the DR+JL (p < .001) and DR (p = .012) compared to JL.

Similar VAS-A scores were observed between the DR+JL and DR conditions (p = .439). Significant time effects for VAS-R, VAS-E, and VAS-A are noted on Figure 4.2.

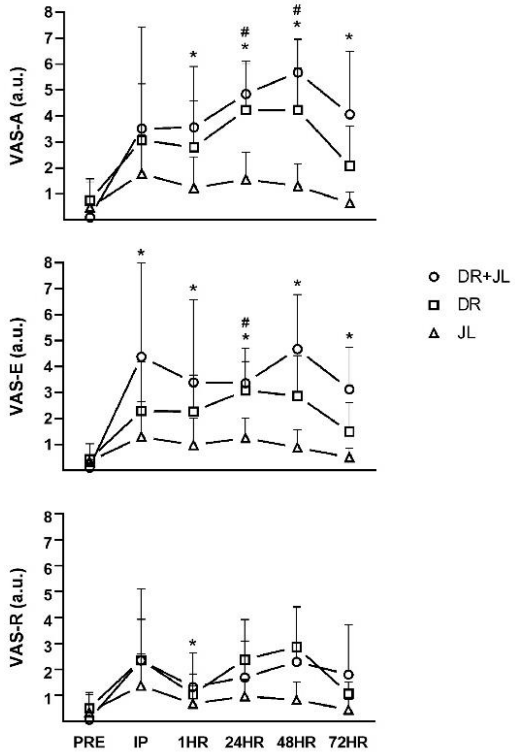


Figure 4.2. Changes in perceived muscle soreness of the lower-body before (PRE) and immediately (IP), 1-hour, 24-hour, 48-hours, and 72-hours after each bout of exercise-induced muscle damage (downhill running plus jumping lunges [DR+JL], downhill running only [DR], and jumping lunges only [JL]).
Data are mean ± SD.
*significantly different from baseline (DR+JL, p < .01)
#significantly different from baseline (DR, p < .05)

Serum creatine kinase. Significant condition*time (p = .005, η_p^2 = 0.276) and main time (p < .001, η_p^2 = 0.322) effects were found for %ΔCK. Compared to baseline, %ΔCK was significantly higher IP and 1HR in the DR+JL (p < .001 for both) and DR (p < .001 and p = .017, respectively) conditions. For the DR+JL condition, %ΔCK was significantly higher compared to baseline at 24HR (p = .025) and a similar trend was observed at 72HR (p = .07). There was no time effect noted for the JL condition (p > .05). Immediately post EIMD, %ΔCK was significantly higher in the DR+JL condition

compared to DR (p = .019) and JL (p < .001) and DR was significantly higher compared to JL (p = .043). At 1HR, %ΔCK was significantly higher in the DR+JL condition compared to DR (p = .015) and JL (p < .001). A similar trend was noted for %ΔCK between DR+JL and JL at 24HR (p = .063). These data and between group effect sizes are shown in Table 4.2. On an individual level, 2, 4, and 6 participants in the JL, DR, and DR+JL conditions, respectively, exhibited physiologically high serum CK levels (> 336 $U \cdot L^{-1}$) at any timepoint.

Table 4.2. Percent change from baseline in creatine kinase for each condition

Timepoints	Downhill Running (DR)	Jumping Lunges (JL)	DR+JL	Between Group Effect Size		
				DR+JL vs DR	DR+JL vs JL	DR vs JL
IP	**16.8 ± 5.4**[a]	6.8 ± 6.4[b]	**28.2 ± 6.8**[c]	1.86	3.24	1.69
1HR	**29.2 ± 14.7**[a]	4.9 ± 6.2[a]	**63.2 ± 26.5**[b]	1.59	3.03	2.15
24HR	271.1 ± 362.7	39.3 ± 54.8	**861.9 ± 885.8**	0.87	1.31	0.89
48HR	139.5 ± 209.2	37.4 ± 116.8	467.4 ± 616.0	0.71	0.97	0.60
72HR	62.3 ± 91.1	36.5 ± 147.1	274.8 ± 305.5	0.94	0.99	0.21

Abbreviations: IP, immediately post exercise-induced muscle damage (EIMD); 1HR, 24HR, 24HR, 72HR, post-EIMD timepoints.
Bold, significantly different than baseline (p < .05)
[abc]Within a row, means without a common superscript differ (p < .05)
ES, Cohen's d effect size (between group difference calculation: d = [(M1-M2)/SDpooled])

Heart Rate and HR-Derived Indices of Autonomic Control

HR and HR-derived data are reported in Table 4.3. During EIMD, HR was significantly higher in the DR+JL (p < .001, d = 3.98) and DR (p < .001, d = 3.41) compared to the JL condition. Similarly, the SI was higher in the DR+JL (p = .017, d = 2.78) and DR (p = .028, d = 1.82) conditions compared to JL. For HR and SI, the differences between DR and DR+JL conditions were moderate (p = .85, d = 0.55) and small (p = 1.00, d = 0.08), respectively. All other variables, RMSSD, LF:HF ratio, and HRV score, were similar between groups (p > .05).

Table 4.3. Comparison of HR and HRV indices between EIMD conditions

	Downhill Running (DR)	Jumping Lunges (JL)	DR+JL
HR (beats·min^{-1})	157.8 ± 15.2[a]	116.6 ± 7.8[b]	166.4 ± 15.9[a]
RMSSD (ms)	113.7 ± 112.4	158.2 ± 159.1	53.9 ± 36.8
LF:HF ratio	0.8 ± 0.4	1.4 ± 1.3	0.7 ± 0.3
HRV Score	84.3 ± 22.8	88.0 ± 28.6	75.6 ± 14.2
Stress Index	1730.2 ± 1194.6[a]	190.7 ± 99.5[b]	1817.0 ± 822.6[a]

Abbreviations: HR, heart rate; HRV, heart rate variability; EIMD, exercise-induced muscle damage; RMSSD, square root of the mean sum of the squared differences between N-N intervals; LF, low frequency; HR, high frequency
Data are mean ± SDs
[ab]Within a row, means without a common superscript differ ($p < .05$)

Protocol Order Effect

There was no protocol order effect on any dependent variable (%Δ CK, $p > .596$, $\eta_p^2 = 0.069$; VAS-R, VAS-E, VAS-A, or any HRV indices, $p > .05$).

Discussion

This study compared the effects of three different EIMD protocols on changes in serum CK and perceived muscle soreness to test the hypothesis that the magnitude of the changes would be greater for the dual-model protocol compared to the single-model without greatly affecting internal workload as measured by cardiac autonomic activity in recreationally-trained men. In agreement with our hypothesis, the dual-model EIMD protocol elicited greater indirect muscle damage than the single-model EIMD protocols. In contrast, HR and HR-derived measures of physical stress were similar between DR+JL and DR; however, internal workload was substantially less in the JL condition.

The presence of muscle damage is classically identified using changes in non-invasive and invasive indirect markers such as perceived muscle soreness and CK activity, respectively [157]. Perceived muscle soreness scores were different based on the type of assessment scale used i.e. rested, extended, or active. There were no notable differences between conditions with a passive assessment of muscle soreness (VAS-R). However, VAS-E and VAS-A results depicted a large degree of soreness from DR+JL

and DR compared to JL. Downhill running alone produced a similar degree of muscle soreness in the VAS-R and VAS-E conditions to JL. To gauge the effect of muscle damage on functional muscular status, it may be more prudent to use extension, flexion, and/or active conditions in lieu of passive standing or sitting positions for perceived muscle soreness.

Regarding serum CK, there was a large variability between individuals and conditions; however, serum CK was consistently elevated for every participant in the DR+JL condition. While the DR only protocol produced a much more blunted CK response (271.1%). This same protocol has been used previously [78,158,159]. In the most recent investigation, the control arm (n = 16) experienced a substantial increase in CK (1006.5%). Whereas the previous investigations were more in line with our results [158,159]. In 6 males, Sorichter et al. [158] found that DR (-16%) at 70% VO_2max elicited a ~290% increase in CK. Both studies used HR to estimate VO_2max and did not report participant average speed during the DR protocol. While the relationship between HR and VO_2max is generally linear, this method has some limitations and may lead to an estimated VO_2max values 10-20% lower than the measured values [160]. Since the mean or median speed during the DR bout was not reported, it is unclear, yet plausible, that the participants from the Mickleborough et al. [78] investigation were at a greater speed, which has been shown to substantially increase CK activity during DR [147]. It is clear from these data that researchers using DR alone may need to adopt more traditional protocols that tend to be longer in duration (median: 40 min) with a -10 to -15% slope (median: -12%) [147]. For example, a more traditional protocol used in our lab consisted of 45 min DR with -10˚ slope at 60% VO_2max elicited an approximately 450% increase in serum CK [161].

Combining DR with an eccentrically-biased plyometric exercise appears to produce an appropriate CK and soreness response to use as a research EIMD protocol.

Plyometric exercise as a EIMD model has been shown to produce increases in CK, perceived muscle soreness, and muscle fiber damage [146,162,163]. However, results are modest relative to DR protocols. Our data suggests that jumping lunges alone do not elicit an appropriate muscle soreness or CK response to be an effective model to study EIMD. Previous investigations have used squat and drop jumps with shorter rest intervals (60 s), which appear to be more appropriate plyometric exercises for single-model EIMD [146,162,163].

Although HR and HRV-indices are typically evaluated during rest, recent evidence suggests that HRV kinetics during or in response to exercise stressors may have considerable potential to monitor training load and training-induced fatigue [164–167]. In the present study, HR and SI was similar between DR+JL and DR; however, both variables were significantly higher than JL alone. While DR+JL produces higher indirect markers of muscle damage compared to DR and JL, it appears that the amount of internal cardiovascular strain is similar to DR.

In a research setting, the use of a dual-model EIMD protocol, including DR and plyometrics, does appear to affect the magnitude of muscle damage compared to DR or plyometric exercise only. However, cardiac sympathetic activity and parasympathetic withdrawal were similar between DR+JL and DR conditions, but more pronounced compared to plyometric exercise alone. Future studies may benefit from utilizing EIMD protocols with more than one eccentric exercise component that may represent feasible muscle damage induced in military and athlete settings.

CHAPTER FIVE

Manuscript Three: The Differential Effects of EPA and DHA on Recovery from Exercise-Induced Muscle Damage

Introduction

Certain sporting and military environments expose individuals to intermittent periods of high-intensity movement such as sprinting, jumping, and tackling combined with the necessity for rapid deceleration and change of direction [86,133–135]. These physiological stressors may lead to exercise-induced muscle damage (EIMD), especially after extended breaks (i.e., summer break or off-season) or new training cycles with eccentric or unaccustomed exercise. EIMD oftentimes leads to acute, yet debilitating, muscle soreness, loss of muscle function, exercise capacity, and force production. The EIMD associated loss in muscle function and increased soreness are important factors to combat for athletes and military personnel as they can contribute to sub-optimal performance and increase susceptibility to injuries. While exercise stress can stimulate muscular adaptations, interventions should be in place to avoid inadequate recovery or maladaptation, especially in situations with multiple sporting events or missions occurring within a short time frame (e.g., tournaments, multi-day combat operations).

Recent reviews have highlighted the utility of nutritional interventions to improve negative sequelae associated with EIMD [168–170]. These reviews similarly acknowledge the potential effectiveness of omega-3 polyunsaturated fatty acid (PUFA) supplementation on parameters of muscle recovery. Our systematic review identified muscle recovery, including the preservation of strength after muscle damage, as one of the most important

roles of omega-3 PUFA supplementation in young, healthy adults [102]. However, questions remain. Due to the heterogeneity of omega-3 PUFA supplementation in studies, it is unclear if the effects on recovery are mediated by the total omega-3 dose or the potential differential effects of the type of long-chain omega-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

While recent investigations have delved into the dose-response relationship of omega-3 PUFA and recovery from EIMD [74,171], there are no studies on the effects of EPA vs DHA on muscle recovery. The long-chain omega-3 PUFAs, EPA and DHA, are the most bioactive constituents and modify cell membranes, such as skeletal and cardiac muscle. While EPA and DHA seem to exert similar effects on inflammation [172], EPA is uniquely associated with improvements in skeletal muscle protein synthesis and breakdown and insulin action [18,173,174]. DHA is preferentially incorporated into myocardium and, thus, may have more potent effects on heart rate and heart rate recovery, not necessarily muscle recovery. As such, markers of muscle recovery such as muscle soreness, strength, power, range of motion, and swelling may be differentially affected by EPA and DHA supplementation.

The purpose of this novel investigation was to examine the differential effects of EPA and DHA on markers of muscle recovery following EIMD – creatine kinase, perceived muscle soreness, strength, range of motion, and swelling. This would be the first study to investigate the differential effects of EPA and DHA on recovery from EIMD.

Methods

Study Design

Using a randomized, double-blind, placebo-controlled experimental design, participants were allocated to one of four groups: EPA+DHA, EPA only, DHA only, or placebo (PL; coconut oil). Participants consumed 4 capsules (4 g) of their assigned supplement daily for 52 days. Phase 1 consists of screening, 6-weeks of supplementation, and baseline muscle function measures, while phase 2 consists of the EIMD protocol and three days of muscle recovery testing. Muscle soreness, venous blood, and indices of muscle function and recovery were collected prior to EIMD, as well as immediately post, 1-, 24-, 48-, 72-hours (H) afterward. A schematic overview of the study design is depicted in Figure 5.1.

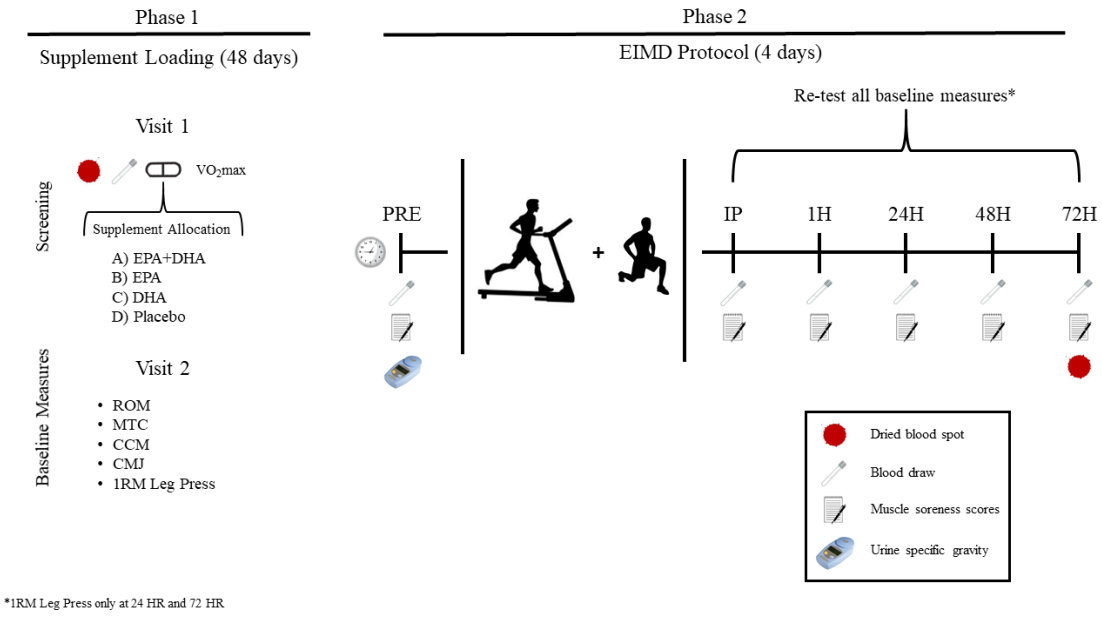


Figure 5.1 Schematic Overview of the Study Design
Abbreviations: ROM, range of motion; MTC, mid-thigh circumference; CCM, calf circumference; CMJ, countermovement jump

Participants

A total of 31 healthy, physically active (self-reported: 3-4 days per week of exercise) males aged 18-35 years were recruited to participate in the present investigation. Following participant withdrawal (n = 1; injury unrelated to the study), 30 participants completed the study (EPA+DHA, n = 8; EPA, n = 8; DHA, n = 7; PL, n = 7).

Individuals were excluded if they 1) had current and/or history of any musculoskeletal or neuromuscular disorders; 2) had any self-reported illness or conditions that may interfere with the study parameters or put the participant at significant risk; 3) had a body fat percentage > 26%; or 4) reported the use of fish oil supplements within the past 6 months or fish intake > 3 servings per week.

This study was approved by the Baylor University Institutional Review Board (Project ID: 1755737-3) and was performed in accordance with the ethical standards of the Declaration of Helsinki. All participants were informed of the experimental procedures, EIMD protocol, and associated risks before providing written informed consent.

Supplementation Protocol

Throughout the duration of the study, in a double-blind manner, participants will randomly be assigned to one of four groups: EPA+DHA, EPA only, DHA only (Carlson Labs, Arlington Heights, IL), and placebo (coconut oil [Jarrow Formulas, Los Angeles, CA]) using a random number generator (www.randomization.com). A third party, not associated with data collection, analysis, or outcome assessment, assigned the supplements a letter (A, B, C or D) to ensure the investigators are blinded to the treatment allocation. Participants will be randomly assigned to orally ingest 4g of EPA+DHA (2g

EPA, 2g DHA), 4g EPA, 4g DHA, or 4g coconut oil capsules, which will start immediately after visit 1 and continue throughout the duration of the study (day 1 to 52). Each participant will be provided with enough of their respective supplement for the entire duration of the study. Weekly email reminders will be sent to reinforce compliance. Participants will also be asked to save their capsule bottles and present it to a researcher at visit 2 and visit 6. Additionally, and most importantly, compliance will be tracked with red blood cell EPA+DHA (the omega-3 index), which is considered the goal standard for tracking long-chain omega-3 intake.

Diet and Activity Controls

Participants will be asked to maintain their normal diet and consume < 2 servings of fish per week. Participants will track their macronutrient intake via MyFitnessPal for 2-days (1 weekday, 1 weekend). Additionally, they will complete a validated omega-3 food frequency questionnaire (18) to track their omega-3 PUFA intake. Both will be completed at the beginning and end of the study. Similar to diet, exercise will not be strictly controlled; however, subjects will be asked to keep their training regimes the same. Subjects will fill out a physical activity questionnaire at visit 1 and at the final visit to determine if training remained consistent.

Anthropometrics

Participants' height and weight will be measured via stadiometer and digital scale at every visit (SECA 703, Hamburg, Germany). A dual energy x-ray absorptiometry (DXA) will be conducted to determine body composition at visit 1 and visit 6.

Aerobic Capacity Assessment

At visit 1, participants will perform a volitional maximal cardiopulmonary exercise test according to the Bruce protocol (19). Participants will be instructed to perform the test for as long as possible to ensure a true maximal attempt. During this session, heart rate (HR) and heart rate variability (HRV) will be monitored. Standard American College of Sports Medicine test termination criteria will be monitored and followed throughout each test. Metabolic gases will be obtained with the Parvo Medics 2400 TrueMax metabolic measurement system (Parvo Medics, Sandy, Utah, USA) on a laboratory treadmill (TrackMaster, Newton, Kansas, USA). The mean coefficient of variation (assessing VO_2max) for this protocol has previously been shown to be 6.5% (range 2%–14%) (19).

Hydration Status

Urine sample was collected pre-EIMD to assess participants' hydration status. Hydration was verified by checking that urine specific gravity (USG) via handheld digital refractometer (MISCO Palm Abbe^{TM} PA201X-093, MISCO, Solon, OH) upon arrival was ≤ 1.02 prior to the EIMD protocol [148]. The refractometer was calibrated with distilled water before each sample. A previous validation study indicated high precision (%CV < 0.01) for the MISCO Palm Abbe^{TM} handheld digital refractometer [149].

Exercise-Induced Muscle Damage Protocol

On visit 3, participants performed an eccentrically biased aerobic exercise test followed by a plyometrics component adapted from two separate protocols (12, 20). On a treadmill modified and positioned to be downhill at a grade of 16%, participants ran for 20 minutes at 70% of their VO_2max. Oxygen consumption will be monitored

continuously, and the treadmill speed will be adjusted accordingly to maintain intensity at 70% of VO_2max. Once complete, the participant rested for two minutes, then began the plyometric section of the protocol that consisted of five sets of 20 jumping lunges with a two-minute rest between each set. Participants refrained from any structured exercise for 48 h and lower-body exercises for 72 h prior to the EIMD protocol and were asked to avoid using any other therapeutic modality (massage, ice, compression, NSAIDs, etc.) and any exercise until the completion of data collection.

Blood and Biochemical Analysis

A venous blood sample was obtained into 10ml vacutainer tubes using a 22-gauge phlebotomy needle inserted into the antecubital vein based on our standard laboratory protocol (26). Creatine kinase will be determined from the venous blood sample as indirect markers of muscle damage used previously (12, 27). Serum blood samples will be taken at PRE and every timepoint after the EIMD protocol.

Muscle Recovery Assessments

Subjective muscle soreness. As an indicator of delayed onset of muscle soreness and the severity of muscle injury, muscle soreness was assessed using a 10-cm visual analog scale (VAS) (0 = no soreness, 10 = worst soreness ever). Participants rated their level of soreness immediately before EIMD and immediately after, then at 1-, 24-, 48-, and 72H post-EIMD by drawing an intersecting line across a continuum line extending from 0 to 10. Participants provided three separate VAS measures at each timepoint: resting (unweighted, sitting), full extension (knee and ankle joint), and active. For the active component, the participant will be asked to squat down to 90˚ from a standing

position, rise to the starting position, and then draw a perpendicular line corresponding to the soreness level. Perceived muscle soreness was then measured in centimeters using a ruler across the scale.

Jump performance and power metrics. Participants' vertical jump performance and power will be assessed by the countermovement jump (CMJ) with no arm swing. The CMJ has been shown to be one of the most reliable methods to measure lower-body power and is related to sprint and 1-RM performance (21). For the CMJ, the participant starts from an upright standing position, makes a preliminary downward movement by flexing at the knees and hips, then immediately extends the knees and hips again to jump vertically up off the ground. There will be no arm swing, hands will be placed on the participants hips for each CMJ. Participants will conduct three CMJs with a 1-minute rest between each attempt. The average (jump height [JH]) and highest jump (jump height peak [JHP]), recorded to the nearest 0.01 cm, will be used for statistical analysis. Additionally, mean and peak power (W) will be calculated based on previously validated equations (22, 23).

Strength. For lower-body strength, participants will perform 1RM leg press (LP) test at visit 2 (baseline), then 24H and 72H post-EIMD. All participant exercise testing and protocols will be completed using a four-point tempo prescription that controls eccentric, amortization, concentric, and lift beginning to standardize repetitions. A tempo of 1-0-1-0 will be used to standardize all repetitions. Additionally, LP foot placement will be recorded and held constant over all testing conditions to maintain consistency. To ensure participants are moving through the full range of motion during each repetition, a

goniometer (Prestige Medical, Northridge, CA) will be used to establish 90˚ of knee flexion on the LP. Any excessive "bouncing" at the intersection of the eccentric and amortization phases will not be counted as a successful repetition. Participant warm-ups were standardized and adapted from the procedures used previously used by our lab. Briefly, subjects will complete 10 repetitions at approximately 50% of their estimated 1RM; subsequently, participants will rest for 2 minutes before completing 5, 3, and 1 repetition(s) at approximately 70%, 80%, and 90% of their estimated 1RM. Load will then be increased conservatively ~5-10% as per lower-body exercise testing NSCA guidelines and the participant will attempt to lift the load for 1 repetition. If the lift is successful, the participant will rest for 3 minutes before attempting the next weight increment, and this procedure will continue until the participant fails to complete the lift. The 1RM will be recorded as the maximum weight that the participant is able to lift for a single repetition. All attempts will be blinded from the participant via weight coverings used previously in our lab for the back squat [116].

Range of motion (ROM). ROM has been shown to be an accurate method of determining the extent of muscle damage and will be measured PRE, IP, 1H, 24, 48H, and 72H post-EIMD. Subjects will be instructed to lay prone on a massage table with both knees fully extended. Subjects will flex their right knee with no assistance from the investigator, and the angle measured with a goniometer (Prestige Medical, Northridge, CA) using universal landmarks (lateral epicondyle of the femur, lateral malleolus and greater trochanter) that were marked with a permanent marker to ensure consistency on subsequent measures. Three measurements will be averaged and reported in degrees. This method for assessing ROM has been validated previously (24).

Mid-thigh and calf circumference. To determine the presence of swelling within the muscle, MTC and calf circumference will be conducted based on a previously established protocol (20). Briefly, mid-thigh circumference will be assessed at the midpoint of the anterior superior iliac spine and SPP of the right leg with an arthrometric tape. While standing, participants will be instructed to equally distribute their weight on both legs and three measurements will be taken. For calf circumference, the largest portion (girth) of the calf will be measured. The measurement site will be marked for consistency.

Statistical Analysis

To determine the effect of supplementation, an ANOVA with repeated measures (separate treatments [4 levels: EPA+DHA, EPA, DHA, Placebo] X time [6 levels: PRE, IP, 1H, 24H, 48H, 72H]), will be conducted for CK, VAS, JH, JHP, MP, PP, while leg press will only have three timepoints (PRE, 24HR, and 72HR). Absolute and percent changes from baseline will be analyzed. Whole blood fatty acids, dietary, and exercise data will be analyzed similarly; however, there are only two timepoints (PRE and POST). In the event of significant group by time interactions, pairwise comparisons with a Bonferroni correction will be used to determine which groups are different. If the assumption of sphericity (Mauchly's test) was violated, the Greenhouse-Geisser correction was used. Effect sizes will be reported as indicated (partial eta squared and Cohen's *d*) and all data will be reported at mean ± standard deviation (SD), unless otherwise specified. The sample size for this project is 28-36, justified by a priori power analysis in G*power using a target effect size (ES) of f = 0.25, alpha of 0.05 and power of 0.80, which determined that 28 subjects (7 per group) were required for participation.

To account for the possibility of an approximately 25% dropout rate, we planned to recruit up to 36 participants (9 per group).

Results

Participant descriptive data are shown in Table 5.1.

Table 5.1 Baseline participant descriptive data

Variables	EPA+DHA (n = 8)	EPA (n = 8)	DHA (n = 7)	PL (n = 7)	*p*-value
Age (*y*)	20.5 ± 2.6	22.6 ± 4.7	19.1 ± 1.2	24.1 ± 7.0	.171
Height (cm)	179.3 ± 5.7	179.9 ± 11.8	183.6 ± 2.1	181.6 ± 5.1	.674
Weight (*kg*)	83.6 ± 17.2	79.6 ± 19.0	78.9 ± 7.6	83.5 ± 11.7	.887
BMI ($kg \cdot m^{-2}$)	26.0 ± 5.1	24.3 ± 3.3	23.4 ± 2.1	25.4 ± 4.2	.566
Body Fat (%)	18.2 ± 5.0	14.1 ± 3.5	14.9 ± 3.1	15.2 ± 3.4	.186
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	40.1 ± 3.7	45.7 ± 4.9	41.7 ± 6.1	42.0 ± 4.1	.147

Hydration Status

Participant's hydration status was similar between conditions (p = .532). The average USG for EPA+DHA, EPA, DHA, and PL was 1.016 ± 0.007, 1.012 ± 0.005, 1.013 ± 0.008, and 1.011 ± 0.008, respectively.

Dietary and Exercise Controls

Descriptive characteristics of dietary and exercise data from PRE to POST are noted in Table 5.2. There were no significant PRE to POST changes or group differences noted for any macronutrient. Overall, DHA was significantly reduced from PRE with a similar trend noted for EPA intake. Exercise sessions, minutes per session, and intensity were similar from PRE to POST and between groups.

Table 5.2 Dietary and Exercise Data

Variables		EPA+DHA	EPA	DHA	PL	Time, *p*-value	GxT *p*-value
Dietary Intake							
Energy (kcal·kg^{-1})	PRE	25.1 ± 8.1	28.4 ± 6.0	23.9 ± 3.5	24.1 ± 3.1	.618	.681
	POST	25.9 ± 11.3	27.3 ± 6.8	23.2 ± 2.4	24.0 ± 3.7		
	p-value	*.489*	*.361*	*.545*	*.681*		
Carbohydrate (g·kg^{-1})	PRE	2.8 ± 1.1	2.9 ± 1.1	2.2 ± 0.6	2.4 ± 0.7	.121	.995
	POST	2.7 ± 1.2	2.8 ± 1.0	2.1 ± 0.5	2.4 ± 0.9		
	p-value	*.385*	*.352*	*.585*	*.409*		
Protein (g·kg^{-1})	PRE	1.4 ± 0.4	1.5 ± 0.5	1.4 ± 0.3	1.5 ± 0.8	.720	.592
	POST	1.5 ± 0.7	1.5 ± 0.4	1.4 ± 0.4	1.5 ± 0.8		
	p-value	*.319*	*.471*	*.678*	*.588*		
Fat (g·kg^{-1})	PRE	0.9 ± 0.4	1.2 ± 0.3	1.0 ± 0.2	0.9 ± 0.1	.835	.619
	POST	1.0 ± 0.5	1.1 ± 0.3	1.0 ± 0.2	1.0 ± 0.1		
	p-value	*.341*	*.576*	*.600*	*.580*		
EPA (mg·d^{-1})	PRE	20.6 ± 14.5	39.0 ± 69.1	21.9 ± 26.4	26.4 ± 19.2	.090	.854
	POST	9.3 ± 10.4	19.5 ± 35.3	19.1 ± 20.5	11.9 ± 18.5		
	p-value	*.397*	*.151*	*.844*	*.316*		
DHA (mg·d^{-1})	PRE	43.7 ± 31.0	87.3 ± 147.9	66.7 ± 62.7	63.9 ± 39.4	.047	.879
	POST	28.9 ± 32.5	42.7 ± 81.2	42.9 ± 40.7	26.8 ± 33.1		
	p-value	*.599*	*.121*	*.431*	*.223*		
Exercise							
Sessions ·wk^{-1}	PRE	4.1 ± 2.0	5.1 ± 1.3	5.0 ± 1.8	4.8 ± 1.6	.709	.202
	POST	4.3 ± 2.1	5.4 ± 1.2	4.2 ± 1.2	4.8 ± 1.6		
	p-value	*.613*	*.401*	*.055*	*1.00*		
Minutes·session^{-1}	PRE	46.9 ± 16.0	53.1 ± 10.0	55.0 ± 13.2	50.7 ± 13.4	.196	.749
	POST	43.8 ± 19.2	51.3 ± 9.9	55.7 ± 7.3	47.9 ± 15.5		
	p-value	*.241*		*.799*	*.314*		
Intensity·session^{-1}	PRE	7.0 ± 1.4	7.3 ± 1.3	6.9 ± 1.5	7.5 ± 1.6	.508	.981
	POST	6.8 ± 1.9	7.0 ± 1.4	6.9 ± 1.9	7.4 ± 1.2		
	p-value	*.657*	*.555*	*1.00*	*.752*		

Blood n-3 PUFA Composition

Baseline EPA, DHA, and O3i status was similar between groups ($p > .05$). Before and after supplementation blood EPA and DHA status are noted on Figure 5.2. There were significant main effects for time ($F = 204.87$, $p < .001$, $\eta_p^2 = 0.887$) and group ($F = 16.62$, $p < .001$, $\eta_p^2 = 0.657$) on the O3i and significant group by time interactions ($F = 23.36$, $p < .001$, $\eta_p^2 = 0.729$). From PRE to POST, the O3i was significantly increased in the EPA+DHA (148.9%, $p < .001$), EPA (96.3%, $p < .001$), and DHA (115.6%, $p < .001$) groups, whereas there was no change in the PL group (2.9%, $p = .876$). After supplementation, all groups had significantly higher O3i compared to PL ($p < .001$).

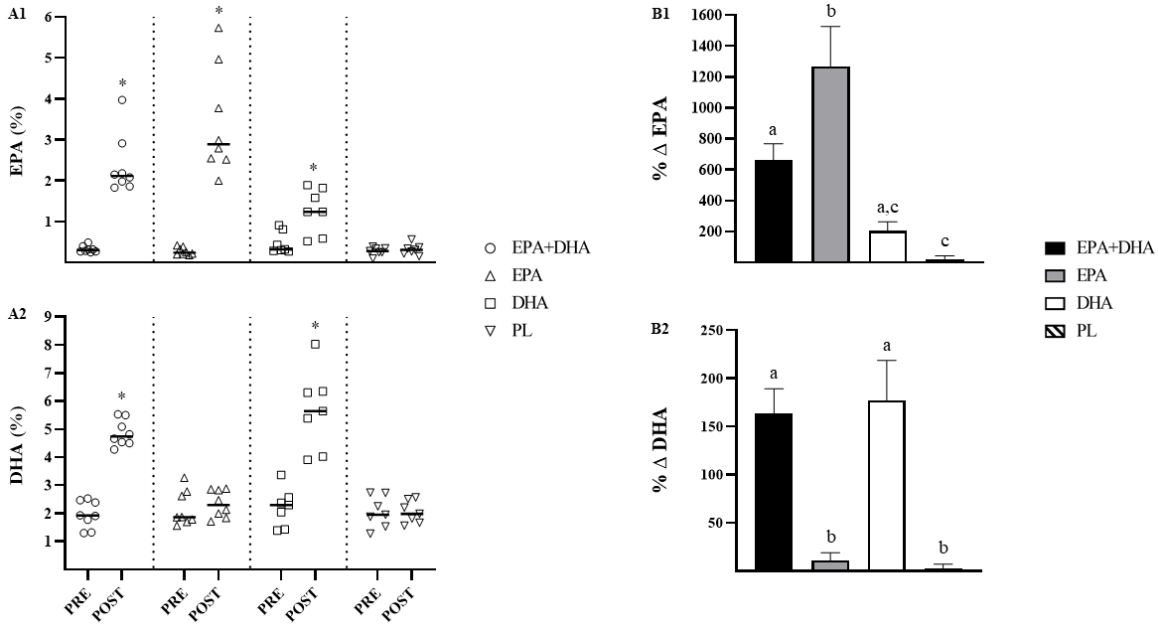


Figure 5.2 Individual participant blood A1) EPA (%) and A2) DHA (%) status before (PRE) and after (POST) supplementation for each group to include the percent change (% Δ) of B1) EPA and B2) DHA. *significantly different than PRE; different subscripts = significant difference between groups

Serum Inflammatory Markers

There was a significant main effect for time (F = 20.07, p < .001, η_p^2 = 0.436); however, no group by time effects were noted (F = 0.483, p = .705, η_p^2 = 0.053). Notably, there were no large between group differences identified compared to PL except after 72H (EPA v PL, d = 0.87). The percent change of CK over time for each group is noted on Figure 5.3.

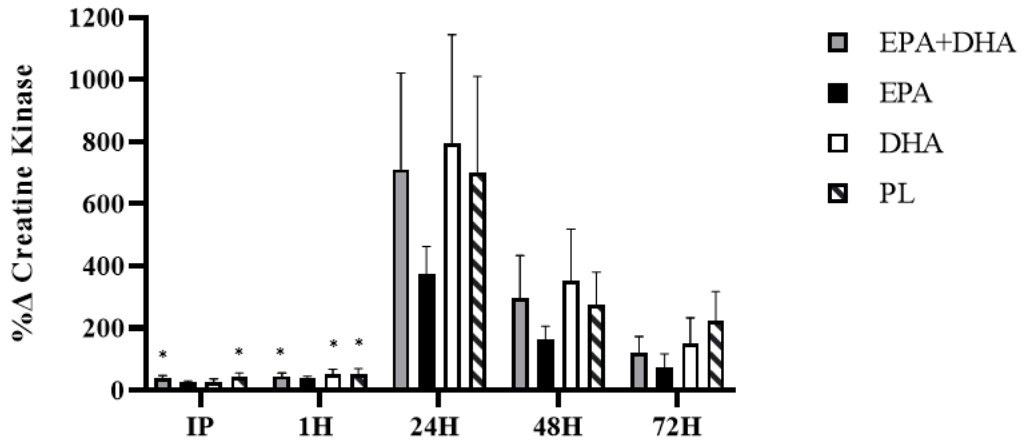


Figure 5.3 Percent change in creatine kinase after exercise-induced muscle damage per group. Data are mean ± SEM; *significantly different than PRE

Muscular Recovery Measurements

Subjective muscle soreness. There were significant main effects for time in VAS-R (F = 25.85, p < .001, η_p^2 = 0.499), VAS-E (F = 32.32, p < .001, η_p^2 = 0.554), and VAS-A (F = 39.82, p < .001, η_p^2 = 0.605) soreness scores. Significant group by time interactions were noted for VAS-E (F = 2.27, p = .021, η_p^2 = 0.207) and VAS-A (F = 3.22, p = .002, η_p^2 = 0.271). All muscle soreness score data are shown in Table 5.3. Briefly, PL was the only group to report significantly higher muscle soreness scores at

72H (v. PRE) in the VAS-E (p = .001) and VAS-A (p < .001) conditions. Of note, in the VAS-A condition, the DHA (d = 2.55) and EPA (d = 1.56) groups were significantly lower than PL at 48 hours (p < .05) and tended to be lower in the EPA+DHA group (p = .143, d = 1.37).

Table 5.3 Muscle Soreness Scores in the Rested (VAS-R), Extended (VAS-E), and Active (VAS-A) Conditions Before and After EIMD

Groups	EPA+DHA	EPA	DHA	PL
		VAS-R		
PRE	0.17 ± 0.35	0.39 ± 0.31	0.46 ± 0.60	0.13 ± 0.26
IP	**4.61 ± 2.39**	**3.67 ± 2.15**	**5.79 ± 1.50**	**4.53 ± 3.10**
1H	**3.24 ± 2.00**	2.09 ± 1.68	3.04 ± 2.10	**3.51 ± 2.86**
24H	2.06 ± 1.68	**4.08 ± 3.33**	3.23 ± 2.85	**4.19 ± 2.50**
48H	1.51 ± 1.50	2.75 ± 3.19	1.91 ± 1.74	**3.79 ± 3.26**
72H	0.91 ± 0.81	2.23 ± 3.09	0.73 ± 0.76	2.01 ± 1.69
		VAS-E		
PRE	0.50 ± 0.89	0.80 ± 0.54	0.93 ± 1.09	0.21 ± 0.28
IP	**4.66 ± 3.23**	**5.68 ± 2.15**	**6.20 ± 2.20**	**4.49 ± 2.79**
1H	**5.61 ± 1.83**	**3.95 ± 2.75**	**5.41 ± 2.10**	**3.77 ± 3.08**
24H	**4.60 ± 2.92**	**5.18 ± 2.77**	**4.56 ± 2.53**	**4.94 ± 2.18**
48H	**4.60 ± 2.84**	**3.98 ± 2.85**	3.43 ± 1.69	**6.53 ± 2.68**
72H	2.70 ± 1.99	2.98 ± 2.98	1.75 ± 1.17	**4.24 ± 1.89**
		VAS-A		
PRE	0.66 ± 1.18	1.28 ± 1.17	0.90 ± 0.69	0.19 ± 0.29
IP	**5.54 ± 3.22**	**5.43 ± 1.50**	**7.30 ± 1.63**	**4.44 ± 2.88**
1H	**5.85 ± 3.52**	3.58 ± 1.55	**5.19 ± 2.00**	**3.89 ± 3.04**
24H	**6.50 ± 3.07**	**6.26 ± 1.81**	**5.86 ± 2.72**	**5.47 ± 2.58**
48H	**5.56 ± 2.37**	**4.78 ± 2.79***	**4.37 ± 1.59***	**8.24 ± 1.44**
72H	2.78 ± 2.09	3.26 ± 2.27	2.50 ± 0.90	**4.83 ± 2.14**

Data are mean ± SD, rounded to the nearest 0.01.
Bold indicates significant difference from PRE (p < .05); *significant difference from PL (EPA, p = .027; DHA, p = .015)

Jump performance and power metrics. There were significant main effects for time on JH (F = 28.80, p < .001, η_p^2 = 0.526), JHP (F = 25.85, p < .001, η_p^2 = 0.499), MP (F = 26.49, p < .001, η_p^2 = 0.505), and PP (F = 28.36, p < .001, η_p^2 = 0.522). JH and JHP was significantly lower at all timepoints except 72H in the EPA+DHA and PL groups, whereas DHA was only lower at 24H (p = .035 and p = .033, respectively) and EPA was

not lower at any timepoint. MP was significantly lower at all timepoints except 72H in the PL and EPA+DHA group; DHA was lower at 24H (p = .041), while EPA was similar to PRE at all timepoints (p > .05). PP was significantly lower at all timepoints (p < .05) except 72H in the PL group, EPA+DHA was lower at IP (p < .001), 1H (p < .001), and 24H (p = .024); DHA was lower IP (p = .036); and EPA was not significantly lower at any timepoint. Significant group by time interactions were noted for JH (F = 2.05, p < .016, η_p^2 = 0.191), JHP (F = 1.86, p = .033, η_p^2 = 0.177), MP (F = 2.3, p = .029, η_p^2 = 0.210), and PP (F = 2.46, p = .013, η_p^2 = 0.221). The percent change data for each variable including main (group, time) and interaction (GxT) effects are noted on Figure 5.4A-D.

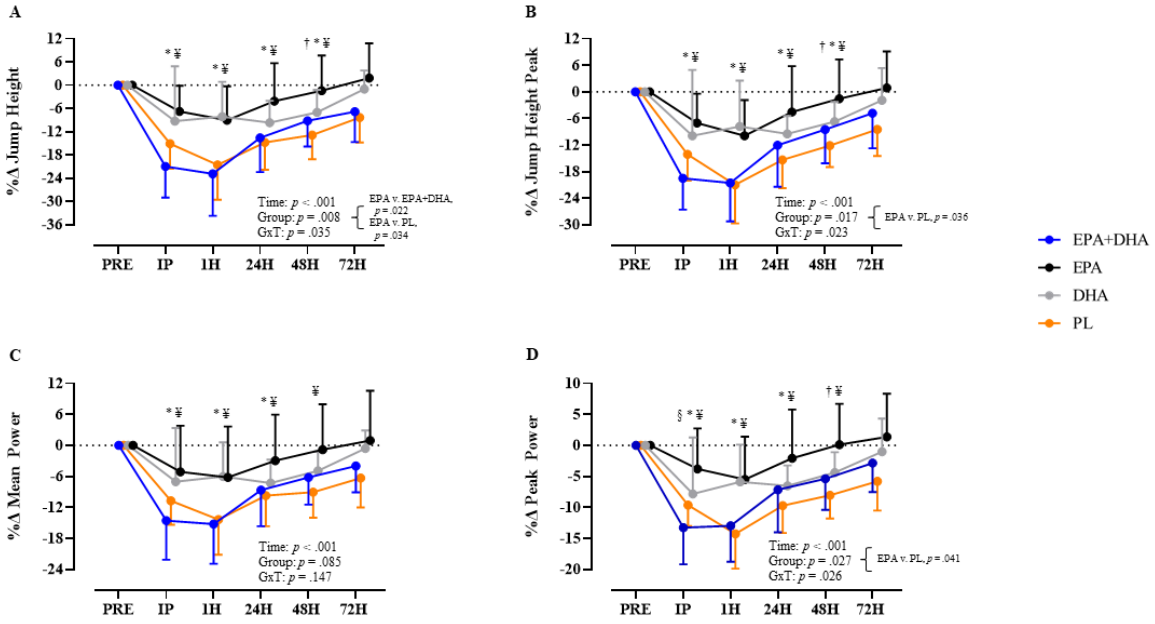


Figure 5.4 Percent change data for jump performance ([A] jump height , [B] jump height peak) and power ([C] mean power, [D] peak power) metrics
Data are mean ± SD. * significantly different than PRE for EPA+DHA; ¥ significantly different than PRE for Placebo (PL); † significant difference between EPA and PL; § significant difference between EPA and EPA+DHA

Strength. There were significant main effects for time (F = 36.55, p < .001, η_p^2 = 0.584) and group by time interactions (F = 3.87, p = .003, η_p^2 = 0.308) on leg press performance. Absolute and percent change leg press data are presented in Table 5.4 and Figure 5.5, respectively.

Table 5.4 Leg Press Performance Data Following Exercise-Induced Muscle Damage

	PRE	**24H**			**72H**		
		Weight (kg)	Δ (kg)	ES (vs PL)	Weight (kg)	Δ (kg)	ES (vs PL)
EPA+DHA	327.0 ± 98.4	**284.7±98.6**	-42.3±32.6[a,b]	0.30	313.9±97.8	-13.1±19.9[a,b]	0.75
EPA	303.1 ± 83.9	292.3±88.4	-10.8±17.0[a]	2.39	310.5±87.2	7.4±13.8[a]	1.71
DHA	298.4 ± 83.1	276.9±79.2	-21.4±22.7[a,b]	1.46	294.5±81.5	-3.9±10.2[a,b]	1.27
PL	354.2 ± 83.0	**304.2±93.9**	-50.0±15.8[b]	--	**322.4±107.8**	-31.8±29.3[b]	--

Bold = significantly different than PRE ($p < .05$); different subscripts within a column = significant difference between groups ($p < .05$); ES, effect size (Cohen's *d*)

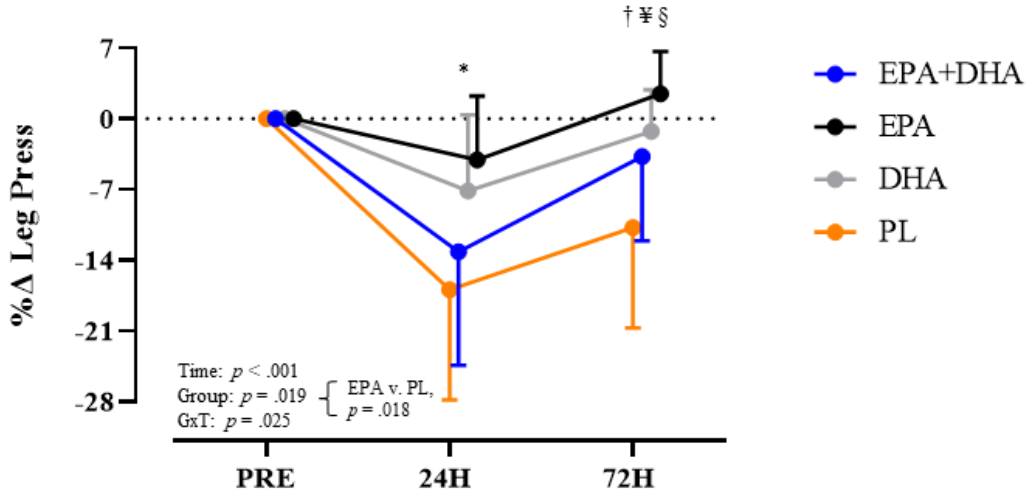


Figure 5.5 Percent change in leg press performance after exercise-induced muscle damage.
Data are mean ± SD. *significantly different than PRE for EPA+DHA and PL;
¥ significantly different than PRE for PL; † significantly different than 24H for all groups; § significantly different between EPA and PL

ROM and circumference measures. The ROM and circumference data are presented in Table 5.5. For ROM, there was a significant effect for time (F = 6.11, p < .001, η_p^2 = 0.190). No group by time interaction was noted (F = 1.01, p = .441, η_p^2 = .105). There was also a significant main effect of time for MTC (F = 5.71, p = .001, η_p^2 = .180) and CC (F = 1.01, p = .441, η_p^2 = .105); however, no group by time effects were identified (MTC: F = 0.95, p = .487, η_p^2 = .099; CC: F = 0.86, p = .567, η_p^2 = .090).

Table 5.5 Range of Motion (ROM) and Localized Muscle Swelling Measures

	EPA+DHA	EPA	DHA	PL	Between Group *p*-value
ROM (°)					
PRE	124.4 ± 16.0	123.5 ± 7.3	123.1 ± 7.1	126.9 ± 7.3	.441
IP	120.5 ± 17.4	119.6 ± 6.4	121.4 ± 8.4	**118.3 ± 6.9**	
1H	121.5 ± 13.8	119.9 ± 5.6	117.9 ± 5.3	120.7 ± 8.8	
24H	120.6 ± 13.4	120.8 ± 4.2	119.6 ± 6.1	124.1 ± 7.8	
48H	120.3 ± 13.1	121.5 ± 5.4	119.6 ± 6.5	122.3 ± 12.2	
72H	122.8 ± 12.1	122.4 ± 6.3	119.4 ± 5.3	125.0 ± 4.3	
MTC (cm)					
PRE	60.7 ± 8.1	60.3 ± 7.2	58.5 ± 3.4	61.1 ± 6.0	.487
IP	62.0 ± 6.8	60.7 ± 7.9	60.0 ± 3.6	62.1 ± 5.5	
1H	61.5 ± 7.4	60.8 ± 7.4	59.1 ± 3.3	62.4 ± 6.1	
24H	61.6 ± 7.7	60.5 ± 7.2	59.6 ± 2.9	62.0 ± 5.6	
48H	61.0 ± 7.7	60.8 ± 7.2	59.9 ± 2.9	62.3 ± 5.8	
72H	61.5 ± 7.8	60.5 ± 7.3	**59.5 ± 2.9**	62.0 ± 6.1	
CC (cm)					
PRE	38.2 ± 3.9	37.6 ± 3.2	37.7 ± 2.1	39.6 ± 4.4	.567
IP	38.1 ± 3.7	**37.0 ± 3.5**	37.5 ± 2.3	39.3 ± 4.7	
1H	38.0 ± 3.9	37.2 ± 3.4	37.3 ± 2.2	39.3 ± 4.7	
24H	38.6 ± 4.1	37.8 ± 3.3	37.8 ± 1.9	**40.3 ± 4.4**	
48H	38.5 ± 3.9	37.7 ± 3.2	38.1 ± 2.0	**40.2 ± 4.2**	
72H	38.4 ± 4.0	37.4 ± 3.1	37.8 ± 2.0	40.0 ± 4.4	

Bold indicates significantly different value from PRE;
Abbreviations: MTC, mid-thigh circumference; CC, calf circumference

Discussion

This is the first study to investigate the potential differential effects of EPA and DHA on strength performance, jump height and power metrics, muscle soreness, range of motion, muscle swelling, and serum muscle damage markers following EIMD. The results of the present study suggest that 4 $g \cdot d^{-1}$ EPA supplementation significantly enhances recovery following EIMD as measured by perceived soreness, jump performance, power metrics, and 1RM leg press compared to PL. Supplementation with DHA significantly attenuated muscle soreness and may preserve leg strength and power. Equivalent dosing of EPA and DHA appears to provide a much more blunted recovery of performance outcomes compared to EPA or DHA alone. The beneficial effects in this

study associated with EPA and DHA appear to not be mediated by mechanisms not associated with improved ROM or muscle swelling as our results indicated no influence on these parameters.

Serum Markers

We observed a large degree of variability in circulating CK levels ranging from 71 to 4235 U/L. While we observed significant increases in CK immediately post EIMD for EPA+DHA and PL and at 1H for EPA+DHA, DHA, and PL; there were no other time effects noted. Some investigations have reported significant influences of n-3 PUFAs on serum markers of muscle damage. A more recent study using a downhill running protocol reported similar levels of CK variability (65-4939 U/L) and no changes in muscle damage markers associated with n-3 PUFA supplementation.[175] However, EIMD protocols using alternate methods to produce muscle damage may lead to a less variable CK response.

While we controlled for prior running history and kept VO_2max and slope consistent during the protocol, DR is complex and the associated CK response may be influenced by running speed, participants' unique running biomechanics, and, even, footwear (for a thorough discussion, see Bontemps et al.[147]). Creatine kinase is one of the most well established indirect markers of muscle damage; however, due to the inconsistency and substantial variation in this measure, CK should not be considered in isolation, especially with DR protocols.

Muscular Recovery Assessments

All groups reported significant increases in VAS muscle soreness, especially in the conditions requiring movement (VAS-E, VAS-A), which suggests the protocol caused substantial muscle damage. Only the PL group reported significantly elevated muscle soreness at 72H in VAS-E and VAS-A conditions. Our study demonstrated that the EPA and DHA groups experienced significantly less soreness at 48H compared to PL. While the difference between the EPA+DHA and PL group did not reach statistical significance at 48H, the effect size was considered large (d = 1.37), and, thus, represents a clinically meaningful between group difference. Our results largely agree with previous studies using EPA-biased [16,74,175], DHA-biased [70,72,73], or equivalent dosing of EPA and DHA.[28,176] For EPA-biased studies, perceived muscle soreness was significantly lower than PL with EPA doses ranging from 1.6 – 2.4 $g \cdot d^{-1}$. [74,175] In the study by Vandusseldorp et al., the group supplemented with the lowest EPA dose (0.8 $g \cdot d^{-1}$) did not experience significantly lower muscle soreness scores, whereas the moderate (1.6 $g \cdot d^{-1}$) and high (2.4 $g \cdot d^{-1}$) EPA dose groups reported significantly lower soreness. Similarly, Kyriakidou et al. reported a significant reduction in soreness after supplementation of 2.1 g EPA daily for four weeks. For DHA-biased or DHA only studies, muscle soreness was significantly lower than PL with DHA doses ranging from 2.0 – 3.0 $g \cdot d^{-1}$.[70,72,73] These results strongly suggest that decreased soreness resulting from EIMD is mediated by the n-3 PUFA dose, not necessarily the type of n-3 PUFA (EPA v DHA) supplemented.

Jump performance and power metrics were not significantly reduced in either the EPA or DHA groups, while mean and peak power in the EPA+DHA was reduced through 24H and up to 48H in the PL group. Jump performance was significantly reduced

through 48H for both the EPA+DHA and PL groups. In a field-based study, Black et al.[176] reported suppressed peak force after 35 days in the PL group; however, the intervention group, taking equal amounts of EPA+DHA, exhibited a 4.6% improvement in peak force. In an EPA-biased study, Vandusseldorp et al.[74] reported that jump height in the group consuming the most amount of EPA (2.4 $g \cdot d^{-1}$) returned to baseline within 1H post-EIMD. A more recent study supplementing 2.1 $g \cdot d^{-1}$ EPA and 0.86 $g \cdot d^{-1}$ DHA found that peak power was only suppressed in the PL group.[175]

Leg strength was not significantly suppressed at 24H or 72H in the EPA or DHA groups. Strength returned to baseline at 72H in the EPA+DHA group; however, strength was significantly suppressed at 24H and 72H in the PL group. Similar studies have not reported significant changes in strength deficits following EIMD compared to PL. However, in a study investigating the varying doses of EPA+DHA on muscle recovery, the highest daily dose (2.4 g EPA, 1.8 g DHA) resulted in the greatest improvement in strength at 72H (Δ: +0.54 Nm) compared to the moderate dose (Δ: -16.0 Nm), low dose (Δ: -8.33 Nm), and PL (Δ: -19.52 Nm) groups.[74]

Conclusion

Seven weeks of fish oil supplementation prior to exercise-induced muscle damage enhances recovery based on physical performance, strength, and perceived soreness. However, 4$g \cdot d^{-1}$ EPA outperformed PL and EPA+DHA on certain metrics, whereas DHA improved perceived soreness and tended to improve other recovery parameters moreso than EPA+DHA or PL.

CHAPTER SIX

Conclusion

In this dissertation I present three manuscripts representing the completion of three independent studies. Two of the studies were experimental interventions investigating the effect of FO n-3 PUFA supplementation on functional muscular outcomes from RET and EIMD. The third study was a pilot investigation on the utility of a dual-model EIMD protocol.

The first manuscript of this dissertation presents novel findings that 3.85 $g \cdot d^{-1}$ FOS improves absolute and relative strength compared to PL, despite similar changes in LBM. Additionally, FOS appeared to reduce FM greater than the RET and PL group.

In the second manuscript we present pilot data on the validity of a dual-model EIMD protocol compared to a standard DR and plyometric protocol. Our data suggests that DR plus JL produces adequate muscle damage for research, whereas DR alone does not provide as consistent results based on CK and muscle soreness scores.

In the final manuscript we explored the potential differential effects of EPA and DHA on muscle recovery following EIMD. Fish oil supplementation enhances recovery based on performance metrics and subjective soreness. Our study suggests that 4 $g \cdot d^{-1}$ EPA provides a superior improvement in power, strength, and soreness; whereas, DHA attenuates soreness during movement at 48H.

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