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Impact of varying doses of omega-3 supplementation on muscle damage and recovery after eccentric resistance exercise

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ABSTRACT

Background: Exercise-induced muscle damage (EIMD) commonly occurs following intense resistance exercise and is associated with decrements in exercise performance and delayed muscle recovery. Thus, practical methods to attenuate EIMD would prove useful to both training and athletic populations. Omega-3 (n-3) supplementation has been shown to mitigate EIMD with evidence of increasing efficacy at higher doses (up to 6 g/day). However, data of its efficacy in trained individuals is limited. Therefore, this study investigated the effects of 6 and 8 g of n-3 supplementation on markers of muscle damage and muscle recovery after eccentric resistance exercise in resistance-trained males.

Methods: Using a double-blind, randomized, placebo-controlled design, 26 resistance trained males (23 ± 4 years; 173.6 ± 20.5 cm; 81.9 ± 9.7 kg; 14.2 ± 3.7% body fat) supplemented with 6 (n=10) or 8 g (n=7) of n-3 polyunsaturated fatty acids, or placebo (n=9) for 33 days. On day 30, participants performed a lower body muscle-damaging eccentric resistance exercise bout. Measures of muscle performance, soreness, and damage were taken pre-exercise on day 30 as well as on days 31–33, including vertical jump height (VJH), perceived muscle soreness (PMS), hip and knee range of motion (ROM), repetitions to fatigue (RTF) at 70% 1-RM, and serum creatine kinase (CK) while participants continued to supplement until day 33.

Results: There were significant differences in VJH, PMS, and serum CK following the muscle-damaging exercise bout compared to pre-exercise ($p<0.05$). However, there were no significant ($p>0.05$) differences between supplementation groups (6 g, 8 g, and placebo) at any time point post-exercise (day 31–33). There were no changes in hip and knee ROM or RTF at any time point or between groups. Vertical jump height and PMS returned to pre-exercise levels despite CK remaining elevated post-exercise.

Conclusions: Thirty-three days of 6 and 8 g of n-3 supplementation did not attenuate EIMD or enhance muscle recovery following muscle-damaging eccentric resistance exercise in resistance-trained males. Further research using various n-3 supplementation durations, doses, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations may be needed to establish its efficacy in attenuating EIMD, which may vary between trained and untrained individuals. Furthermore, while circulating CK is commonly used to assess muscle damage, elevated CK levels may not reflect muscle recovery status following muscle-damaging exercise.

1. Introduction

Exercise-induced muscle damage (EIMD) commonly occurs following unaccustomed and/or intense resistance exercise, particularly exercise with emphasized eccentric components (i.e., eccentric training). EIMD elicits an acute inflammatory response and is associated with a rise in blood creatine kinase (CK) concentrations, delayed-onset muscle soreness (DOMS), decrements in range of motion (ROM), and a

Abbreviations: EIMD, exercise-induced muscle damage; n-3, omega-3; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; VJH, vertical jump height; ROM, range of motion; RTF, repetitions to fatigue; DOMS, delayed-onset muscle soreness; PMS, perceived muscle soreness; CK, creatine kinase.

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reduction in muscle performance (i.e., force production, vertical jump, etc.) [1]. Thus, interventions that attenuate acute inflammation may serve as strategies to mitigate EIMD following intense exercise.

Omega-3 (n-3) polyunsaturated fatty acids, particularly, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known for their anti-inflammatory and anti-nociceptive (pain-inhibiting) properties [2–6]. Given these effects, n-3 supplementation with EPA and DHA has been proposed to mitigate EIMD and facilitate muscle recovery following intense resistance exercise. However, the literature on n-3 supplementation and EIMD is currently inconclusive, which likely stems from variations in dose, ratios of EPA and DHA, duration of supplementation, source of supplementation, as well as training status and exercise protocols employed. Nonetheless, there are emerging data demonstrating that n-3 supplementation attenuates EIMD in response to resistance exercise, particularly at higher doses [7,8].

A large portion of previous investigations on n-3 supplementation and EIMD have used untrained participants [7,9–15]. The effect of n-3 supplementation on EIMD and muscle recovery in trained individuals is understudied, though they likely train at greater intensities and frequencies, and are more likely to perform eccentric training, all of which attribute to EIMD. Additionally, trained populations are most likely to participate in sport and frequent athletic competitions compared to untrained individuals in which more expedient recovery of muscle performance and amelioration of EIMD is imperative. Additionally, it is not clear if n-3 doses greater than those used in previous work (>3.6 g/day) produce more robust and consistent effects. However, VanDusseldorp et al., 2020 [8] showed ~7.5 weeks of 6 g of n-3 supplementation with an EPA to DHA ratio of 2:1 (400:300 mg per 1000 mg pill) attenuated plasma CK and lactate dehydrogenase (LDH) levels, mitigated perceived muscle soreness (PMS), and promoted vertical jump height (VJH) recovery in resistance-trained individuals within 72-hr post-eccentric resistance exercise compared to placebo, 2 g, and 4 g of n-3 supplementation [8]. Previous work in untrained individuals demonstrates ~4 weeks of n-3 supplementation (at lower doses) is capable of mitigating markers of EIMD [10,14,16]. This suggests higher doses (i.e., 6 g/d) of n-3 supplementation may be effective in attenuating EIMD and promoting muscle recovery in resistance-trained individuals following eccentric resistance exercise. Moreover, this may suggest a dose-response relationship between n-3 supplementation and EIMD and muscle recovery of which an upper limit is unknown and that may be realized with shorter supplementation periods (~4 weeks).

This study follows VanDusseldorp et al., 2020 [8] who observed reductions in muscle damage and recovery after an acute bout of eccentric resistance exercise with 7.5 weeks of 6 g of n-3 supplementation consisting of a 2:1 ratio of EPA to DHA in resistance-trained males. Therefore, the purpose of this study was to investigate the effect of 33 days of 6 and 8 g of n-3 supplementation on markers of EIMD and muscle recovery after an acute bout of muscle-damaging eccentric resistance exercise in resistance-trained males. It was hypothesized that 6 and 8 g per day of n-3 supplementation with a 2:1 ratio of EPA to DHA (200:100 mg) would attenuate markers of muscle damage and promote muscle recovery after eccentric resistance exercise and that supplementation with 8 g would produce greater effects vs. 6 g.

2. Methodology

2.1. Participants and selection criteria

Thirty resistance-trained males (23 ± 4 years) volunteered to participate in this double-blind randomized control study. Four participants were dropped from the study for failure to adhere to supplementation or inability to attend trials, resulting in a total of 26 completing the study. “Resistance-trained” was defined as having had engaged in strength or hypertrophy-based resistance training (≥65% 1-RM or ≤ 15 repetitions to fatigue or exhaustion; ≥ 12 sets per muscle group per training session), as in accordance with National Strength and Conditioning Association (NSCA) volume and intensity ranges for hypertrophy [17], at least 3 times per week for a minimum of one year. Participants were instructed to cease current n-3 supplementation and non-steroidal anti-inflammatory drug (NSAID) use 6 weeks prior to and throughout their participation in the study. Additionally, consumption of fatty fish (mackerel, herring, salmon, trout, halibut, tuna, shrimp, and cod) was prohibited for the duration of the study. Participants were asked to refrain from exercise 96-hr prior to the acute exercise bout through 3 days post-exercise. Diet and exercise were not controlled for prior to the start of the 33-day study. Written informed consent was obtained by all participants prior to beginning the study. The current study was approved by the California State University, Long Beach Institutional Review Board (Board Reference #19-352).

2.2. Supplementation

In this double-blind randomized control study, participants were randomly assigned to one of three groups: (1) 6 g daily of n-3 supplementation (6G), (2) 8 g daily of n-3 supplementation (8G), or (3) placebo (PL). The 6G and 8G groups consumed 6 and 8 1000 mg capsules per day, respectively, while the PL group consumed 6 1000 mg capsules per day of conjugated linoleic acid (CLA) oil for 33 days total (Fig. 1). Conjugated linoleic acid was chosen as the placebo, as it has been used as such in VanDusseldorp et al., 2020 [8] and is characteristically similar to the omega-3 capsules. Participants were given written and verbal instructions on supplement duration, number of capsules to be taken per day, and dietary restrictions throughout the course of the study. Supplementation was self-regulated. Omega-3 supplementation was provided by Beast Sports Nutrition (Beast Sports Nutrition, Fort Lauderdale, FL, USA) with a 2:1 ratio of EPA to DHA (200:100 mg per 1000 mg capsule). Conjugated linoleic acid supplements were supplied by Piping Rock (Piping Rock Health Products LLC, Ronkonkoma, NY, USA).

2.3. Familiarization session

On days 21, 22, or 23, participants reported to the Physiology of EXercise and Sport (PEXS) Lab for measurement of height (cm) and weight (kg) using a Seca 216 wall mount stadiometer (Seca, Chino, CA, USA) and Mettler Toledo scale (Mettler-Toledo, LLC, Columbus, OH, USA), respectively. Skinfold measurements were taken in accordance with the American College of Sport Medicine (ACSM) [18] skinfold procedure using a Lange skinfold caliper (Beta Technology, Santa Cruz, CA, USA) to assess body fat percentage. Measurements were taken at the abdomen, thigh, and triceps. The three-site skinfold equation specific for black or white collegiate male and female athletes, ages 18 to 34 was used to determine body fat percentage (Eq. A.1) [19].

Additionally, one repetition-maximum (1-RM) barbell back squat was tested in accordance with the NSCA 1-RM protocol [17], along with repetitions to fatigue (RTF) at 70% 1-RM barbell back squat on the day of familiarization (detailed in measurements section). All squats were performed in a Rogue RM-4 Monster Rack 2.0 with a Rogue Monster Safety Strap System 2.0 (Rogue Fitness, Columbus, OH, USA). For all repetitions, participants were required to reach a squat depth at which mid-thigh was parallel to the floor, as defined by the NSCA [17]. To measure squat depth, a Rogue Monster resistance band (Rogue Fitness, Columbus, Ohio, USA) supported by Rogue Monster Lite/Infinity band pegs (Rogue Fitness, Columbus, Ohio, USA), was placed across the rack at the distance in which the participant’s mid-thigh was parallel to the floor, as determined by the principal investigator.

2.4. Measures

Outcome variables included serum CK, VJH, hip and knee ROM, PMS, and RTF at 70% 1-RM barbell back squat. Hip and knee ROM, VJH, PMS, and a capillary blood draw were taken immediately pre-exercise bout (day 30) and 1d (day 31), 2d (day 32), and 3d post-exercise (day

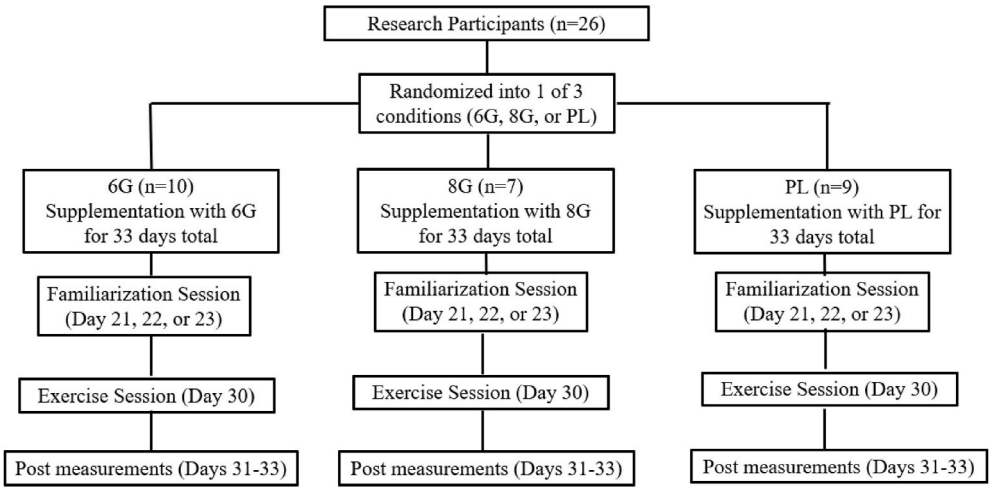

Fig. 1. Overview of Study Design.
PL = placebo; 6G = 6 g n-3 supplementation; 8G = 8 g n-3 supplementation.

33), while RTF was taken at the familiarization session (day 21, 22, or 23) and 3d post-exercise.

2.4.1. Vertical jump height

Vertical jump height was measured using a wall-mounted Vertec (Sports Imports, Columbus, OH, USA) and assessed in accordance with manufacturer instructions for jump height measurement [20]. To obtain standing reach height, participants fully extended their right arm upward and walked through the horizontal vanes. Then, participants performed a countermovement squat jump and were instructed to jump with maximal effort. Three trials were completed. All values were recorded using the highest vane reached. Vertical jump height was determined by taking the difference between the standing reach and highest jump reach values.

2.4.2. Perceived muscle soreness

Perceived muscle soreness was measured to assess DOMS with the vasQ Clinical iPhone application (Apple Incorporated, Cupertino, CA, USA). The application uses a visual analogue scale (VAS) to assess subjective measures that cannot be directly measured. The application displayed a continuous line, ranging from "none" to "extreme", and participants were asked to rate their perceived level of muscle soreness by moving a blue diamond pointer along the continuum. The position of the pointer determined the level of soreness and was expressed as a number between 1 and 100.

2.4.3. Hip and knee range of motion

A goniometer (Baseline Evaluation Instruments, White Plains, NY, USA) was used to measure hip and knee ROM. To assess hip flexion and extension, the axis of the goniometer was aligned with the greater trochanter of the hip joint, with the moving arm and stationary arm parallel with the femur and midline of the pelvis, respectively. Hip flexion was measured with the participant in the supine position and hip extension was measured with the participant in the prone position. From the supine and prone positions, the participant was instructed to lift their leg upward without abduction or adduction at the hip joint, while keeping their pelvis parallel to the table. For both hip flexion and extension, three measurements were taken on each leg and averaged together to determine hip ROM [21,22].

To measure knee flexion and extension the axis of the goniometer was placed over the lateral epicondyle of the knee joint, with moving and stationary arms aligned with the lateral malleolus of the ankle joint and greater trochanter of the hip joint, respectively. Knee flexion was assessed by the participant laying in the prone position and bringing their heel towards their buttocks with no internal or external rotation at the knee joint. Knee extension was measured with the participant in the supine position. The participant was instructed to extend their knee without internal or external rotation at the knee joint. For both knee flexion and extension, three trials were performed on each leg and averaged together to determine knee ROM [21].

2.4.4. Repetitions to fatigue

Participants completed a maximum number of consecutive barbell back squat RTF at 70% 1-RM on the day of familiarization. Fatigue was defined at the point in which the participant exceeded a 1-s pause at the end of the concentric phase in between repetitions or if proper squat depth (mid-thigh parallel to the floor), as defined by the NSCA [17], was not achieved, as confirmed with a band placed at the appropriate level behind the participant. For a repetition to be complete, participants' buttocks needed to make contact with the band on the descent, full extension at the hip and knee on the ascent, and no greater than 1-s pause in between repetitions at the end of the concentric phase. Tempo was controlled by the participant. If the participant did not make contact with the band or exceeded 1-s at the top, they were given a warning and the repetition was not counted. The trial was terminated when the participant failed to lift the load.

2.4.5. Blood samples

A capillary blood sample was taken at the distal portion of the third or fourth finger for each participant using Capiject Safety Lancet (Terumo Medical Corporation, Somerset, NJ, USA) or Medlance Plus 1.8 mm Safety Lanclets (HTL-STREFA, Inc., Marietta, GA, USA) to puncture the skin. A Kim wipe (Kimberly-Clark Worldwide, Inc., Roswell, GA, USA) was used to wipe away the first blood droplet and blood was collected using Lithium Heparin CB 300 μL Microvettes (Sarstedt, Numbrecht, Germany, Europe). Blood samples were centrifuged at 8,000 g for 10 min at 20 °C using a table-top centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) to separate serum. Serum samples were stored at −80 °C until further analysis. Serum CK was analyzed using Abcam CKM Human SimpleStep ELISA Kits according to the manufacturer's instructions (Abcam, Cambridge, MA, USA). A SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA) was used to determine the optical density of each well at an absorbance of 450 nm.

2.5. Exercise session

On day 30, participants completed an acute bout of eccentric resistance exercise. The protocol employed has been used in previous work

and has been shown to produce EIMD [8,23]. The exercise protocol consisted of ten sets of eight repetitions at 70% 1-RM barbell back squat with a 4-s eccentric phase followed by five sets of ten per side body weight split squat jumps upon completion of back squats. The 4-s eccentric phase is within the 3-5 s range for lower body movement eccentric training, as recommended by the NSCA [17]. Participants were given a 5-min warm-up of their choosing. Proper squat depth and requirements for a complete squat repetition were mandatory, as detailed in the familiarization session section. If the participant failed to meet these requirements, the squat repetition was not recorded, and another repetition was completed. The 4-s eccentric phase was timed using Soundbrenner Metronome application (Apple Incorporated, Cupertino, CA, USA) on an iPhone 10. Following each set, participants completed a required 3-min rest period. In the case participants could not complete the exercise protocol at 70% 1-RM, the load was reduced by 10% until the subject could complete the remainder of the protocol. A total of 9 participants completed the protocol at 70% 1-RM, while 10 dropped to 60% and 7 to 50% 1-RM. In the case participants failed to complete the set at the prescribed intensity, an additional 3-min rest was required prior to the 10% drop in intensity. Upon completion of the barbell back squat routine, participants were required to rest 3-min before beginning the body weight split squat jumps. Participants were instructed to refrain from additional recovery methods (ice, anti-inflammatory drugs, massage therapy, etc.) after the exercise session through the 3d post-exercise data collection period.

2.6. Statistical analysis

A univariate one-way repeated measure analysis of variance (ANOVA) was used to determine statistical differences in descriptive measures (i.e., height, weight, body fat percentage, and 1-RM) and volume load (VL) between groups. Mean differences in serum CK, VJH, hip and knee ROM, and PMS between 6G, 8G, and PL at pre-exercise, 1d, 2d, and 3d post-exercise by a 3 (group) x 4 (time) repeated measures ANOVA, while RTF was analyzed by a 3 × 2 repeated measures ANOVA. All data analyses were performed with IBM SPSS Statistics 25 (Armonk, NY, USA). Where a significant main effect or interaction was found, Bonferroni's correction was performed as a post-hoc test. A p-value of <0.05 was regarded as statistically significant. All data is presented as mean ± standard deviation (SD).

3. Results

As depicted in Table 1, there were no differences in participant demographics and individual characteristics between conditions. Original total sample size was 30, with 10 participants in each group (PL, 6G, and 8G); however, 26 participants total completed the study, as 4 were dropped for failing to complete full study procedures, resulting in 9 in PL, 10 in 6G, and 7 in 8G. Total sample size was determined based on VanDusseldorp et al., 2020 [8] and confirmed using G* Power 3.1.9.7 power analysis application (Düsseldorf, Germany, Europe) (see Table 2).

Measurements for all outcome variables at each time point (immediately pre-exercise, familiarization session, 1d, 2d, and 3d post exercise session) were taken for each participant; however, serum CK concentration was not analyzed for one participant in the 6G group due to insufficient sample volume.

Table 1
Participant characteristics.

Variables Group	PL (n=9)	6G (n=10)	8G (n=7)	p-value
Age (years)	22.9 ± 3.2	22.7 ± 2.6	24.6 ± 5.1	0.536
Height (cm)	177.4 ± 9.3	176.1 ± 4.0	165.1 ± 5.1	0.454
Weight (kg)	83.4 ± 11.6	80.5 ± 7.1	82.8 ± 11.3	0.819
Body Fat (%)	15.3 ± 3.5	14.3 ± 3.1	12.5 ± 4.5	0.359

Values are mean ± SD; PL= placebo; 6G = 6 g n-3 supplementation; 8G = 8 g; n-3 supplementation; cm = centimeters; kg = kilograms.

Table 2
Participant 1-RM and volume load.

Variables Group	PL	6G	8G	p-value
Back Squat 1-RM (kg)	137.7 ± 25.0	140.8 ± 17.2	124.2 ± 24.0	0.337
Volume Load (kg)	7,137.5 ± 1,178.6	7,401.1 ± 1,027.9	6,681.1 ± 1,290.1	0.460

Values are mean ± SD; PL = placebo; 6G = 6 g n-3 supplementation; 8G = 8 g n-3 supplementation; kg = kilograms; 1-RM = 1-Repetition Maximum.

A main effect for time was observed for VJH. Vertical jump height significantly decreased 1d after exercise (p=0.009). There was a significant increase in VJH at 2d and 3d compared to 1d (p=0.02), as well as 2d to 3d (p=0.002) (Fig. 2). There was no statistically significant difference in VJH between 6G, 8G, and PL groups immediately pre-exercise, 1d, 2d or 3d post exercise ($p>0.05$; Fig. 2).

There was a main effect for time for PMS, however there were no statistical differences between groups at any time point. There was a statistically significant increase in PMS from pre-exercise at 1d, 2d, and 3d post-exercise ($p<0.05$). Perceived muscle soreness was significantly lower at 3d compared to 1d and 2d ($p<0.05$), however was still elevated from pre ($p<0.05$; Fig. 3).

There were no significant differences in hip or knee ROM at any time point or between 6G, 8G, or PL groups ($p>0.05$; Figs. 4 and 5). There were no significant differences in number of barbell back squat RTF at 70% 1-RM between familiarization compared to 3d post-exercise or between groups ($p>0.05$; Fig. 6) (see Fig. 7).

There was a significant increase in serum CK from pre-exercise to 1d, 2d, and 3d post-exercise in 6G, 8G, and PL groups ($p<0.001$) indicating muscle damage in response to the exercise protocol. However, there were no differences between supplementation groups ($p>0.05$).

4. Discussion

The current study investigated the effects of 33 days of 6 and 8 g of n-3 supplementation with a 2:1 ratio of EPA to DHA on VJH recovery, PMS, serum CK, and hip and knee ROM in response to intense eccentric resistance exercise. This study follows VanDusseldorp et al. [8] who observed reductions in muscle damage and recovery of muscle performance after an acute bout of eccentric resistance exercise with 7.5 weeks of 6 g of n-3 supplementation consisting of a 2:1 ratio of EPA to DHA in resistance-trained males using the same eccentric resistance exercise protocol. It was hypothesized that both 6 and 8 g of n-3 supplementation

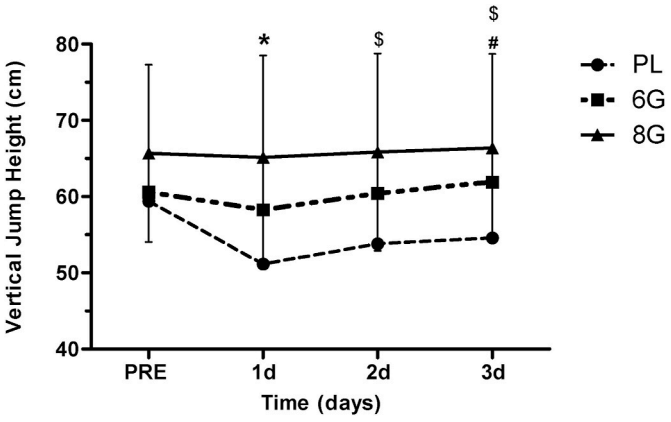

Fig. 2. Vertical jump height (mean ± SD) pre-exercise, 1d, 2d, and 3d post-exercise following 30 days of 6 or 8 g of n-3 supplementation, or placebo (n=26). (*) significant difference from pre (p=0.009). ($) significant difference from 1d post (p=0.02). (#) significant difference from 2d post (p=0.002).

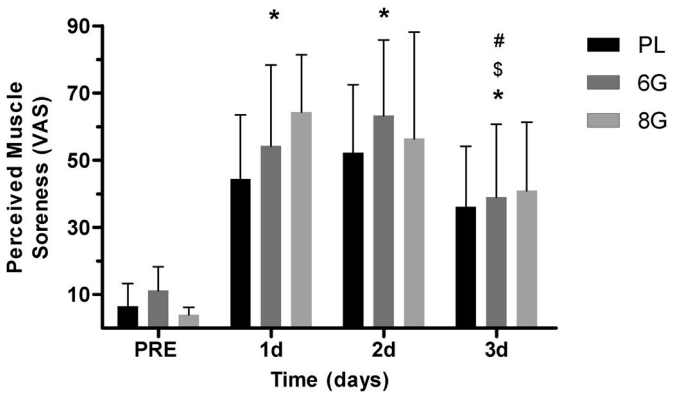

Fig. 3. Perceived muscle soreness (mean ± SD) pre-exercise, 1d, 2d, 3d post-exercise following 30 days of 6 or 8 g of n-3 supplementation, or placebo (n=26). (*) significant difference from pre ($p<0.05$). ($) significant difference from 1d ($p<0.05$). (#) significant difference from 2d ($p<0.05$).

would mitigate markers of muscle damage and promote muscle recovery and 8 g of n-3 supplementation would produce a more pronounced effect. However, neither 6 nor 8 g resulted in any effect on markers of muscle damage or muscle recovery. These data only add to the inconclusive literature on the effect of n-3 supplementation on EIMD, particularly amongst resistance-trained populations.

Exercise-induced muscle damage is associated with decrements in force production resulting from disruption of the sarcomere and impaired excitation-contraction coupling [24]. Eccentric resistance exercise has been shown to impair force generating capacity up to 50-65% compared to pre-exercise values [25,26]. Limited data exists on the effects of n-3 supplementation on mitigating decrements in force production following resistance exercise. This was one of three studies, including VanDusseldorp et al., 2020 [8] and Jakeman et al., 2017 [27], to examine the effects of n-3 supplementation on jump performance following an acute bout of muscle-damaging exercise. A single post-exercise dose of n-3 supplementation with high amounts of EPA (750 mg EPA, 50 mg DHA per dose) was shown to attenuate decrements in squat jump performance compared with supplementation lower in EPA (150 mg EPA, 100 mg per dose) and a placebo in physically active males [27]. In VanDusseldorp et al., 2020 [8], VJH returned to pre-exercise values at 48-hr post-exercise for all groups, which is consistent with the current study showing the return of VJH values at 2d post-exercise for all supplement groups. This suggests that EIMD did not attenuate muscle performance 2d post muscle-damaging exercise in resistance-trained men. Further, the shorter recovery period observed with VJH, compared with PMS and circulating CK, implies that EIMD symptoms may vary in severity and duration post muscle-damaging exercise.

Delayed onset muscle soreness is a common symptom of EIMD and has been shown to persist for days following muscle-damaging exercise [1]. Given the reported anti-nociceptive properties of n-3 supplementation, studies have examined the effects of n-3 supplementation on DOMS after eccentric resistance exercise and produced conflicting results. In the present study, significant increases in PMS from pre-exercise through 3d post-exercise were demonstrated in all groups, however no differences between groups were found at any time point. These results are supported by Gray et al., 2014, DiLorenzo et al., 2014, and Jakeman et al., 2017 who found n-3 supplementation to be ineffective for reducing PMS after eccentric resistance exercise in physically active [11,27] and untrained males [10]. However, other studies have shown reductions in PMS with n-3 supplementation following muscle-damaging exercise in untrained individuals [7,9,12,14–16], while VanDusseldorp et al., 2020 [9] reported reduced PMS in resistance-trained males. Variation in exercise protocols and more specifically, muscle groups utilized during exercise, as well as differences in methods of assessing muscle soreness may have produced these mixed results. Moreover, variation in supplement dosage, duration, and EPA and DHA concentrations, as well as differences in exercise protocols and training status may also have contributed.

Muscle stiffness and swelling, as a result of EIMD, have been shown to impede ROM following exercise performance [28,29]. This study sought to evaluate the efficacy of n-3 supplementation in attenuating reductions in ROM and was the first to assess hip and knee ROM. No changes in hip and knee ROM occurred following muscle-damaging exercise in this study. The current literature has produced mixed results regarding the effects of n-3 supplementation on ROM. Some investigations have shown n-3 supplementation attenuating decrements in elbow ROM following eccentric exercise protocols targeting elbow flexors and biceps brachii [9,12,14–16], whereas other studies have shown such interventions to be ineffective at preventing reductions in elbow ROM [10,30,31]. None of these works were completed in

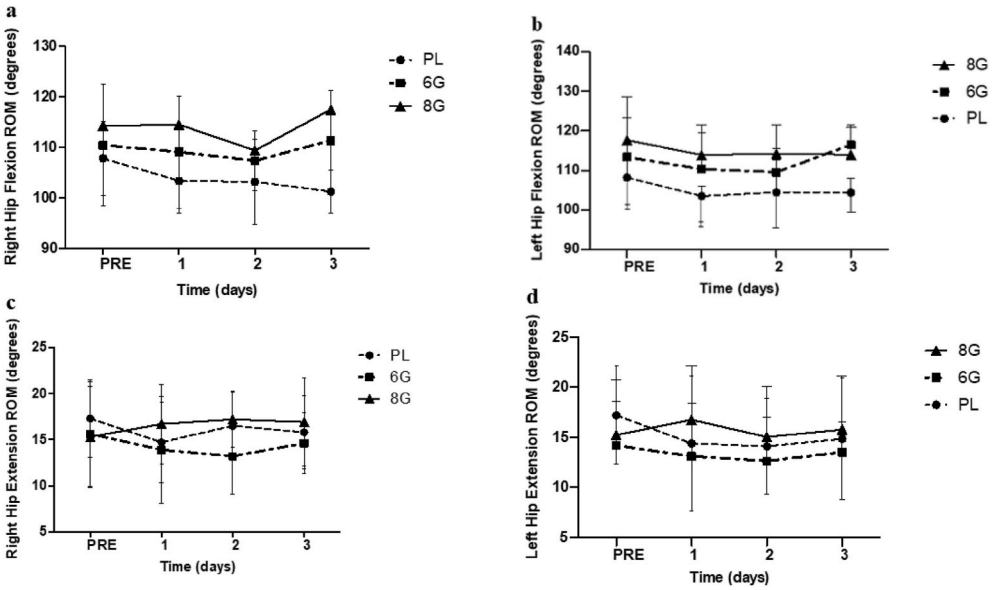

Fig. 4. Hip ROM (mean ± SD) pre-exercise, 1d, 2d, and 3d post-exercise following 30 days of 6 or 8 g of n-3 supplementation, or placebo (n=26). **(a)** Right hip flexion, **(b)** left hip flexion, **(c)** right hip extension, **(d)** left hip extension.

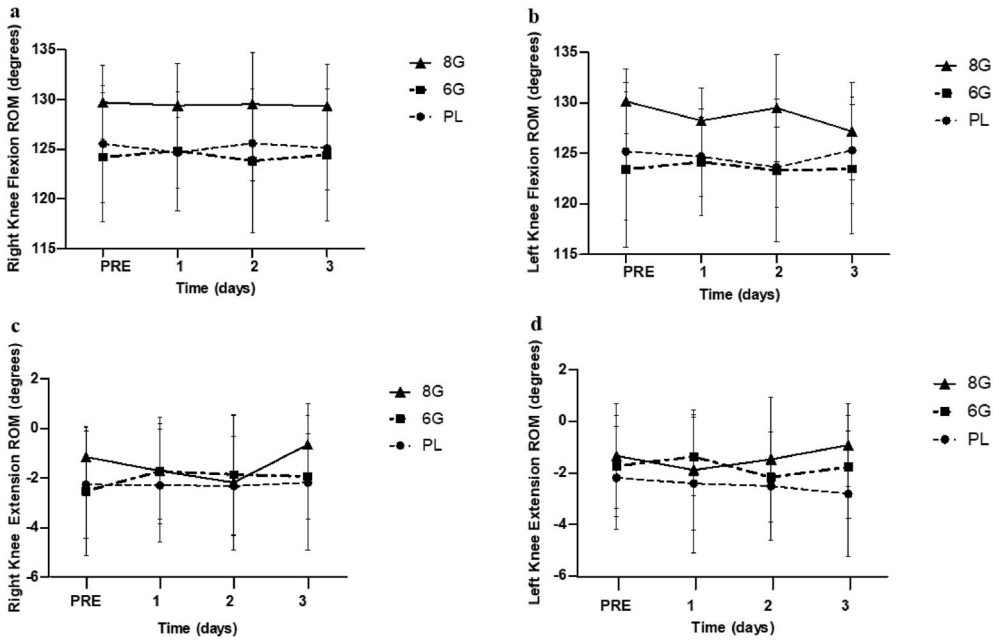

Fig. 5. Knee ROM (mean ± SD) pre-exercise, 1d, 2d, and 3d post-exercise following 30 days of 6 or 8 g of n-3 supplementation, or placebo (n=26). **(a)** Right knee flexion, **(b)** left knee flexion, **(c)** right knee extension, **(d)** left knee extension.

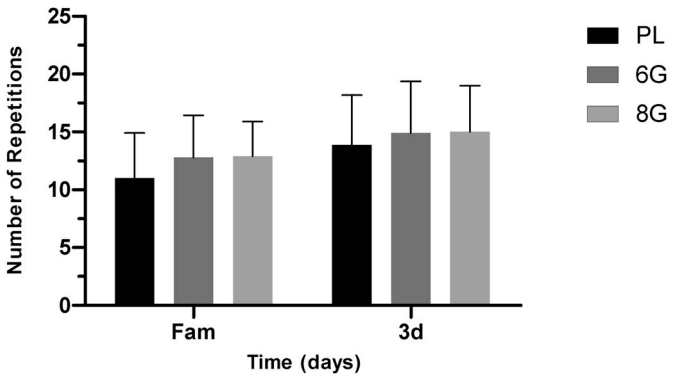

Fig. 6. Number of barbell back squat repetitions to fatigue (mean ± SD) on day of familiarization (Fam) and 3d post-exercise following 30 days of 6 or 8 g of n-3 supplementation, or placebo (n=26).

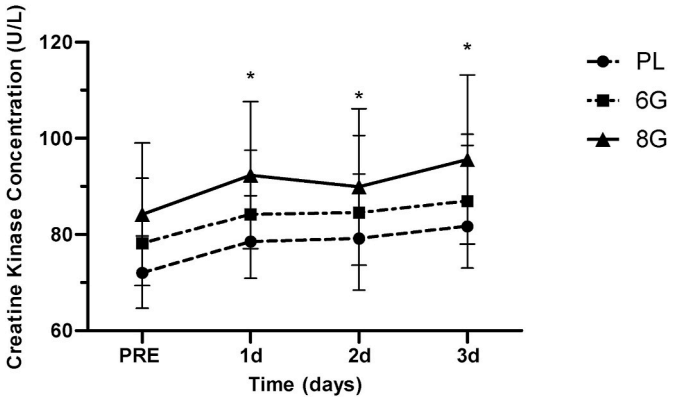

Fig. 7. Serum CK (mean ± SD) pre-exercise, 1d, 2d, 3d post-exercise following 30 days of 6 or 8 g of n-3 supplementation, or placebo (n=25). (*) significant difference from pre ($p<0.001$).

resistance-trained populations. Additional studies are needed to elucidate the effects of n-3 supplementation on preserving ROM after muscle-damaging exercise given that impaired ROM may compromise subsequent performance.

Exercise-induced muscle damage is associated with a decrease in force production after eccentric resistance exercise [32]. Number of repetitions to fatigue has been used to assess muscle fiber type percentage as well as work capacity and muscular endurance after resistance exercise [33,34]. However, RTF has not been employed as a measure to assess the severity of EIMD nor in the evaluation of n-3 supplementation on mitigating symptoms of EIMD. This was the first study to investigate the effects of n-3 supplementation on RTF at 70% 1-RM barbell back squat following eccentric resistance exercise. There were no differences between pre-exercise and 3d post-exercise RTF or between supplement groups. Symptoms of EIMD begin to subside two days after exercise [1,35], as supported in the current study (i.e., VJH) and, therefore, may not have negatively affected number of RTF at day three. Nonetheless, RTF was not measured at previous time points, as to not confound other measures.

Serum CK is an established biomarker of muscle damage and is commonly used to assess EIMD. The few studies investigating the effects of n-3 supplementation on blood CK (i.e., plasma and/or serum) have produced conflicting results [10,15,16,27,36]. No differences in CK levels were observed between placebo, experimental, or control groups at all time points (pre-exercise, immediately post, 24-hr, and 48-hr post-exercise) following 30 days of supplementation in Tarbitian et al., 2011 [17]. VanDusseldorp et al., 2020 [8] reported no significant differences in CK from placebo at 24-hr, 48-hr, and 72-hr post-exercise after 7.5 weeks of 6 g of n-3 supplementation, though 72-hr approached significance ($p=0.055$) and LDH was lower compared to placebo at 72-hr post-exercise in resistance-trained males. However, CK was significantly different between 6 g and 2 g at 24-hr. The current study did not observe any differences between supplement groups post-exercise compared to pre-exercise after 33 days of supplementation in resistance-trained males. Interestingly, lower doses of n-3 (1.8 and 2 g per day) for similar supplementation durations (28 and 30 days) resulted in reduced circulating CK levels following eccentric-based resistance exercise [10,16]. However, these studies [10,16] used untrained individuals. Creatine kinase response to exercise has been shown to be highly variable [1,37,38], and is contingent on training familiarity and

level, as well as analysis of serum or plasma [39–41]. These findings suggest n-3 supplementation dose and duration may affect EIMD distinctly between trained and untrained individuals.

Primary differences between VanDusseldorp et al., 2020 [8] and the present study were supplementation duration (~53 days vs. 33 days) and source of supplementation. Marked increases in EPA and DHA composition in cellular membranes have been reported from 4 to 12 weeks of n-3 supplementation [42], which may improve supplement efficacy and, thus, explain the positive results observed in VanDusseldorp et al., 2020 [8]. Additionally, the n-3 supplementation used by VanDusseldorp et al., 2020 [8] was sourced from MusclePharm (MusclePharm Corporation, Burbank, CA, USA) which is third party verified by Informed-Choice, whereas n-3 supplementation used in the current study (Beast Sports Nutrition) is not. Third party verification substantiates supplement quality, inspects manufacturing processes, and ensures labeling is accurate and in accordance with regulations. A of lack of third-party certification cannot ensure that the quality of n-3 supplementation used in the present study is equivalent to VanDusseldorp et al., 2020 [8], which serves as a limitation of the current study. Additionally, both supplementation and maintenance of a training routine meeting the "resistance-trained" requirement were self-regulated throughout the duration of the study, thereby, making it difficult to ensure consistency in consumption and training, respectively. Moreover, a familiarization session for RTF was not performed, whereby, a lack of an effect induced by the exercise session may stem from a learning effect.

There has been variability in n-3 supplement doses ranging from 0.8 to 6 g, while both 2:1 or 3:1 ratio of EPA to DHA have been frequently used and shown to be effective for moderating EIMD [8,12,14–16]. Moreover, the majority of previous work has used untrained individuals. Adding to this, exercise protocols varied in their target musculature (i.e., upper vs. lower body). This study sought to investigate the effects of higher dosages of n-3 supplementation on EIMD following muscle-damaging exercise in resistance-trained individuals using an established exercise protocol. However, while the previous studies have demonstrated ~4 weeks of ~2 g of n-3 supplementation to be effective in mitigating EIMD in untrained individuals, we observed no such effect in trained individuals with 3 to 4 times the dosage (6 and 8 g). Additionally, despite 6 g of n-3 having been shown to reduce EIMD after ~ 7.5 weeks of supplementation, neither 6 or 8 g of n-3 for 33 days affected markers of EIMD and muscle recovery. These data may suggest trained and untrained individuals may respond differently to n-3 supplementation and distinct dosing protocols may be needed for each of these populations to elicit effects on EIMD.

Interestingly, CK levels remained elevated 3d post-exercise, while VJH values returned to pre-exercise values 2d post and PMS was lower 3d post-exercise compared to 1d and 2d post. These results show that markers of EIMD may persist up to 3d following muscle-damaging exercise; however, severity of symptoms may vary at different time points. Similarly in VanDusseldorp et al., 2020 [8], VJH returned to pre-exercise values 48-hr post-exercise in all groups (PL, 2G, 4G, 6G) and PMS was similar to pre-exercise values at 48-hr post, while CK remained elevated in PL, 2G, and 4G up to 72-hr post-exercise in VanDusseldorp et al., 2020. Additionally, other studies have also shown the return of muscle performance, despite CK levels remaining elevated [10,15,27]. This suggests that circulating CK concentrations may not reflect recovery status of muscle performance following muscle-damaging exercise [10,15,16] and should be interpreted with caution. Future research should work to further clarify this relationship.

5. Conclusion

This study is the second study, in addition to VanDusseldorp et al. [8], to test the effects of n-3 supplementation on EIMD in resistance-trained individuals and the first to investigate the efficacy of an 8 g daily dose. Our double-blind randomized control study demonstrated that 33 days of 6 and 8 g of n-3 supplementation with 2:1 ratio of EPA to DHA did not mitigate symptoms of EIMD following an acute bout of eccentric resistance exercise. While our study further validates the muscle-damaging resistance exercise protocol developed by VanDusseldorp et al. [8], our results add to the currently inconclusive literature on n-3 supplementation and EIMD resulting from muscle-damaging resistance exercise. However, taken together with previous work in trained and untrained individuals, it may be that these populations respond differently to n-3 supplementation and require distinct n-3 dosing protocols to yield effects on EIMD. Further research is needed focusing on duration, dosage, and source, as well as EPA and DHA concentrations in order to establish the effectiveness of n-3 supplementation in mitigating muscle damage and facilitating muscle recovery in trained populations. Furthermore, consistent with other studies, our data show that, while circulating CK is commonly used to assess muscle damage, elevated CK levels may not be indicative of muscle recovery status following muscle-damaging exercise.

CRediT authorship contribution statement

Lauren M. Visconti: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. **Joshua A. Cotter:** Project administration, Resources, Writing – review & editing. **Evan E. Schick:** Project administration, Resources, Writing – review & editing. **Noah Daniels:** Investigation. **Frederick E. Viray:** Investigation. **Carson A. Purcell:** Investigation. **Cate B.R. Brotman:** Investigation. **Karen E. Ruhman:** Investigation. **Kurt A. Escobar:** Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendices.

$$\%BF = 8.997 + 0.2468(\Sigma 3SKF) - 6.343(gender^{a}) - 1.998(race^{b}), \quad \text{Eq. (A.1)}$$

[a]Male athletes = 1; female athletes = 0
[b]Black athletes = 1; white athletes = 0

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