

Research article

Effects of a High Protein and Omega-3-Enriched Diet With or Without Creatine Supplementation on Markers of Soreness and Inflammation during 5 Consecutive Days of High Volume Resistance Exercise in Females

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Abstract

We examined if two different dietary interventions affected markers of soreness and inflammation over a 5-day high-volume resistance training protocol in females that resistance-trained 8 weeks prior. Twenty-eight females (age: 20 ± 1 yr; body mass: 63.5 ± 1.6 kg, height: 1.67 ± 0.01 m) completed 4 weeks of pre-training (weeks 1-4) followed by a subsequent 4-week training period along with a dietary intervention (weeks 5-8). Dietary interventions from weeks 5-8 included: a) no intervention (CTL, $n = 10$) b) a higher-protein diet supplemented with hydrolyzed whey protein (50 g/d) and omega-3 fatty acids (900 mg/d) (DI, $n = 8$), and c) the DI condition as well as creatine monohydrate (5 g/d) (DI+C, $n = 10$). During week 9, participants resistance-trained for five consecutive days whereby 8 sets of 10 target repetitions at 70% one repetition maximum (1RM) were performed each day for bench press, back squat, deadlift, and hip-thrusters with the intent of eliciting muscle soreness and inflammation. Prior to and 24 h following each of the 5 bouts muscle soreness (DOMS) was assessed via questionnaire, and fasting blood was obtained and analyzed for serum cortisol, interleukin-6 (IL-6) and C-reactive protein (CRP). No group*time (G*T) or time effects were observed for training volume over the 5-d overreaching protocol. Furthermore, no group*time (G*T) or time effects were observed for serum cortisol, IL-6 or CRP, and DOMS actually decreased in all groups 24 h following the fifth day training bout. This study demonstrates that, regardless of protein, omega-3 fatty acid and/or creatine supplementation, 5 days of consecutive resistance training does not alter perceived muscle soreness, training volume, and/or markers of inflammation in novice resistance-trained females.

Key words: Whey protein, creatine monohydrate, muscle soreness, inflammation.

Introduction

Overreaching is posited to occur with high-volume and high-frequency resistance or endurance training with inadequate recovery. Resistance exercise-induced overreaching or overtraining has been theorized to potentially disrupt hormonal status (i.e., reduce testosterone and insulin-like levels and increase cortisol and catecholamine levels), reduce strength and power, and result in increased levels of inflammation (Fry and Kraemer, 1997; Fry et al., 1994; 1998; 2006; Goto et al., 2013; Steinacker et al.,

2004).

One prevailing issue in overreaching and overtraining research is the putative lack of laboratory-based resistance training protocols which promote an 'overreaching'-like signature as discussed above. In this regard, some researchers have questioned if overreaching or overtraining is a true physiological phenomena (Halson and Jeukendrup, 2004). Notwithstanding, putative resistance-training induced overreaching protocols include: a) one-day eccentric resistance exercise protocols which lead to appreciable increases in markers of muscle damage and inflammation as well as significant decrements in force or power production (Howatson et al., 2012; Kerksick et al., 2013), b) consecutive three-day bouts of eccentric resistance training which, like one-day eccentric training protocols, lead to the aforementioned decrements in performance and increases in inflammation (Willoughby et al., 2003), or c) 4-6 weeks of higher-volume/frequency heavy resistance exercise training (Ratamess et al., 2003; Volek et al., 2004). Indeed, while these laboratory-based protocols are not characteristic of long-term overreaching or overtraining that may be observed in athletes, these 'accelerated' protocols lead to acute changes in muscle damage and inflammation biomarkers which may be observed with chronic overtraining.

A prevailing hypothesis is that nutritional factors can mitigate indices of resistance exercise-induced overreaching and, in this regard, several studies have examined the effects of amino acid supplementation on markers of overreaching. For instance, an investigation by Ratamess and colleagues (2003) determined that essential (EAA) supplementation during 4 weeks of high-volume resistance training reduced rate of fatigue during a post-intervention 20-repetition jump squat test. Howatson et al. (2012) also reported that short-term branched chain amino acid (BCAA) supplementation was better able to preserve muscle strength following a short-term muscle damage protocol. Thus, there is evidence to also suggest that amino acid supplementation can attenuate the effects of resistance training-induced overreaching in a laboratory setting, and this may be related to either an enhancement in post-exercise anabolic processes in skeletal muscle (Campbell et al., 2007; Tipton et al., 1999) and/or alterations in circulating BCAA: tryptophan ratios which pre-

vents excessive serotonin production and reduces the potential 'central' fatigue that can accompany overreaching (Blomstrand, 2001).

Beyond the potential beneficial effects that amino acid supplementation (or higher protein diets) may exert on overreaching-like symptoms, other nutritional modulators may mitigate overreaching. For instance, it stands to reason that creatine supplementation may mitigate overreaching given that vast research evidence has shown creatine supplementation to be beneficial for increasing strength and power (Buford et al., 2007; Kreider et al., 2010; Terjung et al., 2000). Furthermore, a study by Volek et al. (2004) reported that creatine supplementation prevented power and strength losses over a 6-week overreaching protocol.

Omega-3 fatty acid supplementation also carries promise in preventing overreaching given that supplementation has been shown to reduce post-exercise muscle inflammation (Bloomer et al., 2009; Corder et al., 2016). Specifically, it has been posited that chronic omega-3 fatty acid supplementation displaces omega-6 fatty acid content in muscle cell membrane structures which, in turn, reduces the pro-inflammatory prostaglandin response to exercise (Lenn et al., 2002). Notwithstanding, beyond investigations which have reported that omega-3 fatty acid supplementation reduces the acute post-exercise inflammatory response, no studies to our knowledge have examined if chronic supplementation improves recovery during laboratory-based high-volume consecutive-day resistance training protocols.

Therefore, the primary purpose of this investigation was to examine if two different dietary interventions prevented increases in muscle soreness and/or serum inflammation markers in females over a 5-day high volume resistance training protocol. These dietary interventions included: a) a higher-protein diet supplemented with omega-3 fatty acids, and b) a higher-protein diet supplemented with omega-3 fatty acids as well as creatine monohydrate. Notably, the intent of the 5-day consecutive training protocol was to elicit an accelerated overreaching response. We hypothesized that both diets would reduce the aforementioned markers during the 5-day protocol compared to control participants, and further hypothesized that the creatine-supplemented group would have the most optimal response during the week 9 training period (i.e., the least soreness and sustainment of lifting volume, lowered inflammatory markers).

Methods

Participants

Twenty-eight apparently healthy non-resistance trained females (age: 20 ± 1 yr; body mass: 63.5 ± 1.6 kg, height: 1.67 ± 0.01 m) volunteered for the 9-week study. Pre-study questionnaires were administered to ensure that participants had not been taking any nutritional supplements for the past 6 months or had any prior experience with a structured resistance training program prior to enrolling in the study (i.e., did not participate in a team-based and/or self-structured strength and conditioning program in college or high school). Medical screening was implemented

to ensure participants were free of any potential orthopedic or medical issues that could be aggravated by the study protocol. Each participant was verbally informed of study as well as the potential risks of the investigation and signed an informed consent in accordance with the University of Mary Hardin-Baylor's Institutional Review Ethics Committee and Helsinki Declaration. It should be noted that our *a priori* rationale for studying an untrained subject pool was to ensure that relative training status prior to the week 9 high-volume training stimulus was similar between intervention groups. Alternatively stated, we posited that studying trained subjects or athletes would have led to a substantial variation in training age as well as weekly training volume, and these phenomena may have led to a more heterogeneous response to the week 9 training stimulus.

Experimental design

The study was conducted as a randomized 'open label' controlled experimental design whereby participants were aware of which treatment group they were assigned to. Dependent variables included: body composition, upper and lower body one-repetition-maximum (1RM), overreaching protocol performance (4 exercises, 8 total sets, 10 repetitions per set), and select blood markers related to overreaching [cortisol, C-reactive protein (CRP), and interleukin-6 (IL-6)].

To determine the effects of dietary intervention on performance, body composition and biological markers of overreaching, three experimental groups were used:

- 1) no dietary intervention (CTL)
- 2) dietary intervention without creatine (DI)
- 3) dietary intervention plus creatine (DI+C)

All participants completed 4 weeks of pre-training (weeks 1-4) followed by a subsequent 4-week training period along with the dietary intervention (weeks 5-8). We attempted to use a high-volume 'overreaching' training protocol during week 9 which involved five consecutive days of lifting. Exercises during these 5 days stressed both the large upper and lower body muscle groups while minimizing rest between sets, reps, and workouts. A more detailed schematic of the study design is presented in Figure 1.

Pre-training testing (T1) included a battery of tests in the following order: a) hydration testing via urine refractometry, b) resting energy expenditure (REE) assessment using indirect calorimetry (Parvo Medics, Sandy, UT), and c) 1RM testing for bench press, deadlift, back squat, and hip-thrusters. After T1 testing, participants were instructed to report to the laboratory for a 4-week pre-training period (weeks 1-4) described in detail below. Notably, REE was only assessed at T1 in order to prescribe a target Caloric intake for the DI and DI+C groups and this is described in greater detail below.

At the end of the four week pre-training period, participants reported to the laboratory for a battery of tests including the following (noted as T2): a) hydration testing via urine refractometry, b) body composition assessment via dual energy x-ray absorptiometry (DEXA), and c) 1RM lifts for bench press, deadlift, back squat, and hip-

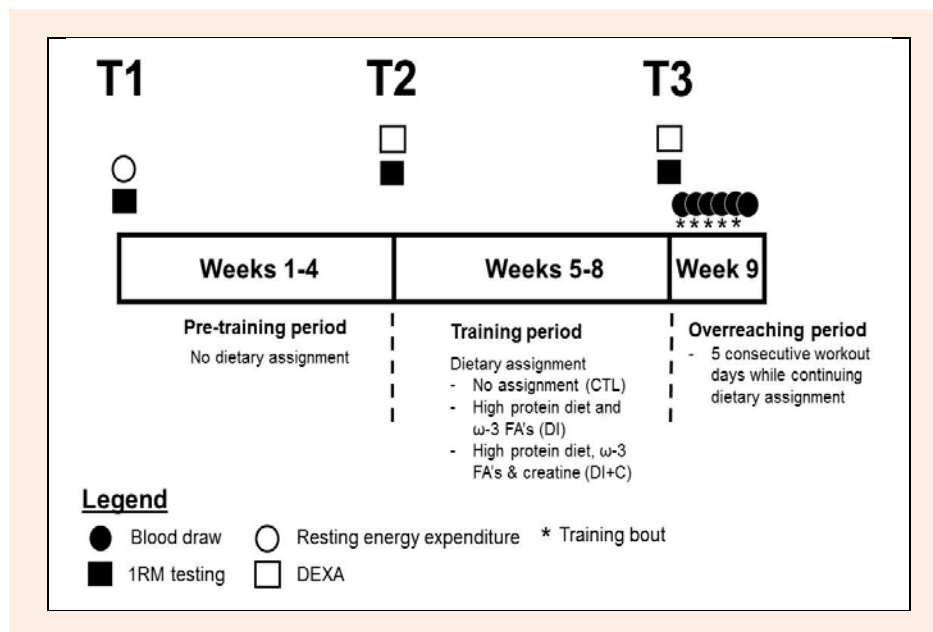


Figure 1. Study design. T1 occurred prior to the intervention whereby resting energy expenditure and 1RM testing occurred. Subjects then engaged in a 4-week pre-training period with no dietary assignment. T2 occurred after week 4 whereby 1RM testing was re-assessed and body composition was assessed via DEXA. Subjects then engaged in a 4-week training period (weeks 5-8) with a dietary assignment which included either: a) no assignment (CTL), b) a high-protein diet with omega-3 fatty acid supplementation (DI), or c) a high-protein diet with omega-3 fatty acid and creatine supplementation (DI+C). T3 occurred after week 8 whereby 1RM testing and body composition was re-assessed. During week 9, subjects performed a 5-d overreaching protocol, and blood was obtained and subjective soreness was assessed 24 h following each bout.

thrusters in order to increase loads during training period 2. The four week dietary intervention for all three groups also started through weeks 5-8 and diets were designed by researchers in the Human Performance Laboratory. Moreover, after T2 testing participants were instructed to report to the laboratory for a 4-week training period (weeks 5-8) described in detail below.

At the conclusion of week 8, a third testing session (T3) occurred and included a battery of the following tests: a) a donation of a venous blood sample following an overnight fast, b) 1RM for bench press, deadlift, back squat and hip-thrusters, and c) body composition assessment via DEXA.

After T3, and at the beginning of week 9, subjects reported to the lab in an overnight-fasted state for 5 consecutive days between the hours of 5 AM and 8 AM. Subjects performed an overreaching workout described in greater detail below. Notably, venous blood samples and a visual analog scale for muscle soreness (DOMS) were obtained prior to workouts.

Body composition assessments

Total-body lean body mass (LBM) and fat mass (FM) was determined using a DEXA (Discovery QDR, Hologic, Inc., Bedford, MA). Before the DEXA scan, body mass was determined using TANITA body composition analyzer (Tanita Corporation of America, Inc., Arlington Heights, IL, USA) and height was determined using a SECA stadiometer (SECA North American, Chino, CA, USA). Subjects were then aligned on the DEXA table and instructed to lay completely still in a supine position for the 6-min duration of the test. The scan was analyzed by trained lab personnel. For DEXA measurements, previous

test-retest reliability in our lab are as follows: fat mass: intra-class correlation coefficient (ICC) = 0.998; lean mass: ICC = 1.00.

1RM testing

1RM tests were determined for bench press, squat, deadlift, and hip-thruster, and testing as well as proper technique was maintained as outlined by the National Strength and Conditioning Association (McGuigan, 2015). Notably, the bench press was performed on a barbell bench press rack. A Smith machine was used for squat testing to help overcome improper technique that could potentially lead to lower-back injury associated with free-weight squat, and a successful repetition was counted once the upper thighs were parallel with the floor at the bottom position; this being visually confirmed by a laboratory technician. Deadlift 1RM testing was performed using a TB-1 Rogue Trap Bar (Rogue Fitness, Columbus, Ohio), and a successful repetition was counted when the plates touched the floor during the eccentric phase and the participant stood completely upright after the concentric phase; this being visually confirmed by a laboratory technician. A hip-thruster is one continuous movement that involves a front squat immediately followed by an overhead push press. Participants were instructed to hold the barbell while in a front rack position (holding the bar in a flexed position at the sternoclavicular joint, with the triceps parallel to the floor) while performing a front squat. Once the knee and hip joints reached the end range of motion (upper thigh parallel to the ground), participants were instructed to stand up while pushing the bar overhead. A completed rep was counted once the bar was completely overhead with elbows in full extension.

Table 1. Workout schematic for weeks 1-8.

Weeks 1-4 (no dietary intervention)			Weeks 5-8 (with dietary intervention)			
Day 1 (sets*target reps)	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4
Bench Press Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Cable Cross-Over Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Incline Bench Press Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Bench Press Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Incline DB Press Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	DB Flies Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Skull Crushers Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8
Triceps Pushdown Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Seated Calf Raises Week 1&2: 2*25-30 Week 3: 3*15-20 Week 4: 4*10-14	Skull Crushers Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Triceps Pushdown Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Preacher Curls Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Russian Twists Week 1&2: 3*20-30 Week 3: 3*15-20 Week 4: 4*10-14	Bent-over Rear Lateral Raises Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8
DB Bent Over Row Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	1-Arm High Cable Curl Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Wide-grip Pull-Down Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Seated Cable Rows Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Front Raises Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	1-Arm Cable Curls Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Hip-thrusters Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8
Barbell Curl Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Deadlift Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Straight Arm Pull-Down Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Barbell Curl Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Squat Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	1-Arm Cable Lateral Raises Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Knee Raises Week 1&2: 3*25-30 Week 3: 3*25-30 Week 4: 3*25-30
Squat Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Plank Week 1&2: 3*1 min Week 3: 3*1 min Week 4: 3*1 min	Hip-thrusters Week 1&2: 3*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Lying Leg Curls Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Lat Pulldown Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Deadlift Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Cable Rotations Week 1&2: 3*20-30 Week 3: 3*15-20 Week 4: 4*10-14
Lying Leg Curls Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Leg Press Calf Raises Week 1&2: 2*25-30 Week 3: 3*15-20 Week 4: 4*10-14	Russian Twists Week 1&2: 3*20-30 Week 3: 3*15-20 Week 4: 4*10-14	V-Ups Week 1&2: 3*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Knee Tucks Week 1&2: 3*25-30 Week 3: 3*25-30 Week 4: 3*25-30	Reverse Grip Pulldown Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Seated Leg Curls Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8
DB Shoulder Press Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Weighted Crunches Week 1&2: 3*15-19 Week 3: 3*15-20 Week 4: 4*6-8	Weighted Crunches Week 1&2: 3*25-30 Week 3: 3*15-20 Week 4: 4*6-8	DB Shoulder Press Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	3-way Calf Raises Week 1&2: 3*25-30 Week 3: 3*15-20 Week 4: 4*6-8	Oblique Crunches Week 1&2: 3*20-30 Week 3: 3*15-20 Week 4: 4*10-14	1-Arm Cable Front Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8
Machine chest flies Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Standing Pull-Down Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	DB Lateral Raise Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8	Standing Calf Raises Week 1&2: 2*25-30 Week 3: 3*15-20 Week 4: 4*10-14			
		DB Upright Rows Week 1&2: 2*12-15 Week 3: 3*9-11 Week 4: 4*6-8				

DB, dumbbell

Resistance training protocols

Before the start of the dietary intervention, subjects participated in a 4-week pre-training protocol (weeks 1–4) as described above. This period helped ensure each subject entered the training and dietary intervention in an equally-trained state as has been previously employed by Ratamess et al. (2003). Pre-training consisted of a 3-day per week upper and lower split-body workout and is presented in Table 1. The second training protocol (weeks 5–8) required participants to workout four times a week for four weeks. This program also included a split-body workout and is presented in Table 1 as well. All training sessions were performed in the Human Performance Laboratory at the University of Mary Hardin-Baylor in order to ensure training compliance and to monitor proper technique and weight progression.

Nutritional prescription and dietary analysis

Prior to the T1 laboratory visit, participants were instructed to record all food intakes for seven days. Following T1 testing, subjects were randomly assigned into one of the three testing groups: CTL, DI, & DI+C. For those in the group CTL, they were given a photocopy of their diet log and instructed to eat the same meals for the weeks 5–9. A dietary intervention was implemented for the both the DI and DI+C group and recommended kilocalorie intakes were prescribed based off the 1.15*REE results from T1. Researchers gave each subject an individualized diet that provided an adequate number of servings for whole grains, vegetables, fruits (especially those high in antioxidants), and dairy based on their recommendations. Daily protein recommendations were assigned using a total daily intake of 1.8 g*kg⁻¹ of bodyweight. Two scoops of hydrolyzed whey protein isolate (serving size: 1 scoop 29.9 g; 110 kilocalories, 25 g protein, 0 g carbohydrate, 0 g fat; Dymatize Nutrition, Dallas, TX) were provided each day for both the DI and DI+C groups. Subjects were given a list of approved foods and foods to avoid, during the intervention. Also those in the DI+C and DI groups were instructed to drink at least a gallon of water a day. Fish-oil capsules high in omega-3 fatty acids (Nature Made, Mission Hills, CA), which provided 540 mg/d eicosapentaenoic acid and 360 mg/d of docosahexaenoic acid, were also provided to the DI and DI+C groups. Participants in the DI+C group were given 5 g/d of micronized creatine monohydrate (Dymatize Nutrition) with their supplemental protein. Finally, a second diet log was administered at the end of the study whereby participants were instructed to record all food intakes for seven days prior to the T3 visit. T1 and T3 diets logs were entered into a nutrition informatics software program (ESHA Research, Salem, OR) for nutritional assessment in order to obtain kilocalorie intakes, macronutrient intakes and omega-3 and -6 fat intakes.

Overreaching protocol

During week 9, participants remained on their respective diets and performed a putative overreaching protocol over 5 consecutive days. The training protocol consisted of 4 exercises (bench press, deadlift, squat and hip-thruster)

performed for 8 sets of 10 target repetitions (or until failure) per exercise at 70% of the subjects 1RM with two-minute rest periods between each set. Requirements for a completed repetition were the same as for the 1RM efforts previously outlined. If a participant did not execute proper form for a repetition, that lift was considered a “no rep”, and therefore was not counted in the total number of reps for that set. Training volume was recorded for each workout in order to assess between-group differences. Moreover, subjects were told to refrain from the use of any non-steroidal anti-inflammatory or analgesic drugs, the use of ice, or any other workouts during the overreaching protocol.

Blood sample analysis and delayed onset of muscle soreness (DOMS) assessments

On blood collection days, serum blood was collected from the antecubital vein in 7.5 ml serum separator tubes. After collection, blood was centrifuged for 15 min at 3,500*g at room temperature. Blood serum was aliquoted into 2 ml pre-labeled microcentrifuge tubes, and stored at -20C for batch analysis. Serum markers were analyzed in duplicate using colorimetric enzyme-linked immunoassays (cortisol and CRP, ALPCO Diagnostics, Salem, NH; IL-6, Cayman Chemical Company, Ann Arbor, Michigan; CK, BioVision, Inc. Headquarters, Milpitas, CA) according to manufacturer’s instructions.

For DOMS assessments, data collection occurred as previously reported (Kephart et al., 2016). Briefly, participants were asked to mark a perpendicular line through a visual straight line scale which was 100 mm in length whereby their mark represented how sore they were at that moment. The researcher explained that the most left aspect indicated no soreness at all, whereas the most right aspect indicates the most soreness that the participant has ever experienced.

Hydration assessment

Hydration status was assessed weekly and, importantly, prior to DEXA testing. Briefly, participants were asked to provide a urine sample (≥ 1 fluid ounce) upon arrival to the laboratory. Urine was analyzed by a laboratory assistant using a digital hand-held urine specific gravity “pen” refractometer (PEN-Wrestling, ATAGO U.S.A., Inc., Bellevue, WA). The pen was calibrated using manufacturer guidelines. The pen was dried with a paper towel and then used to analyze the urine sample by pressing the start button and then submerging the tip of the pen into the sample until a reading was displayed on the screen. Adequate hydration status was defined as 1.006–1.028 ppm urine specific gravity. Given that all participants were adequately hydrated during each week as well as prior to DEXAs, these data were not reported.

Statistical analysis

Mixed factorial-way group by time repeated measures ANOVAs were used to analyze nutritional variables, body composition, blood markers, DOMS scores and overreaching training volume. If significant group*time interactions existed, the model was further decomposed by: a)

performing between-group comparisons at each time point using independent samples t-tests, and b) variables at post-intervention time points were compared between groups using one-way ANOVAs with Bonferroni *post hoc* tests. Data were analyzed using statistical software (SPSS Version 21.0; IBM, Somers, NY) and significance was set at an alpha level of $p < 0.05$.

Results

Training compliance as well as nutritional differences between treatments from baseline to post-intervention

Training compliance during the course of the study was 94% across all subjects. Self-reported nutritional data prior to and following the intervention are presented in Table 2. There were group*time interactions for protein intake ($p = 0.025$) whereby: a) the DI and DI+C groups increased intakes from pre-study to week 8, and b) week 8 protein intakes were greater in the DI and DI+C groups compared to the CTL group ($p < 0.05$). There were group*time effects for omega-3 fat intake ($p < 0.001$) whereby: a) the DI and DI+C groups increased intakes from pre-study to week 8, and b) week 8 protein intakes were greater in the DI and DI+C groups compared to the CTL group ($p < 0.05$). There were no group*time interactions for self-reported kilocalorie intakes ($p = 0.97$), self-reported carbohydrate intakes ($p = 0.55$), self-reported fat intakes ($p = 0.35$) or self-reported omega-6 fat intakes ($p = 0.30$).

Body composition changes between treatments from weeks 4-8

There were no group*time interactions for DEXA fat mass ($p = 0.72$; Figure 2) or DEXA lean body mass ($p = 0.14$; Figure 2). There were significant time effects whereby all groups experienced: a) significant decrease in fat mass ($p = 0.015$; CTL: -0.39 kg; DI: -0.35 kg; DI+C: -0.68 kg), and b) a significant increase in lean mass ($p < 0.001$; CTL: $+0.38$ kg; DI: $+1.35$ kg; DI+C: $+0.96$ kg).

Strength changes between treatments from weeks 4-8

There was no significant group*time interaction for 1RM

bench press ($p = 0.28$; Figure 3), 1RM deadlift ($p = 0.61$; Figure 3), 1RM squat ($p = 0.96$; Figure 3) or 1RM hip-thruster ($p = 0.10$; Figure 3). There were significant time effects whereby all groups experienced: a) a significant increase in 1RM bench press ($p < 0.001$; CTL: $+3.9$ kg; DI: $+2.0$ kg; DI+C: $+4.5$ kg), b) a significant increase in 1RM deadlift ($p = 0.003$; CTL: $+3.8$ kg; DI: $+7.7$ kg; DI+C: $+8.5$ kg), c) a significant increase in 1RM squat ($p < 0.001$; CTL: $+10.1$ kg; DI: $+10.5$ kg; DI+C: $+9.3$ kg), and d) a significant increase in 1RM hip-thruster ($p < 0.001$; CTL: $+2.8$ kg; DI: $+5.1$ kg; DI+C: $+2.5$ kg).

Table 2. Self-reported nutritional data prior to and following the intervention. Data are means (\pm SD).

Variable	CTL	DI	DI+C	Significance
kcal/d				
Pre-study	1580 (103)	1970 (124)	1651 (172)	Time $p=0.55$
Week 8	1554 (76)	1920 (200)	1527 (98)	G*T $p=0.97$
Protein/d (g)				
Pre-study	74 (8)	87 (8)	72 (7)	Time $p=0.006$
Week 8	70 (7) ^a	130 (8) ^{*b}	113 (12) ^{*b}	G*T $p=0.025$
CHO/d (g)				
Pre-study	201 (14)	241 (25)	193 (25)	Time $p=0.55$
Week 8	200 (19)	235 (24)	174 (13)	G*T $p=0.92$
Fat/d (g)				
Pre-study	56 (6)	76 (10)	68 (8)	Time $p=0.008$
Week 8	54 (2)	55 (7)	48 (3)	G*T $p=0.35$
ω-3/d (g)				
Pre-study	.8 (.1)	.7 (.2)	.7 (.2)	Time $p < 0.001$
Week 8	.7 (.1) ^a	2.1 (.2) ^{*b}	1.8 (.1) ^{*b}	G*T $p < 0.001$
ω-6/d (g)				
Pre-study	5.1 (1.1)	5.2 (1.2)	4.8 (1.0)	Time $p=0.40$
Week 8	6.0 (1.2)	4.7 (.7)	3.1 (1.1)	G*T $p=0.30$

Food logs were acquired prior to the pre-training phase and following the training phase at week 8. Abbreviations: ω -3, omega-3 fatty acids; ω -6, omega-6 fatty acids; G*T, group*time interaction. Symbols and superscripts: *, within-group change from pre-study value; different superscript letters indicate between-group significance at week 8.

Volume lifted, DOMS scores and blood markers of overtraining during week 9

Interestingly, there was no time or group*time interaction ($p = 0.12$ and $p = 0.83$, respectively) for volume lifted on days 1-5 during the week 9 overreaching protocol (Figure 4). There was no group*time interaction ($p = 0.81$) for

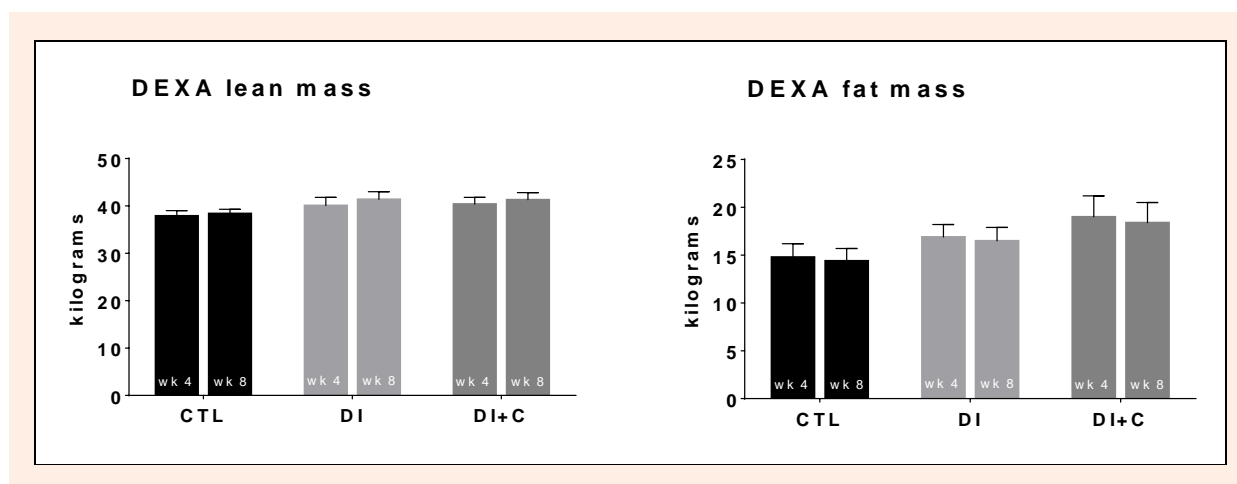


Figure 2. Body composition changes from weeks 5 to 8. Lean mass increased (panel a) and fat mass decreased (panel b) in all groups over the 4-week monitoring period. However, there were no group*time interactions.

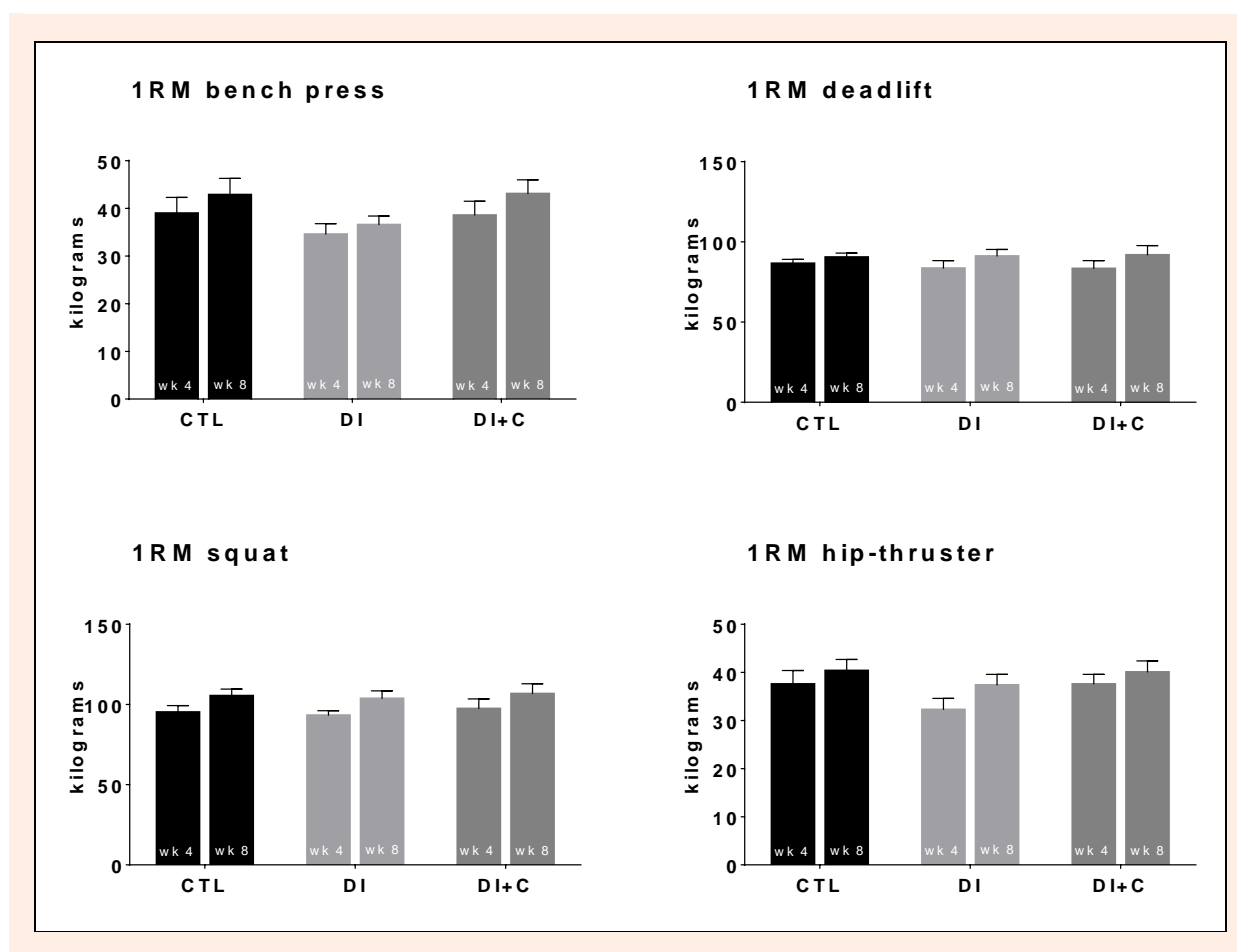


Figure 3. Strength changes from weeks 5 to 8. 1RM bench press increased (panel a), 1RM deadlift increased (panel b), 1RM squat increased (panel c) and 1RM hip-thruster increased (panel d) in all groups over the 4-week monitoring period. However, there were no group*time interactions.

DOMS scores on days 1-5 during the week 9 overreaching protocol, although there was a significant time effect ($p < 0.001$) whereby all groups experienced a decrease in DOMS scores 24 hours following Day 5 compared to all other training days (Figure 4). There were no time or group*time interactions for cortisol ($p = 0.27$ and $p = 0.35$, respectively; Figure 4), CRP ($p = 0.63$ and $p = 0.28$, respectively; Figure 4), or IL-6 ($p = 0.16$ and $p = 0.68$, respectively; Figure 4). Notably, intra- and inter-plate coefficients of variation for each assayed marker were as follows: cortisol: 5.2% and 4.6%, respectively, CRP: 4.3% and 5.9%, respectively, IL-6: 3.1% and 2.5%, respectively.

Discussion

Here, we report two major findings which include: a) under our current experimental design, it appears that 4 weeks of a dietary intervention with added whey protein and omega-3 fat supplementation with or without added creatine does not affect body composition or muscle strength in females, and b) the employed 5-d resistance training protocol during week 9 did not cause a decrease in training volume, an increase in DOMS and/or an increase in select blood markers associated with inflammation and, thus, is not a viable overreaching protocol.

Our lack of significant between-group findings for body composition and strength changes during weeks 4-8

were likely due to the relatively short nature of this intervention. In this regard, we have reported that 8 weeks of whey protein supplementation increases DEXA lean body mass in female collegiate basketball players compared to a non-supplemented group (Taylor et al., 2016). Other studies have also reported that longer-term supplementation (10+ weeks) with whey protein (Volek et al., 2013) and/or creatine (Aguiar et al., 2013) increases lean body mass and strength in females, although some evidence exists suggesting that creatine supplementation may not be efficacious due to gender-related differences regarding creatine uptake mechanisms (Ferguson and Syrotuik, 2006). Notwithstanding, our 4-week nutrition intervention protocol was established in accordance with past overreaching studies which have used similar protocols. Specifically, Ratamess et al. (2003) as well as Kraemer et al. (2006) examined the effects of amino acid supplementation over a 4-week resistance training overtraining protocol in men. Both studies reported that the overreaching protocol did not decrease, but rather increased 1RM bench press and squat regardless of supplementation. Volek et al. (2004) employed a similar 4-week resistance training overtraining protocol to test the effects of creatine supplementation on overtraining recovery. Again, these authors reported that, regardless of supplementation, 1RM bench press and squat increased throughout the intervention. As stated previously, the intent of the 5-day training protocol during week 9 was to elicit muscle soreness

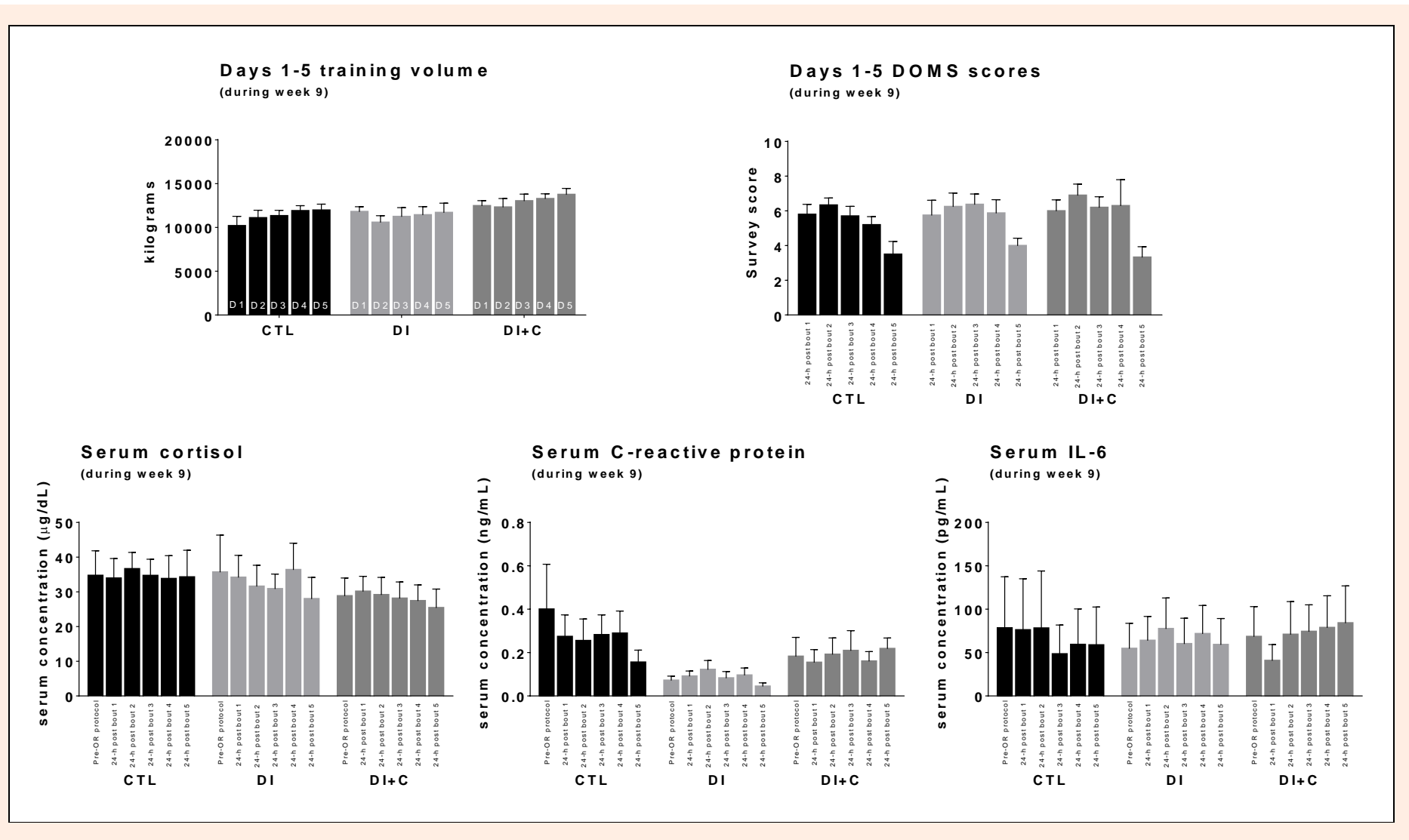


Figure 4. Training volume, muscle soreness and serum markers of overreaching during week 9. Training volume was not altered within or between groups during week 9 (panel a). Interestingly, subjective delayed onset of muscle soreness scores (DOMS) decreased in all groups during week 9 (panel b). Select serum markers of overreaching (cortisol, panel c; C-reactive protein, panel d; interleukin-6, panel e) were not altered within or between groups during week 9.

and/or increase inflammation-related markers (i.e., ‘overreaching’), and we hypothesized that the DI and/or DI+C groups would experience potential improvements in these markers. However, our current data along with the aforementioned resistance training ‘overreaching’ laboratory protocols suggest that strength actually increases; specifically, participants in our study experienced a non-significant ($p = 0.12$) increase in training volume over the 5-day overreaching intervention. What is interesting to note, however, is that the Volek et al. and Ratamess et al. studies reported that jump power decreased with each respective overreaching protocol. Moreover, we have previously reported that three consecutive days of heavy squats (10 sets of 5 repetitions at 85% 1RM) significantly reduced peak torque during a rapid knee extensor assessment protocol (120°/sec) when comparing pre- to post-intervention values (Kephart et al., 2016). Therefore, these data collectively suggest that laboratory-based resistance training overreaching protocols may promote significant neuromuscular fatigue related to the rate of force development rather decreasing the ability to generate an absolute amount of force. However, in the current study we did not utilize tests to assess the rate of force development, so applying this interpretation to our data is limited.

We also report that, regardless of dietary intervention, our 5-d training protocol during week 9 actually decreased DOMS scores and did not alter circulating markers associated with overtraining (i.e., cortisol) and inflammation (IL-6 and CRP). Indeed, the aforementioned study by Volek et al. (2004) also reported that circulating cortisol levels were elevated after one week of the employed overtraining protocol, albeit levels decreased below pre-training levels by week 4 of training. Moreover, the Kraemer et al. (2006) study reported that circulating cortisol levels were unaffected during the 4-week overtraining protocol. Neither study examined circulating IL-6 or CRP levels; both of these biomarkers being associated with low-grade systemic inflammation (Calle and Fernandez, 2010). Therefore, our data seems to be the first data to our knowledge suggesting that the attempted 5-d overreaching protocol does not seem to affect these markers.

This study has noteworthy limitations. First, menstrual cycle phase was not accounted for in the tested participants. Collecting this data would have been interesting given that high circulating levels of estrogen during the late follicular and luteal phases may have correlated with reductions in circulating DOMS and/or serum overtraining markers; a phenomena which has been reported previously in females with eccentric resistance exercise protocols (Kendall and Eston, 2002). Second, while we speculate that our protocol may have reduced power output, we did not assess a metric of power in the current study. Therefore, future research protocols implementing laboratory-based overreaching models with nutritional modulation should focus on power output as well as other performance-based tests. Third, it remains possible that our participants did not achieve an overreaching status given their relative novice training status; alternatively

stated, athletes with years of high-volume training are likely a more suitable group of subjects to address research questions related to nutrition and training stress. Finally, it should be noted that had the DI and/or DI+C groups improved strength and/or week 9 training volume relative to the CTL group, then the ‘open-label’ design and/or the lack of a placebo-control group in the current study may have confounded these positive results. Thus, again, longer-term, double-blinded, placebo-controlled studies implementing a similar study design in athletes is warranted.

Conclusion

Our study, along with other similar laboratory-based resistance training overreaching studies, demonstrate the difficulty in promoting an ‘overreached’ state in participants over a 5-d to 4-week period. Specifically, our findings suggest that our attempt to elicit overreaching during week 9 with 5 consecutive days of high-volume resistance training did not affect circulating biomarkers associated with increased stress or inflammation, and may facilitate positive training adaptations (i.e., lowered DOMS and increases in strength). Notwithstanding, reports from other studies suggest that the rate of force development is impaired with laboratory-based overreaching protocols, and this phenomena is sports-relevant given that many athletic endeavors require a rapid rate of force development (i.e., sprinting, jumping, exerting force against an opponent, etc.). Therefore, future research should continue to examine if team-based training protocols elicit an overreaching signature related to power decrements and determine whether nutritional modulation and/or unloading periods can help mitigate these effects.

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References

- Aguiar, A.F., Januario, R.S., Junior, R.P., Gerage, A.M., Pina, F.L., do Nascimento, M.A., Padovani, C.R. and Cyrino, E.S. (2013) Long-term creatine supplementation improves muscular performance during resistance training in older women. *European Journal of Applied Physiology* **113**, 987-996.
- Blomstrand, E. (2001) Amino acids and central fatigue. *Amino Acids* **20**, 25-34.
- Bloomer, R.J., Larson, D.E., Fisher-Wellman, K.H., Galpin, A.J. and Schilling, B.K. (2009) Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: a randomized, placebo controlled, cross-over study. *Lipids in Health and Disease* **8**, 36.
- Buford, T.W., Kreider, R.B., Stout, J.R., Greenwood, M., Campbell, B., Spano, M., Ziegenfuss, T., Lopez, H., Landis, J. and Antonio, J. (2007) International Society of Sports Nutrition position stand: creatine supplementation and exercise. *Journal of the International Society of Sports Nutrition* **4**, 6.
- Calle, M.C. and Fernandez, M.L. (2010) Effects of resistance training on the inflammatory response. *Nutrition Research and Practice* **4**, 259-69.
- Campbell, B., Kreider, R.B., Ziegenfuss, T., La Bounty, P., Roberts, M.,

- Burke, D., Landis, J., Lopez, H. and Antonio, J. (2007) International Society of Sports Nutrition position stand: protein and exercise. *Journal of the International Society of Sports Nutrition* **4**, 8.
- Corder, K.E., Newsham, K.R., McDaniel, J.L., Ezekiel, U.R. and Weiss, E.P. (2016) Effects of Short-Term Docosahexaenoic Acid Supplementation on Markers of Inflammation after Eccentric Strength Exercise in Women. *Journal of Sports Science and Medicine* **15**, 176-183.
- Ferguson, T.B. and Syrotuik, D.G. (2006) Effects of creatine monohydrate supplementation on body composition and strength indices in experienced resistance trained women. *Journal of Strength & Conditioning Research* **20**, 939-946.
- Fry, A.C. and Kraemer, W.J. (1997) Resistance exercise overtraining and overreaching. Neuroendocrine responses. *Sports Medicine* **23**, 106-129.
- Fry, A.C., Kraemer, W.J. and Ramsey, L.T. (1998) Pituitary-adrenal-gonadal responses to high-intensity resistance exercise overtraining. *Journal of Applied Physiology* (1985) **85**, 2352-2359.
- Fry, A.C., Kraemer, W.J., Van Borselen, F., Lynch, J.M., Triplett, N.T., Koziris, L.P. and Fleck, S.J. (1994) Catecholamine responses to short-term high-intensity resistance exercise overtraining. *Journal of Applied Physiology* (1985) **77**, 941-946.
- Fry, A.C., Schilling, B.K., Weiss, L.W. and Chiu, L.Z. (2006) beta2-Adrenergic receptor downregulation and performance decrements during high-intensity resistance exercise overtraining. *Journal of Applied Physiology* (1985) **101**, 1664-1672.
- Goto, K., Shioda, K. and Uchida, S. (2013) Effect of 2 days of intensive resistance training on appetite-related hormone and anabolic hormone responses. *Clinical Physiology Functional Imaging* **33**, 131-136.
- Halson, S.L. and Jeukendrup, A.E. (2004) Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Medicine* **34**, 967-981.
- Howatson, G., Hoard, M., Goodall, S., Tallent, J., Bell, P.G. and French, D.N. (2012) Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study. *Journal of the International Society of Sports Nutrition* **9**, 20.
- Kendall, B. and Eston, R. (2002) Exercise-induced muscle damage and the potential protective role of estrogen. *Sports Medicine* **32**, 103-123.
- Kephart, W.C., Mumford, P.W., McCloskey, A.E., Holland, A.M., Shake, J.J., Mobley, C.B., Jagodinsky, A.E., Weimar, W.H., Oliver, G.D., Young, K.C., Moon, J.R. and Roberts, M.D. (2016) Post-exercise branched chain amino acid supplementation does not affect recovery markers following three consecutive high intensity resistance training bouts compared to carbohydrate supplementation. *Journal of the International Society of Sports Nutrition* **13**, 30.
- Kerksick, C.M., Roberts, M.D., Dalbo, V.J., Kreider, R.B. and Willoughby, D.S. (2013) Changes in skeletal muscle proteolytic gene expression after prophylactic supplementation of EGCG and NAC and eccentric damage. *Food and Chemical Toxicology* **61**, 47-52.
- Kraemer, W.J., Ratamess, N.A., Volek, J.S., Hakkinen, K., Rubin, M.R., French, D.N., Gomez, A.L., McGuigan, M.R., Scheett, T.P., Newton, R.U., Spiering, B.A., Izquierdo, M. and DiGuardi, F.S. (2006) The effects of amino acid supplementation on hormonal responses to resistance training overreaching. *Metabolism* **55**, 282-291.
- Kreider, R.B., Wilborn, C.D., Taylor, L., Campbell, B., Almada, A.L., Collins, R., Cooke, M., Earnest, C.P., Greenwood, M., Kalman, D.S., Kerksick, C.M., Kleiner, S.M., Leutholtz, B., Lopez, H., Lowery, L.M., Mendel, R., Smith, A., Spano, M., Wildman, R., Willoughby, D.S., Ziegenfuss, T.N. and Antonio, J. (2010) ISSN exercise & sport nutrition review: research & recommendations. *Journal of the International Society of Sports Nutrition* **7**, 7.
- Lenn, J., Uhl, T., Mattacola, C., Boissonneault, G., Yates, J., Ibrahim, W. and Bruckner, G. (2002) The effects of fish oil and isoflavones on delayed onset muscle soreness. *Medicine & Science in Sports & Exercise* **34**, 1605-1613.
- McGuigan, M. (2015) Principle of Test Selection and Administration. In: *Essentials of Strength and Conditioning*. Eds: Haff, G.G. and Triplett, N.T. 4th edition. Human Kinetics.
- Ratamess, N.A., Kraemer, W.J., Volek, J.S., Rubin, M.R., Gomez, A.L., French, D.N., Sharman, M.J., McGuigan, M.M., Scheett, T., Hakkinen, K., Newton, R.U. and DiGuardi, F. (2003) The effects of amino acid supplementation on muscular performance during resistance training overreaching. *Journal of Strength & Conditioning Research* **17**, 250-258.
- Steinacker, J.M., Lormes, W., Reissnecker, S. and Liu, Y. (2004) New aspects of the hormone and cytokine response to training. *European Journal of Applied Physiology* **91**, 382-391.
- Taylor, L.W., Wilborn, C., Roberts, M.D., White, A. and Dugan, K. (2016) Eight weeks of pre- and postexercise whey protein supplementation increases lean body mass and improves performance in Division III collegiate female basketball players. *Applied Physiology Nutrition & Metabolism* **41**, 249-254.
- Terjung, R.L., Clarkson, P., Eichner, E.R., Greenhaff, P.L., Hespel, P.J., Israel, R.G., Kraemer, W.J., Meyer, R.A., Spriet, L.L., Tarnopolsky, M.A., Wagenmakers, A.J. and Williams, M.H. (2000) American College of Sports Medicine roundtable. The physiological and health effects of oral creatine supplementation. *Medicine & Science in Sports & Exercise* **32**, 706-717.
- Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle, D., Jr. and Wolfe, R.R. (1999) Postexercise net protein synthesis in human muscle from orally administered amino acids. *American Journal of Physiology* **276**, E628-34.
- Volek, J.S., Ratamess, N.A., Rubin, M.R., Gomez, A.L., French, D.N., McGuigan, M.M., Scheett, T.P., Sharman, M.J., Hakkinen, K. and Kraemer, W.J. (2004) The effects of creatine supplementation on muscular performance and body composition responses to short-term resistance training overreaching. *European Journal of Applied Physiology* **91**, 628-637.
- Volek, J.S., Volk, B.M., Gomez, A.L., Kunces, L.J., Kupchak, B.R., Freidenreich, D.J., Aristizabal, J.C., Saenz, C., Dunn-Lewis, C., Ballard, K.D., Quann, E.E., Kawiecki, D.L., Flanagan, S.D., Comstock, B.A., Fragala, M.S., Earp, J.E., Fernandez, M.L., Bruno, R.S., Ptolemy, A.S., Kellogg, M.D., Maresh, C.M. and Kraemer, W.J. (2013) Whey protein supplementation during resistance training augments lean body mass. *Journal of American College of Nutrition* **32**, 122-135.
- Willoughby, D.S., McFarlin, B. and Bois, C. (2003) Interleukin-6 expression after repeated bouts of eccentric exercise. *International Journal of Sports Medicine* **24**, 15-21.

Key points

- We examined if two different dietary interventions (higher protein and omega-3 supplementation, or higher protein and omega-3 supplementation with creatine supplementation) affected muscle soreness and inflammation markers over a 5-day high-volume resistance training protocol in females that resistance-trained 8 weeks prior.
- Neither dietary intervention affected training volume, muscle soreness and inflammation markers over the 5-d consecutive training period.
- More research is needed in order to determine if the dietary interventions employed herein affects athletes which may experience overreaching-like symptoms over a training season.

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