

The time course of short-term hypertrophy in the absence of eccentric muscle damage

Matt S. Stock¹ · Jacob A. Mota² · Ryan N. DeFranco³ · Katherine A. Grue³ ·
A. Unique Jacobo⁴ · Eunhee Chung⁵ · Jordan R. Moon⁶ · Jason M. DeFreitas⁷ ·
Travis W. Beck⁸

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Abstract

Background It has been proposed that the increase in skeletal muscle mass observed during the initial weeks of initiating a resistance training program is concomitant with eccentric muscle damage and edema.

Purpose We examined the time course of muscle hypertrophy during 4 weeks of concentric-only resistance training.

Methods Thirteen untrained men performed unilateral concentric-only dumbbell curls and shoulder presses twice per week for 4 weeks. Sets of 8–12 repetitions were performed to failure, and training loads were increased during each session. Subjects consumed 500 ml of whole milk

during training. Assessments of soreness, lean mass, echo intensity, muscle thickness, relaxed and flexed arm circumference, and isokinetic strength were performed every 72 or 96 h.

Results Soreness, echo intensity, relaxed circumference, and peak torque data did not significantly change. Significant increases in lean mass, muscle thickness, and flexed circumference were observed within seven training sessions. Lean mass was elevated at tests #7 (+109.3 g, $p=.002$) and #8 (+116.1 g, $p=.035$), with eight different subjects showing changes above the minimal difference of 139.1 g. Muscle thickness was elevated at tests #6 (+0.23 cm, $p=.004$), #7 (+0.31 cm, $p<.001$), and #8 (+0.27 cm, $p<.001$), with ten subjects exceeding the minimal difference of 0.24 cm. There were no changes for the control arm.

Conclusion In individuals beginning a resistance training program, small but detectable increases in hypertrophy may occur in the absence of eccentric muscle damage within seven training sessions.

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✉ Matt S. Stock
matt.stock@ucf.edu

¹ Department of Health Professions, University of Central Florida, Health and Public Affairs Bldg I, Room 258, 4364 Scorpis Street, Orlando, FL 32816-2205, USA

² Department of Exercise and Sport Science, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA

³ Department of Kinesiology and Sport Management, Texas Tech University, Lubbock, TX, USA

⁴ Honors College, Texas Tech University, Lubbock, TX, USA

⁵ Department of Kinesiology, Health, and Nutrition, University of Texas at San Antonio, San Antonio, TX, USA

⁶ American Public University System, Charles Town, WV, USA

⁷ Applied Neuromuscular Physiology Laboratory, Oklahoma State University, Stillwater, OK, USA

⁸ Department of Health and Exercise Science, University of Oklahoma, Norman, OK, USA

Keywords Muscle mass · Lean mass · Soreness · Concentric · Torque · Force

Abbreviations

ANOVA Analysis of variance
A.U. Arbitrary units
DXA Dual energy X-ray absorptiometry
1RM One-repetition maximum

Introduction

Many research groups have examined the time course at which hypertrophy occurs when untrained subjects begin

a resistance training program. Historically, muscle hypertrophy has been considered a slow process that typically begins once neural adaptations have subsided (Moritani and deVries 1979). Some studies, however, have reported significant changes in muscle size in response to only a few weeks of training. Seynnes et al. (2007) used magnetic resonance imaging to demonstrate significant increases in vastus lateralis fascicle length and quadriceps femoris cross-sectional area within 10 and 20 days of training, respectively. Similarly, DeFreitas et al. (2011) reported a significant increase in thigh muscle cross-sectional area in response to only two training sessions, with additional increases shown 3 weeks into the training program, as well as each week thereafter. Significant increases in lean mass following four training sessions for women engaged in low volume training have also been reported, with several subjects demonstrating large changes (Stock et al. 2016). Collectively, although the body of literature is reasonably small, there are data to suggest that increases in muscle size may occur early in a training program (DeFreitas et al. 2011; Seynnes et al. 2007; Stock et al. 2016; among others).

When untrained subjects partake in unaccustomed exercise, significant muscle damage occurs (Warren and Palubinskas 2008). It is generally accepted that a single bout of high force, eccentric muscle actions produce substantial tissue damage (Warren et al. 1999). In contrast, a single bout of concentric or isometric muscle actions results in considerably less muscle damage (Friden et al. 1986; Gibala et al. 1995; McCully and Faulkner 1985). For this reason, investigators that seek to study muscle injury almost always utilize eccentric modes of training. As described in a review by Warren et al. (1999), evidence of damage includes delayed onset muscle soreness, elevated intracellular enzymes in the blood (e.g., creatine kinase, lactate dehydrogenase, and myoglobin), loss of calcium homeostasis, decreased joint range of motion, and edema. Interestingly, the decline in muscular force/torque is considered one of the most valid and reliable indirect markers of exercise-induced muscle damage (Warren et al. 1999). The signs of muscle damage typically subside within 10 days (Nosaka and Clarkson 1995), and their magnitude lessens with each subsequent bout of training (McHugh 2003). Based on these facts, designing short-term training investigations involving eccentric muscle actions aimed at increasing muscle strength or size can be methodologically challenging. This is particularly true because many of the same measurement tools (e.g., force/torque assessments, electromyography, ultrasonography, limb circumferences, and magnetic resonance imaging) can be used to track both improvements as a result of training and functional declines with muscle damage. These issues were thoroughly described and examined in a recent study (Damas

et al. 2016). Damas et al. (2016) attempted to distinguish the training-induced increases in muscle size due to edema-induced swelling from the changes due to muscle hypertrophy. Ten untrained men performed bilateral leg press and leg extension exercise twice per week for 10 weeks, and measurements were performed following 3 and 10 weeks of training. Based primarily on changes in the ratio of ultrasonography-derived echo intensity to cross-sectional area, Damas et al. (2016) proposed that changes observed in muscle size within the first 3 weeks of training should be attributable to edema-induced swelling. The notion that edema may still be present several weeks following the initiation of a novel training program would suggest that previously published results demonstrating significant increases in muscle size may have depicted an inaccurate interpretation of the time course of muscular adaptations to short-term training.

To our knowledge, all of the previous studies that have attempted to quantify changes in muscle hypertrophy in response to short-term resistance training have included eccentric muscle actions. Thus, it is conceivable that previous findings may have been influenced by at least a minor degree of muscle damage. An alternative means of assessing the time course of muscle hypertrophy in the absence of edema-induced swelling is to avoid eccentric muscle actions. Since both concentric and isometric training have been shown to result in increases in muscle size in untrained subjects (Housh et al. 1996; Kubo et al. 2001), these modes have the advantage of providing an effective training stimulus while largely avoiding the negative consequences associated with muscle damage. Therefore, the purpose of this study was to examine the time course of skeletal muscle hypertrophy in response to only 4 weeks (eight sessions) of unilateral concentric-only training. To examine a more precise time course, testing was performed 72 or 96 h following every training session. It was hypothesized that training-induced increases in muscle size and strength would be demonstrated within 4 weeks.

Methods

Subjects

Before data collection, an a priori power analysis was performed for dual energy X-ray absorptiometry (DXA) arm lean mass using the recommendations described by Beck (2013) for a within-subjects design. These calculations were performed using G*Power software (version 3.1.4; Heinrich Heine University, Düsseldorf, Germany) and hypothesized effect sizes based on previously unpublished work from our laboratory. As a result of this analysis, 16 men were originally enrolled and began participating in

this study. Two subjects dropped out because of its time demands. One subject reported neck pain during the initial training session that persisted for several days, and was removed from the investigation for precautionary reasons. Thirteen healthy men (mean \pm SD age = 23 ± 4 years; mass = 75.8 ± 12.2 kg; height = 176.0 ± 9.4 cm; body-fat = $22.8 \pm 7.8\%$) who were not engaged in resistance training during the previous 6 months completed the study. Potential subjects were not able to participate if they were affected by neuromuscular or metabolic disease. Furthermore, men with recent upper-body musculoskeletal pain or injury were not able to participate. This study and its procedures were approved by the Texas Tech University Human Research Protection Program (approval #2016-70). All subjects read, understood, and signed an informed consent form prior to participation. The subjects agreed to refrain from upper-body resistance training throughout the duration of the study. Upon enrollment, random assignment was used to determine which arm would be trained. Out of the 13 subjects, six trained their dominant arm and seven trained their non-dominant arm.

Testing and training schedules

The subjects visited the laboratory on ten separate occasions within a 5-week period. The first visit to the laboratory served as a familiarization and unilateral one repetition maximum (1RM) strength testing session. The following eight visits to the laboratory involved testing followed by training. In other words, the pretest was followed by training session #1, test #1 was followed by training session #2, test #2 was followed by training session #3, etc. The tenth visit to the laboratory involved testing only (test #8). Visits to the laboratory were separated by 72 or 96 h. Each subject visited the laboratory on Monday and Thursday, Tuesday and Friday, or Wednesday and Saturday. Testing for each subject occurred at the

same time of day (± 1 h) throughout the study. All testing occurred in the morning hours (6:00–9:30am) following a 10-h fast. Testing was scheduled such that the subjects visited the laboratory as soon as possible upon awakening to ensure minimal physical activity prior to each assessment. The subjects provided a small urine sample upon arrival to each laboratory visit. Urine specific gravity was measured via a handheld refractometer (MASTER-URC/NM, ATAGO U.S.A., Inc., Bellevue, WA, USA) to ensure normal hydration levels (urine specific gravity range = 1.001–1.029) (Armstrong 2005). On five occasions out of the 130 study visits, urine specific gravity was >1.029 . When this occurred, the subjects consumed 500 ml of water and waited a minimum of 30 min before providing an additional urine sample to verify adequate hydration. Test–retest reliability statistics for each of the dependent variables were determined using the calculations described by Weir (2005), and have been presented in Table 1. The testing procedures have been described below in the order in which the subjects performed them. Investigators were blinded to training and control arms during data analysis. The investigator that performed data collection was blinded to the majority of training and control arms, but did occasionally assist with training and spotting when other investigators were unavailable.

Delayed onset muscle soreness

Upon arrival to the laboratory, the subjects completed a Likert scale to indicate their current level of upper-body delayed onset muscle soreness for the entire training arm and shoulder. The scale had values of 0 and 10 that represented the complete absence of soreness and extremely severe pain, respectively. Each subject clearly understood the Likert scale, and was asked by an investigator to carefully ponder his response.

Table 1 Test–retest reliability statistics for the dependent variables examined in the present study

	Lean mass (g)	Echo intensity (A.U.)	Muscle thickness (cm)	Relaxed circumference (cm)	Flexed circumference (cm)	Concentric peak torque (Nm)
Test Mean \pm SD	3008.3 \pm 427.3	76.9 \pm 17.0	2.97 \pm 0.47	30.8 \pm 3.1	34.3 \pm 3.3	52.3 \pm 12.6
Re-test Mean \pm SD	3046.5 \pm 419.6	76.2 \pm 13.3	2.97 \pm 0.48	30.8 \pm 3.0	34.3 \pm 3.4	53.2 \pm 12.1
Cohen's <i>d</i>	0.09	0.04	0.01	0.01	0.01	0.07
ICC (model 2, 1)	0.983	0.852	0.968	0.986	0.996	0.845
SEM	50.2	6.0	0.09	0.4	0.2	5.0
SEM (%)	1.7	7.9	3.0	1.2	0.7	9.5
MD	139.1	16.7	0.24	1.0	0.6	13.9

These data were collected from the control arm of the 16 subjects that began the study

ICC intraclass correlation coefficient, SEM standard error of mean, MD minimal difference needed to be considered real

DXA

The subjects completed one total body scan using the DXA (Lunar Prodigy Primo, GE Healthcare, Madison, WI, USA) during each of the ten testing sessions. All scans were performed by a trained technician that had completed university radiation training. DXA quality assurance testing was performed every 24–48 h throughout the study. Following data collection, custom regions of interest were created with the manufacturer-provided software's (enCORE 2011, GE Healthcare, Wauwatosa, WI, USA) polygon function for the arms of each subject. Regions were created to provide as much data for the trained musculature as possible while avoiding unaffected tissues. At the distal end, the region began at the intersection between the ulna and humerus. Medially, the region of interest was drawn superiorly between the humerus and torso until the axilla was reached. Special care was taken to ensure that the lateral chest was not included in the region of interest. Upon reaching the axilla, a line was drawn medially at roughly a 45–60° angle (depending on humerus length and torso width) until it reached the ribcage. A line which wrapped around the lateral surface of the ribcage was then drawn. Once the line met the intersection of the ribcage and the superior border of the clavicle, it continued directly superiorly, thereby including much of the trapezius. Collectively, each region of interest was created to include as much of the humerus, shoulder girdle, and trapezius as possible, while attempting to exclude tissue from the forearm and chest. For each arm, the custom region of interest from the initial visit to the laboratory was copied to each subsequent scan. Therefore, while regions of interest may have differed between individuals because of anatomical dissimilarities, the same region was used repeatedly for each subject. In the event that a subject's positioning on the table differed a small amount among scans, a study investigator manually adjusted the region of interest. The same investigator performed all of the DXA analyses.

Elbow flexor ultrasonography

Following each DXA scan, transverse ultrasound images were taken of both arms using methods similar to those described by Caresio et al. (2015) and Jenkins et al. (2015b). Ultrasound measurements were performed with a portable B-mode imaging device (GE Logiq e BT12, GE Healthcare, Milwaukee, WI, USA) and a multi-frequency linear-array probe (12 L-RS, 5–13 MHz, 38.4-mm field of view; GE Healthcare, Wauwatosa, WI, USA). All assessments were performed while the subjects rested in the supine position and the humerus abducted at 90°. Images were taken on the anterior surface of the arm at 66% of the distance from the acromion process to the cubit fossa. To

ensure trial-to-trial consistency, the subjects were oriented such that the olecranon process of the arm being tested was at the end of the wooden table. The forearm was fully extended at the elbow. Special care was taken to ensure that the humerus was located at the middle of the ultrasound image. Following each laboratory visit, the image location was marked with a permanent marker, and in the majority of cases, was still visible during the subsequent testing session. Ultrasound settings were optimized (Frequency: 10 MHz, Gain: 58Db, Dynamic Range: 72) and kept consistent. Image depth varied between 4.0 and 6.0 cm depending on muscle size, but each subject's depth was constant throughout the study. The ultrasonography probe was covered with water-soluble transmission gel (Aquasonic 100 ultrasound transmission gel, Parker Laboratories, Inc., Fairfield, NJ, USA). Three images were taken at each site. The same investigator performed all of the ultrasound measurements. Figure 1 displays an example of the data collection and analysis procedures for one subject.

Images were digitized and analyzed with Image J software (version 1.46, National Institutes of Health, Bethesda, MD, USA). Echo intensity analyses were performed using the rectangle function. The largest region of interest possible for the biceps brachii muscle was used for analysis (Jenkins et al. 2015b). The brachialis muscle was not included (Jenkins et al. 2015b). Echo intensity was assessed by computer-aided gray-scale analysis using the histogram function. The mean echo intensity values were determined as the corresponding index of muscle quality ranging between 0 and 255 arbitrary units ([A.U.] black=0; white=255). Muscle thickness was also quantified using the procedures described by Jenkins et al. (2015b). After scaling each image from pixels to cm, muscle thickness for the elbow flexors was quantified by drawing a vertical line from the adipose tissue–muscle interface to the muscle–bone interface in the middle of the image. Unlike the echo intensity measurements, the muscle thickness assessments included both the biceps brachii and brachialis (Jenkins et al. 2015b). For both ultrasonography variables, the mean value from three images has been reported.

It is worth noting that panoramic imaging was also performed in an attempt to quantify muscle cross-sectional area. This task was met with great difficulty for the subjects that were lean and with little arm lean mass. As such, the panoramic images demonstrated insufficient reliability to be considered as part of this investigation's analyses.

Arm circumference

Relaxed arm circumference (cm) was determined with a cloth tape at 66% of the distance from the acromion process of the scapula to the cubit fossa. Measurements were taken with the arm relaxed at the side of the torso. Flexed

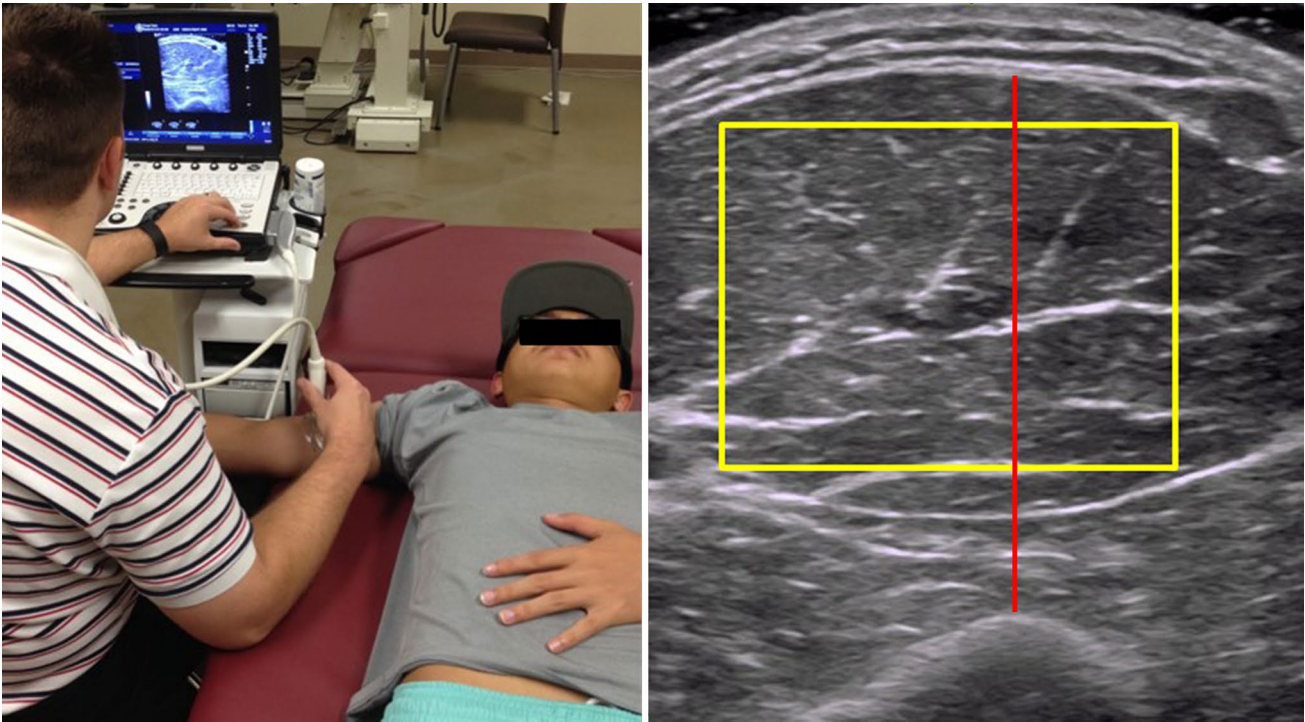


Fig. 1 An example of the data collection (*left*) and image analysis (*right*) procedures for the determination of echo intensity and muscle thickness. The *rectangle* corresponds to the region of interest used to quantify echo intensity. The *vertical line* corresponds to the measure-

ment of muscle thickness. These analyses were performed using the procedures described previously by Caresio et al. (2015) and Jenkins et al. (2015b)

circumference (cm) was quantified as the maximum girth of the arm while the subject flexed at an elbow joint angle of 90°. Subjects were verbally encouraged to “squeeze as hard as possible” during the measurement.

Maximal concentric strength testing

Concentric isokinetic strength testing for both elbow flexor muscle groups was performed with an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, Shirley, NY, USA). Concentric testing was performed rather than isometric maximal voluntary contractions in an attempt to mimic what the subjects performed during training. All data collection sessions began with a warm-up of ten sub-maximal muscle actions. Testing involved five consecutive maximal concentric isokinetic muscle actions. Following each muscle action, passive extension was used to slowly return to the starting position. Each muscle action was performed at a velocity of 90°/second through a full 90° range of motion (0° = full extension). The order in which each arm was tested was determined randomly. Strong verbal encouragement was provided throughout the study. During each subject’s initial visit to the laboratory, the dynamometer’s settings were recorded to ensure consistency. The concentric torque signals were digitized at a sampling rate of

1926 Hz (a preset commercial hardware device frequency) and acquired with EMG works software (version 4.1.7, Delsys Inc., Natick, MA, USA).

The torque signals were processed using custom written software (LabVIEW 8.5, National Instruments, Austin, TX, USA). The raw signals were scaled to appropriate units (Nm) and filtered using a zero phase shift, fourth-order Butterworth filter with a 10-Hz low-pass cutoff frequency. For each muscle action, the torque signal was gravity-corrected by subtracting the limb weight’s baseline torque value from the duration of the signal. Concentric peak torque (Nm) was calculated as the highest single data point during the constant velocity portion of the range of motion. For each testing session, the mean of the two highest values was used for data analysis; the remaining three were ignored.

Concentric training

Following testing, the subjects performed unilateral concentric-only dumbbell biceps curls and shoulder presses with the help of a study investigator (Fig. 2). The dumbbell curls were performed with a preacher bench. Each repetition began with the research investigator gently placing the dumbbell in the subject’s training hand. The training arm was fully extended. Care was taken to not drop the weight



Fig. 2 Examples of the training and spotting techniques utilized for the concentric-only biceps curl (*top*) and shoulder press (*bottom*) exercises. On the *left*, the subject is being gently handed the dumbbell

by a study investigator. The *right images* display the subject returning his hand to the starting position to perform the next concentric-only repetition

in the subject's hand to ensure that the musculotendinous unit did not lengthen. The subject then curled the dumbbell in a controlled manner (~2 s). Upon reaching a full range of motion, the study investigator removed the dumbbell from the subject's hand and returned it to the starting position. The subject also returned his empty hand to the starting position, and this process was performed repeatedly. The dumbbell shoulder press exercise was performed in a similar manner. The shoulder press exercise was performed seated. The subject began with his non-training hand on his hip, and an investigator carefully placed the loaded dumbbell in the subject's open palm, which was just outside of the acromion process. The subject then pressed the dumbbell superiorly as high as possible. Upon reaching

full extension, the study investigator removed the dumbbell from the subject's hand. The investigator then lowered the dumbbell while the subject returned his empty hand back to the starting position. This process was repeated. The subjects were provided strong verbal encouragement throughout training.

During the initial visit to the laboratory, 1RM testing was performed for the two previously described exercises using the step-by-step procedures described by the National Strength and Conditioning Association (Baechle and Earle 2008). The goal of this process was to determine the heaviest external load that each subject could concentrically lift for one repetition through a full range of motion. A 3-min rest period was provided between 1RM attempts. Prior to

1RM testing, the subjects were instructed to concentrically perform a repetition as soon as the external load was placed in the training hand in an attempt to limit eccentric activity of the involved musculature. 1RM strength was not used as a dependent variable in this study, and was only assessed during the initial visit to the laboratory for the purpose of setting initial training loads. The training protocol used in the present study was similar to that utilized by DeFreitas et al. (2011). During the first four training sessions, the subjects performed five sets of each exercise. Six sets of each exercise were performed during the final 2 weeks. The initial exercise loads for the first training session were based on 70% of the 1RM. All sets were performed to volitional failure. A 90-s rest period was allowed between sets. Throughout the study, the external loads were adjusted such that the subjects performed between 8 and 12 repetitions per set. For example, if during the fourth set of the dumbbell shoulder press exercise a subject could only perform six repetitions, the external load was reduced so that additional repetitions could be performed during the subsequent set. The external loads were increased throughout the study in an effort to consistently provide a greater training stress. The use of 0.113, 0.227, 0.340, and 0.430-kg fractional plates (Rogue Fitness HQ, Columbus, OH, USA) allowed for small adjustments in training load.

During each training session, the subjects consumed 500 ml of whole milk, which provided 600 calories and 32 grams of protein. Whole milk was provided to the subjects as a means of increasing muscle protein synthesis (Elliot et al. 2006) while also attempting to create a caloric surplus. Due to the volume of the beverage consumed, subjects were allowed to drink their milk before, during, and/or after their training session. The subjects were not allowed to exit the laboratory until all 500 ml were consumed in the presence of a study investigator.

Dietary analyses

The subjects kept a detailed 5-day food log that required them to record all of the foods and beverages consumed. The 5 days were determined at random, but included at least one weekend day. The subjects were instructed to be as specific as possible, including details such as item brand names and the method of food preparation. When feasible, the subjects were asked to measure their food and beverage quantities. The subjects were instructed to continue their normal ad libitum diet, and to keep their caloric intake consistent throughout the study. We also asked the subjects to keep their caffeine consumption (or lack thereof) before testing consistent. The subjects were frequently reminded to complete their food logs by members of the research team. When food logs were collected on training days, the milk consumed during the training

session was included in the day's dietary analyses. Food logs were analyzed for estimated total calories, protein, carbohydrates, and fats using online software (MyFitnessPal, Inc., San Francisco, CA, USA).

Statistical analyses

For each dependent variable that involved bilateral testing, mean differences were analyzed with two-way (time [pretest, test #1, test #2, test #3, test #4, test #5, test #6, test #7, test #8] × arm [training, control]) repeated measures analyses of variance (ANOVAs). Four separate repeated measures ANOVAs were used to evaluate the increase in training volume and load for the two exercises. When appropriate, follow-up analyses included additional one-way repeated measures ANOVAs across time, paired sample *t* tests between arms, and Bonferroni pairwise comparisons. The partial eta squared (η^2) statistic was reported for each repeated measures ANOVA, with values of 0.01, 0.06, and 0.14 corresponding to small, medium, and large effects, respectively (Stevens 2007). Furthermore, for the dependent variables that demonstrated significant ANOVAs, Pearson product moment correlations (*r*) between the change scores were evaluated at each time point. An alpha level of $p \leq .05$ was used to determine statistical significance. These statistical procedures were performed using SPSS software (version 22.0, IBM SPSS Inc., Chicago, IL, USA). For a second analytical approach, data were interpreted on an individual subject basis. Specifically, the minimal difference needed to be considered real statistic was used to determine change scores from the pretest data that were above and beyond what could be expected due to testing error (Weir 2005). For data demonstrating significant changes for the training arm, univariate scatterplots illustrating the test score for each subject were designed using the Microsoft Excel spreadsheets provided by Weissgerber et al. (2015).

Results

Means and SDs for each arm have been shown in Table 2 for each of the study's dependent variables.

Delayed onset muscle soreness

All 13 subjects reported a complete absence of delayed onset muscle soreness for the training arm by circling zero on the Likert scale upon arriving to the laboratory.

Table 2 Mean \pm SD values for each dependent variable for the nine testing sessions

Variable	Arm	Pretest	Test #1	Test #2	Test #3	Test #4	Test #5	Test #6	Test #7	Test #8
Lean mass (g)	T	3071.0 \pm 452.5	3080.8 \pm 465.9	3079.7 \pm 470.1	3116.4 \pm 420.5	3158.6 \pm 427.7	3125.4 \pm 452.9	3168.8 \pm 452.9	3180.3 \pm 465.8*	3187.1 \pm 438.7*
	C	3091.1 \pm 579.9	3085.5 \pm 601.7	3077.2 \pm 578.2	3080.5 \pm 602.4	3089.3 \pm 557.6	3097.2 \pm 573.6	3112.4 \pm 590.4	3100.2 \pm 589.7	3101.8 \pm 614.6
Echo intensity (A.U.)	T	75.5 \pm 10.2	79.4 \pm 12.1	73.9 \pm 10.9	78.0 \pm 10.2	74.1 \pm 9.5	77.3 \pm 16.0	79.5 \pm 13.0	74.4 \pm 14.3	78.4 \pm 14.3
	C	74.2 \pm 11.5	76.9 \pm 12.6	76.9 \pm 9.5	75.6 \pm 9.1	76.2 \pm 12.1	73.5 \pm 8.3	78.6 \pm 16.9	76.9 \pm 17.0	76.2 \pm 13.3
Muscle thickness (cm)	T	3.12 \pm 0.39	3.16 \pm 0.38	3.20 \pm 0.36	3.21 \pm 0.37	3.21 \pm 0.38	3.27 \pm 0.39	3.35 \pm 0.36**†	3.43 \pm 0.36**†	3.39 \pm 0.35**†
	C	3.13 \pm 0.47	3.11 \pm 0.43	3.10 \pm 0.42	3.15 \pm 0.44	3.15 \pm 0.42	3.17 \pm 0.43	3.14 \pm 0.44	3.15 \pm 0.42	3.15 \pm 0.43
Flexed circumference (cm)	T	34.0 \pm 3.0	34.0 \pm 3.3	34.1 \pm 3.2	34.1 \pm 2.9	34.4 \pm 3.0	34.4 \pm 3.0	34.5 \pm 3.0*	34.7 \pm 3.0**†	34.6 \pm 3.1*
	C	34.3 \pm 3.4	33.9 \pm 3.6	34.0 \pm 3.4	34.1 \pm 3.5	34.0 \pm 3.3	34.0 \pm 3.3	33.7 \pm 3.7	34.0 \pm 3.5	33.9 \pm 3.4
Relaxed circumference (cm)	T	30.4 \pm 2.4	30.5 \pm 2.4	30.4 \pm 2.3	30.7 \pm 2.3	30.5 \pm 2.2	30.4 \pm 2.3	30.6 \pm 2.2	30.8 \pm 2.5	30.7 \pm 2.5
	C	30.8 \pm 3.0	30.3 \pm 2.7	30.5 \pm 2.6	30.5 \pm 2.7	30.2 \pm 2.5	30.4 \pm 3.0	30.1 \pm 2.5	30.1 \pm 2.7	30.2 \pm 2.6
Concentric peak torque (Nm)	T	52.1 \pm 9.0	55.0 \pm 8.5	53.8 \pm 7.9	54.6 \pm 7.4	55.5 \pm 8.5	53.7 \pm 8.7	55.6 \pm 10.6	54.2 \pm 8.0	56.2 \pm 7.9
	C	53.2 \pm 12.1	54.8 \pm 12.3	53.7 \pm 11.0	54.7 \pm 11.0	54.3 \pm 9.3	54.5 \pm 11.8	55.4 \pm 9.6	54.1 \pm 8.9	52.1 \pm 8.3

Data have been provided for both arms. As described within the “Methods” section, lean mass was assessed using DXA-based custom regions of interest that included the shoulder girdle, humerus, and trapezius, but not the chest or forearm. Subjective assessments of muscle soreness have not been displayed because the subjects reported a 0 at each time point

T training arm, C control arm

*Significantly greater than the pretest

†Significantly greater than the control arm

DXA lean mass

The results from the two-way repeated measures ANOVA indicated that there was a significant time \times arm interaction ($F=3.41, p=.012$). A repeated measures ANOVA for the training arm was statistically significant ($F=5.82, p<.001, \eta^2=0.326$), and the Bonferroni pairwise comparisons indicated that the mean lean mass values at test #7 (+109.3 g, $p=.002$) and test #8 (+116.1 g, $p=.035$) were significantly greater than that for the pretest. The mean percentage increase in lean mass at these time points was 3.5 and 3.8%, respectively. The repeated measures ANOVA for the control arm was not statistically significant ($F=0.39, p=.858, \eta^2=0.032$). The paired samples t tests for the training arm versus the control arm were not significant at any of the nine time points. As can be observed in Table 2, the mean differences between arms ranged from 20.1 to 85.3 g. Univariate scatterplots displaying individual subject change scores for the training arm have been displayed in Fig. 3, with data points above or below the horizontal line indicative of change that exceeded the minimal difference needed to be considered real (139.1 g). Eight out of the 13 subjects (61.5%) demonstrated an increase in the training arm that exceeded 139.1 g during at least one of the testing sessions. The number of subjects that showed increases above the minimal difference at each testing session was as follows:

test #1 (0), test #2 (1), test #3 (1), test #4 (4), test #5 (4), test #6 (7), test #7 (5), test #8 (6).

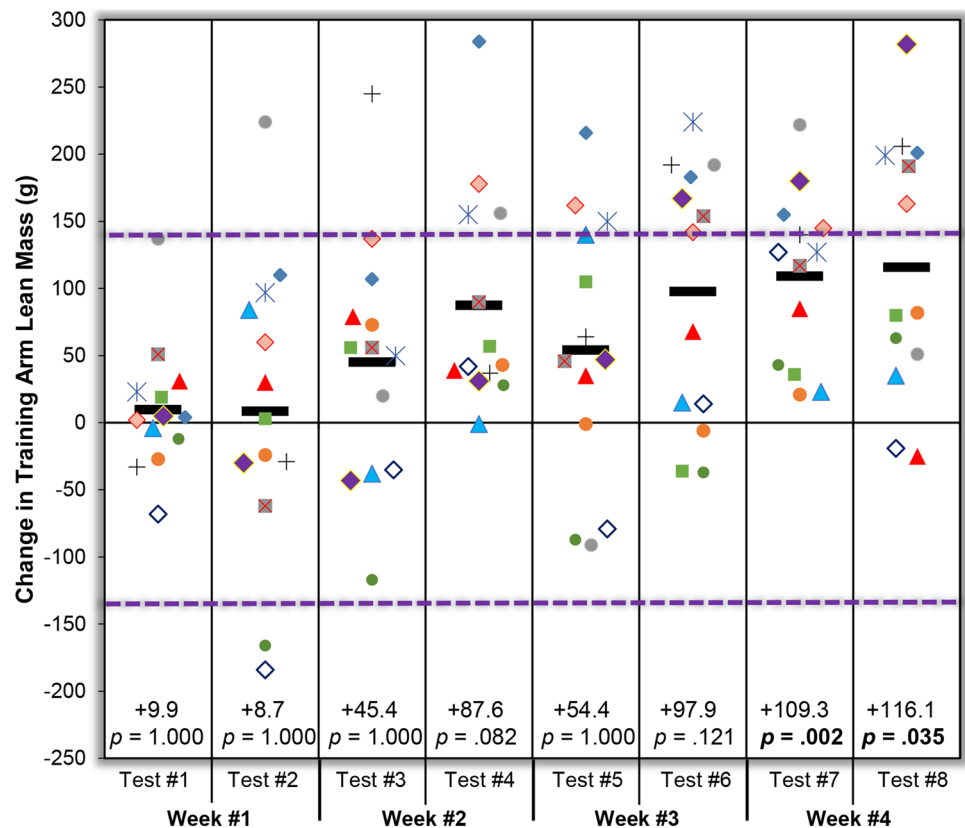
Biceps brachii echo intensity

The results from the two-way repeated measures ANOVA indicated that there was no time \times arm interaction ($F=0.62, p=.681$), as well as no main effects for time ($F=0.40, p=.763$) or arm ($F=0.18, p=.676$). Ten out of the 13 subjects (76.9%) demonstrated changes that exceeded the minimal difference needed to be considered real (16.7 A.U.) during at least one of the testing sessions, six of which were increases and four of which were decreases. The number of subjects that showed changes (increases/decreases) greater than the minimal difference at each testing session was as follows: test #1 (1/0), test #2 (0/1), test #3 (0/0), test #4 (0/0), test #5 (3/1), test #6 (1/0), test #7 (1/2), test #8 (0/0).

Muscle thickness

The results from the two-way repeated measures ANOVA indicated that there was a significant time \times arm interaction ($F=11.48, p<.001$). A repeated measures ANOVA for the training arm was statistically significant ($F=30.52, p<.001, \eta^2=0.718$), and the Bonferroni pairwise comparisons indicated that the mean muscle

Fig. 3 Lean mass (g) univariate scatterplots displaying individual subject change scores (versus the pretest) at each testing interval. The formatting of each subject's data points has been displayed consistently over time. Values above or below the purple line correspond to changes that exceed the minimal difference needed to be considered real (139.1 g). The thick black line corresponds to the mean value at that time point. The numerical values at the bottom of the graph display the mean difference (g) at each time point (versus the pretest), as well as the p value from the corresponding Bonferonni pairwise comparison. Means and SDs for the control arm have been displayed in Table 2



thickness values at test #6 (+0.23 cm, $p = .004$), test #7 (+0.31 cm, $p < .001$), and test #8 (+0.27 cm, $p < .001$) were significantly greater than that for the pretest. The mean percentage increase in muscle thickness at these time points was 7.4, 9.9, and 8.7%, respectively. The repeated measures ANOVA for the control arm were not statistically significant ($F = 0.81$, $p = .503$, $\eta^2 = 0.063$). As displayed in Table 2, paired samples t tests comparing muscle thickness between arms demonstrated a significant mean difference at test #6 ($p = .041$), test #7 ($p = .006$), and test #8 ($p = .011$). Univariate scatterplots displaying individual subject change scores for the training arm have been displayed in Fig. 4, with data points above the horizontal line indicative of change that exceeded the minimal difference needed to be considered real (0.24 cm). Ten out of the 13 subjects (76.9%) demonstrated an increase in the training arm that exceeded 0.24 cm during at least one of the testing sessions. The number of subjects that showed increases above the minimal difference at each testing session was as follows: test #1 (0), test #2 (1), test #3 (1), test #4 (0), test #5 (4), test #6 (3), test #7 (10), test #8 (9).

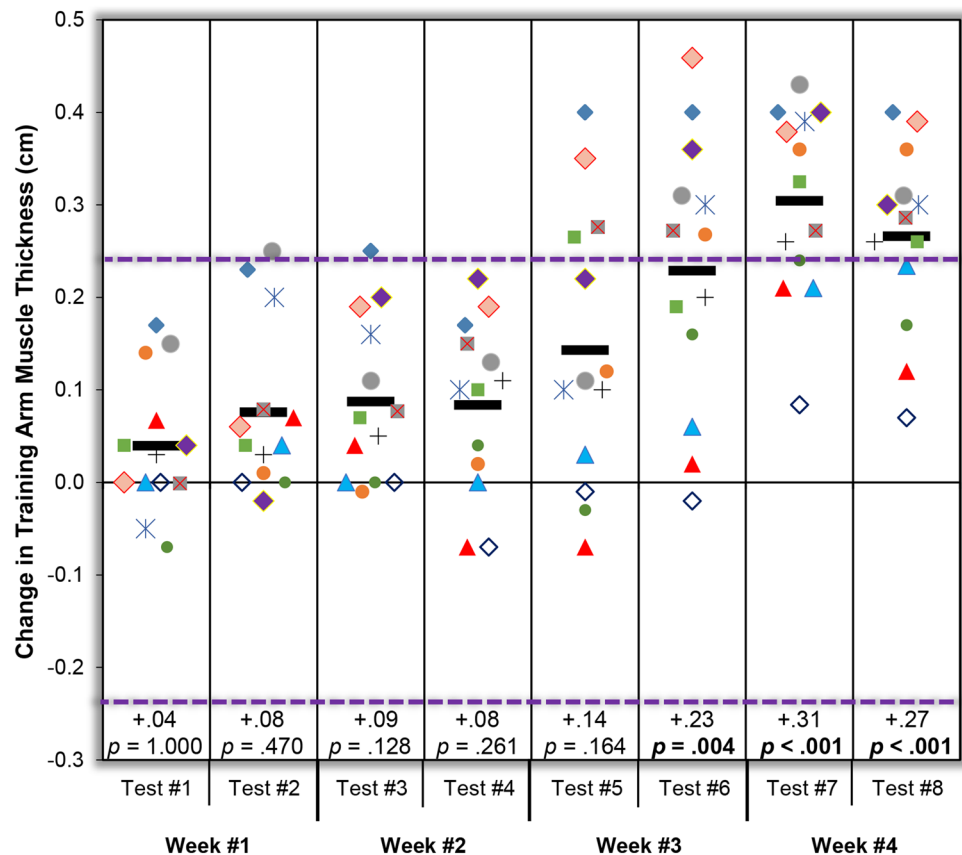
Relaxed arm circumference

The results from the two-way repeated measures ANOVA indicated that there was no time \times arm interaction ($F = 3.41$, $p = .054$), as well as no main effects for time ($F = 0.61$, $p = .660$) or arm ($F = 0.804$, $p = .388$). Five out of the 13 subjects demonstrated an increase in the training arm that exceeded the minimal difference of 1.0 cm during at least one of the testing sessions. The number of subjects that showed increases above the minimal difference at each testing session was as follows: test #1 (1), test #2 (0), test #3 (0), test #4 (1), test #5 (0), test #6 (3), test #7 (1), test #8 (3).

Flexed arm circumference

The results from the two-way repeated measures ANOVA indicated that there was a significant time \times arm interaction ($F = 8.99$, $p < .001$). A repeated measures ANOVA for the training arm was statistically significant ($F = 10.74$, $p < .001$, $\eta^2 = 0.472$), and the Bonferroni pairwise comparisons indicated that the mean flexed arm circumference values at test #6 (+0.52 cm, $p = .031$), test #7 (+0.67 cm, $p = .004$), and test #8 (+0.60 cm, $p = .047$) were significantly greater than that for the pretest. The mean percentage

Fig. 4 Muscle thickness (cm) univariate scatterplots displaying individual subject change scores (versus the pretest) at each testing interval. The formatting of each subject's data points has been displayed consistently over time. Values above the purple line correspond to changes that exceed the minimal difference needed to be considered real (0.24 cm). The thick black line corresponds to the mean value at that time point. The numerical values at the bottom of the graph display the mean difference (cm) at each time point (versus the pretest), as well as the p value from the corresponding Bonferroni pairwise comparison. Means and SDs for the control arm have been displayed in Table 2



increase in flexed arm circumference at these time points was 1.5, 2.1, and 1.8%, respectively. The repeated measures ANOVA for the control arm was not statistically significant ($F=0.71, p=.576, \eta^2 = 0.056$). Paired samples t tests comparing flexed arm circumference between arms at each time point demonstrated a significant mean difference at test #7 ($P=.039$). As displayed in Table 2, the mean differences between arms ranged from 0.06 to 0.66 cm. Univariate scatterplots displaying individual subject change scores for the training arm have been displayed in Fig. 5, with data points above or below the horizontal line indicative of change that exceeded the minimal difference (0.6 cm). Ten out of the 13 subjects (76.9%) demonstrated an increase that exceeded 0.6 cm during at least one of the testing sessions. The number of subjects that showed increases greater than the minimal difference at each testing session was as follows: test #1 (0), test #2 (2), test #3 (1), test #4 (3), test #5 (3), test #6 (5), test #7 (8), test #8 (5).

Concentric peak torque

The results from the two-way repeated measures ANOVA indicated that there was no time \times arm interaction ($F=1.21, p=.409$), as well as no main effects for time ($F=1.16, p=.338$) or arm ($F=0.01, p=.981$). For the training arm, only one out of the 13 subjects (7.7%) showed an increase

in peak torque that exceeded the minimal difference of 13.9 N m. The number of subjects that showed changes (increases/decreases) greater than the minimal difference at each testing session was as follows: test #1 (1/0), test #2 (1/0), test #3 (1/0), test #4 (1/0), test #5 (3/0), test #6 (1/1), test #7 (0/0), test #8 (1/0).

Changes in training load as a measure of strength increase

Both exercises exhibited a significant increase in training volume throughout the study (biceps curls $F=28.89, p<.001, \eta^2 = 0.707$; shoulder press $F=15.48, p<.001, \eta^2 = 0.563$). The increase in volume was largely brought about by the use of heavier loads, and the repeated measures ANOVA for the mean training load used during each training session was significant for both exercises [Fig. 6 (biceps curls $F=113.86, p<.001, \eta^2 = 0.905$; shoulder press $F=38.38, p<.001, \eta^2 = 0.762$)]. During the initial set of the final training session, seven subjects were able to train with an external load greater than their original 1RM for at least ten repetitions for both exercises. Another five subjects trained with loads greater than their original 1RM for the biceps curl exercise, but not the press. Only one subject was unable to use his original 1RM loads in training for both exercises, though

Fig. 5 Flexed arm circumference (cm) univariate scatterplots displaying individual subject change scores (versus the pretest) at each testing interval. The formatting of each subject's data points has been displayed consistently over time. Values above or below the purple line correspond to changes that exceed the minimal difference needed to be considered real (0.6 cm). The thick black line corresponds to the mean value at that time point. The numerical values at the bottom of the graph display the mean difference (cm) at each time point (versus the pretest), as well as the p value from the corresponding Bonferonni pairwise comparison. It is important to note that the highest level of resolution of each circumference measurement was in millimeters. Means and SDs for the control arm have been displayed in Table 2

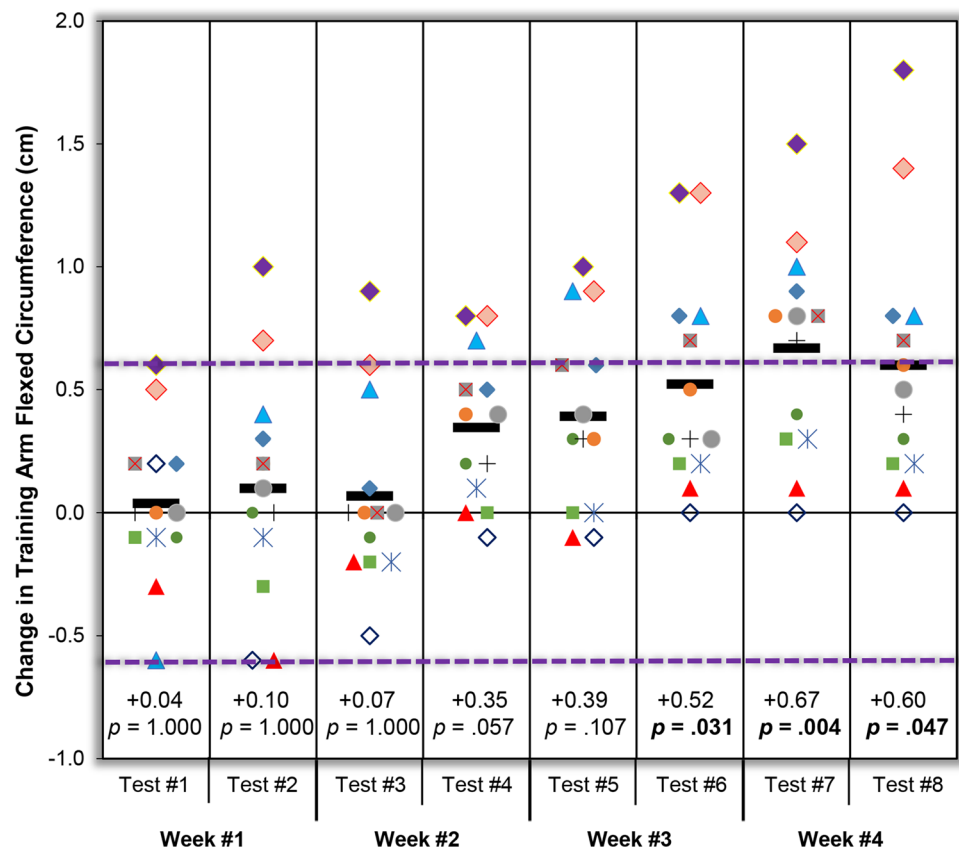
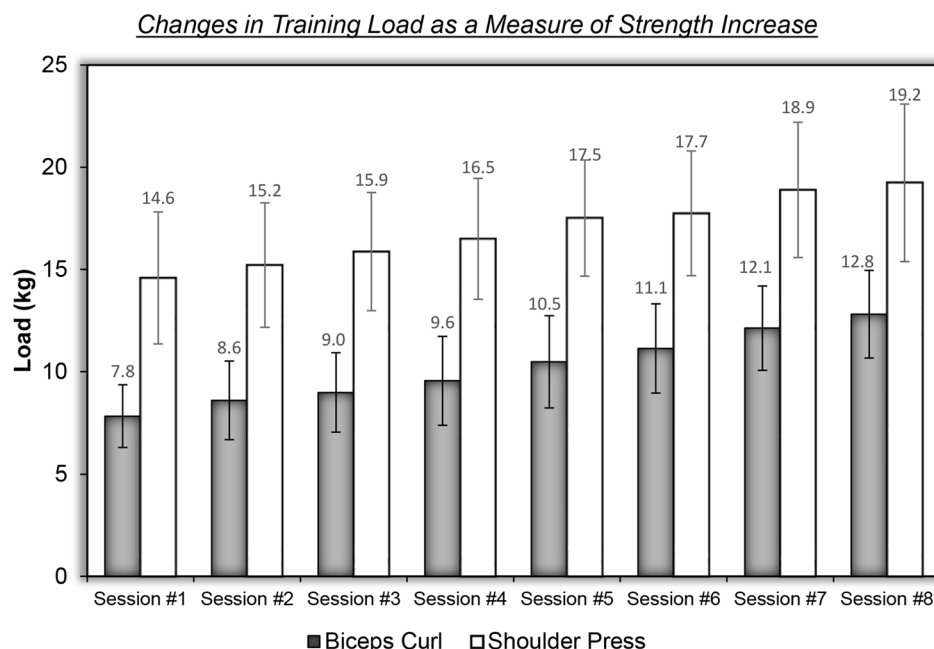


Fig. 6 Mean \pm SD training loads (kg) for each exercise during the eight concentric-only training sessions. As each training session's load was modified on a set-by-set basis to keep the number of repetitions between 8 and 12, the value represents the mean load used across all sets. For both exercises, the repeated measures ANOVA were significant ($p < .001$). The Bonferroni pairwise comparisons have been displayed below the graph. Many of the subjects were able to use their original 1RM as a submaximal training load during the final training session



Biceps Curl Load Marginal Mean Pairwise Comparisons

Session #1 < Sessions #3-8; Session #2 < Sessions #4-8; Session #3 < Sessions #5-8; Session #4 < Sessions #5-8; Session #5 < Sessions #6-8; Session #6 < Sessions #7-8; Session #7 = Session #8

Shoulder Press Load Marginal Mean Pairwise Comparisons

Session #1 < Sessions #4-8; Session #2 < Sessions #4-8; Session #3 < Sessions #5-8; Session #4 < Sessions #5, 7-8; Session #5 < Sessions #7-8; Session #6 < Sessions #7-8; Session #7 = Session #8

he came close (original 1RM curl = 13.6 kg, final training load = 12.3 kg for eight repetitions; original 1RM press = 22.7 kg, final training load = 21.4 kg for nine repetitions). The ability to add progressively more weight to the training loads during each training session demonstrates an alternative means of quantifying the rapid strength increases observed in this study.

Correlations among change scores

As significant mean increases were observed for lean mass, muscle thickness, and flexed arm circumference, Pearson product moment correlations (r) between their change scores were further evaluated at each of the eight testing sessions following the pretest. As can be inferred from Table 3, the correlations tended to be stronger for the muscle thickness analyses. In addition, stronger correlations were observed during the latter stages of the study, possibly

Table 3 Pearson r values for the correlations between change scores for the dependent variables demonstrating significant mean increases

	Test #1	Test #2	Test #3	Test #4	Test #5	Test #6	Test #7	Test #8
Muscle thickness change score (cm)								
Lean mass change score (g)	0.322	0.785*	0.265	0.530	0.615*	0.599*	0.417	0.661*
Flexed Arm Circumference Change Score (cm)								
Lean mass change score (g)	-0.073	0.408	-0.032	0.209	0.345	0.297	0.311	0.635*
Muscle thickness change score (cm)								
Flexed Arm Circumference Change Score (cm)	0.092	-0.046	0.465	0.630*	0.504*	0.666*	0.573*	0.597*

Each cell displays the r value corresponding to the changes versus the pretest value for the 13 subjects. Data are from the training arm only

* $p < .05$

due to more subjects showing changes, thereby increasing data resolution. Nonetheless, some of the results showed weak associations between change scores of the significant dependent variables.

Dietary analyses

The 5-day food log analyses indicated that the mean \pm SD number of calories consumed per day was 2223 ± 774 , with values ranging from 582 to 3763. The mean \pm SD (range) grams of protein consumed each day was 95 ± 42 (17–202). The mean \pm SD grams of carbohydrate and fat consumed each day was 254 ± 98 (86–491) and 91 ± 45 (20–215), respectively.

Discussion

Previous authors that have examined short-term hypertrophic adaptations have included eccentric muscle actions as part of their study's training program (DeFreitas et al. 2011; Seynnes et al. 2007; Stock et al. 2016; among others). Since eccentric muscle actions produce considerably greater muscle damage than concentric muscle actions (Friden et al. 1986; Gibala et al. 1995; McCully and Faulkner 1985), it is conceivable that previous measurements of muscle size may have been influenced by edema-induced muscle swelling, particularly in investigations with frequent testing sessions (DeFreitas et al. 2011; Krentz and Farthing 2010; Seynnes et al. 2007; Stock et al. 2016). Thus, the purpose of this study was to examine the precise time course of short-term muscle hypertrophy during a training program specifically designed to avoid eccentric muscle damage. Our findings demonstrated significant increases in lean mass, muscle thickness, and flexed arm circumference within seven training sessions, but no evidence of training-induced muscle damage was observed (at least at the group mean level). It is important to note, however, that because assessments were not performed 24 or 48 h following each training session, it is conceivable that muscle damage occurred and recovered, or was undetectable, at the time of testing. Thus, we caution that the results of the present study are only applicable to the testing intervals utilized and the measures employed.

Recent work by Damas et al. (2016) led to the conclusion that increases in short-term muscle cross-sectional area were concomitant with edema. Indeed, we concede that many measures that are used to assess muscle size (such as relaxed arm circumference, muscle thickness, cross-sectional area, and muscle volume) are likely to be influenced by edema following eccentric exercise. To overcome these inadequacies, it has been proposed that echo intensity of ultrasonography images represents a valid measure of

edema following exercise-induced muscle damage (Nosaka and Sakamoto 2001). Like other measures, echo intensity has been shown to be affected by training, though it typically increases with muscle damage (Nosaka and Sakamoto 2001) and decreases as a result of improved muscle quality (Wilhelm et al. 2014). In the present study, biceps brachii echo intensity showed no significant increases as a result of concentric-only training, and this was in line with the results for relaxed arm circumference and concentric peak torque. Assuming that echo intensity is the most appropriate non-invasive assessment tool for examining exercise-induced muscle damage, the major difference between the present study and the work of Damas et al. (2016) is the lack of an increase in this variable following concentric-only training.

A novel aspect of the present investigation was the frequency of testing, which allowed for the analysis of adaptations following individual training sessions, rather than weeks or months. Studies that have examined the short-term hypertrophic and/or neuromuscular adaptations to training have typically performed testing every one (DeFreitas et al. 2011; Lixandrao et al. 2016; Stock et al. 2016), two (Abe et al. 2000; Jenkins et al. 2015a; Moritani and deVries 1979), or four (Boone et al. 2015) weeks. In addition, longer duration studies that have performed frequent testing have reported adaptations following 4 weeks of training (Baroni et al. 2013; Staron et al. 1994). Other important studies have utilized varying intervals (Damas et al. 2016; Seynnes et al. 2007). Since the signs and symptoms of muscle damage are greatest following the initial bout of injury and then subside during additional bouts (McHugh 2003), data collection performed within the first week of training seems particularly important. Interestingly, when eccentric exercise is performed frequently, symptoms of muscle damage persist. In a study involving maximal eccentric exercise every other day for 20 days, Krentz and Farthing (2010) reported a prolonged decrease in strength but increased muscle thickness, the latter of which was attributed to edema. The fact that strength may not fully recover following frequent eccentric exercise (Krentz and Farthing 2010), but peak torque was unaffected in the present study, provides further support for the notion that the concentric-only training protocol did not induce muscle damage. An additional advantage of the frequency of testing used in the present study was that it allowed for a better understanding of precisely when given adaptations occurred. For example, Damas et al. (2016) reported increased echo intensity relative to cross-sectional area following 3 weeks of training. The next testing session, which revealed decreased echo intensity and increased cross-sectional area, was not performed for another 7 weeks. Thus, from weeks three through ten, it is unclear precisely when these changes took place. This appears to be an important

issue because in the present investigation, the first significant increase in lean mass (still with no elevation in echo intensity) was observed following seven training sessions, which was just after the 3-week measurements employed in previous studies (Damas et al. 2016; DeFreitas et al. 2011; Seynnes et al. 2007).

One of the perplexing observations of the present study was that concentric isokinetic peak torque was unaffected by the training program. The fact that concentric peak torque did not increase was surprising given that studies have generally shown increases in muscular strength within 4 weeks of training (Abe et al. 2000; Boone et al. 2015; Moritani and deVries 1979; Seynnes et al. 2007). We propose two interrelated explanations for the fact that isokinetic peak torque did not improve. First, the training program used in this study was consistent with the recommendations for increasing muscle size (but not strength) put forth by the National Strength and Conditioning Association (Baechle and Earle 2008). Had heavier external loads and longer rest periods been utilized, it is possible that the training arm would have demonstrated greater increases in concentric peak torque. A second and more likely explanation was the study's lack of training (dumbbell) versus testing (isokinetic) specificity. The issue of task specificity in measuring changes in muscular strength was first well exemplified by the work of Rutherford and Jones (1986). In a series of experiments, these authors (Rutherford and Jones 1986) reported nearly a 200% increase in the training load after 12 weeks, but there was only a 15–20% increase in isometric force. Rutherford and Jones (1986) speculated that learning and coordination played a significant role in the movement specificity of training adaptations. Furthermore, the mean training volume nearly doubled for both exercises as a result of linearly increasing the external loads during each training session, and most subjects were able to use their original 1RM as a training load by the final training session. This demonstrates that muscular strength did increase as a result of training, but the ability to detect such a change is dependent on factors inherent to testing specificity.

Another aspect that makes the present study unique is our reliance not only null hypothesis significance testing, but application of the minimal difference needed to be considered real statistic, which was originally emphasized by Weir (2005). As its name implies, this statistic is designed to be used to infer whether a change is large enough to be considered above and beyond a measurement's testing error. Unlike common test–retest reliability measures with limited practicality, such as the intraclass correlation coefficient and the coefficient of variation, the minimal difference statistic can be directly applied to an individual subject's change score. Of particular concern is the fact that researchers can observe significant group mean differences,

but have only a small number of subjects (or none) exceed the minimal difference needed to be considered real. Stock and Thompson (2014) reported significant increases in peak torque in men following 10 weeks of barbell deadlift training, but only two out of 17 subjects (11.7%) showed changes that exceeded the minimal difference. This illustrates not only the complexity of data interpretation, but also the importance of making inferences based on a given laboratory's testing error.

There are several additional details that require further discussion. First, this study's training program was designed to promote improvements in muscle size and strength for the musculature of the upper arm and shoulder. To examine changes in lean mass, custom regions of interest were created following the DXA scans to ensure that the data reflected all of the muscle tissue activated during training. In contrast, the echo intensity, muscle thickness, arm circumference, and peak torque measurements did not involve the elbow extensors or the shoulder musculature, so it is not entirely clear if the degree of muscle damage differed as a result of the dumbbell curls versus shoulder presses. It is important to note, however, that the Likert scale was designed to inquire about the level of soreness for the muscles surrounding both joints, and all of the subjects circled a 0 for the duration of the study. Although the use of Likert scales following eccentric exercise has inherent limitations (Nosaka et al. 2002), the fact that none of the subjects noted even a small amount of delayed onset muscle soreness suggests that additional measures of muscle damage aimed at the elbow extensors and shoulder musculature may have provided similar results as those obtained for the biceps brachii. Furthermore, Warren and Palubinskas (2008) reported significantly greater strength loss following eccentric exercise for the elbow flexors versus the leg extensors despite a lower number of muscle actions, which is in agreement with injury susceptibility data reported by Jamurtas et al. (2005). These findings would suggest that had muscle damage occurred in the present study, the highly susceptibility elbow flexor muscle group would have likely been affected. A second issue that is inherent to many training studies is that dietary controls are difficult to implement, and underreporting has been reported in investigations that have examined the validity of food logs (Hutchesson et al. 2015). In the present study, the food log results showed that nutrient consumption varied widely, with several subjects reporting protein intake far too low to optimize muscle protein synthesis (e.g., one subject reported consuming 17 grams of protein for one of his food log days). It is possible that dissimilarities in the subjects' diets may have contributed to the variability in the responsiveness to training. Similarly, as many of the subjects were undergraduate

students with little interest in exercise or nutrition and the study occurred during the latter portion of an academic semester, changes in emotional stress, dietary patterns, and sleep habits may have contributed to the degree of within-subject variance for some of the dependent variables. Third, the echo intensity analyses performed in this study included the biceps brachii, but not the brachialis. This approach was taken to remain consistent with the methods described previously by Jenkins et al. (2015b). Although we speculate that inclusion of the brachialis in the echo intensity analyses would not have affected the present study's findings, this hypothesis currently remains untested. Finally, as can be inferred from Figs. 3, 4 and 5, a moderate degree of variability in the subjects' responsiveness to training was observed. Although the variability in these responses may seem substantial, the results from other studies have indicated that this may be omnipresent in resistance training data (Bamman et al. 2007). Previous work concerning the topic of adaptations to short-term training from our laboratory has shown the presence of "responders" and "non-responders," with one female subject demonstrating a 2.9kg increase in gynoid+leg lean mass in response to six training sessions (Stock et al. 2016). For readers that are skeptical of the degree of within-subject variability displayed in Figs. 3, 4 and 5, attention should be focused only on the data points above the horizontal lines.

In summary, the results of the present study showed significant increases in lean mass, muscle thickness, and flexed arm circumference within seven bouts of concentric-only training, yet no changes in echo intensity, relaxed arm circumference, muscle soreness, and peak torque were observed. Although testing was not performed 24 or 48 h following each training bout, these data have provided evidence that detectable short-term muscle hypertrophy might occur in the absence of eccentric muscle damage. This conclusion is not only based on mean differences, but also by examining data points that exceeded the minimal difference needed to be considered real for each dependent variable. Using this approach, it was determined that at least eight subjects showed changes that should be considered meaningful. It is important to note that the present study's observed changes could be considered small, and provide further support for the notion that large increases in skeletal muscle mass may take many months or years of dedicated training. As replication studies are critical to the advancement of science, we hope that investigators will perform experiments with similar methodology in the future.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

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