Whey Protein Does Not Enhance the Adaptations to Elbow Flexor Resistance Training

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ABSTRACT

ERSKINE, R. M., G. FLETCHER, B. HANSON, and J. P. FOLLAND. Whey Protein Does Not Enhance the Adaptations to Elbow Flexor Resistance Training. *Med. Sci. Sports Exerc.*, Vol. 44, No. 9, pp. 1791–1800, 2012. **Purpose**: It is unclear whether protein supplementation augments the gains in muscle strength and size observed after resistance training (RT) because limitations to previous studies include small cohorts, imprecise measures of muscle size and strength, and no control of prior exercise or habitual protein intake. We aimed to determine whether whey protein supplementation affected RT-induced changes in elbow flexor muscle strength and size. **Methods**: We pair-matched 33 previously untrained, healthy young men for their habitual protein intake and strength response to 3-wk RT without nutritional supplementation (followed by 6 wk of no training) and then randomly assigned them to protein (PRO, $n = 17$) or placebo (PLA, $n = 16$) groups. Participants subsequently performed elbow flexor RT 3 $d \cdot wk^{-1}$ for 12 wk and consumed PRO or PLA immediately before and after each training session. We assessed elbow flexor muscle strength (unilateral 1-repetition maximum and isometric maximum voluntary force) and size (total volume and maximum anatomical cross-sectional area determined with magnetic resonance imaging) before and after the 12-wk RT. **Results**: PRO and PLA demonstrated similar increases in muscle volume (PRO 17.0% ± 7.1% vs PLA 14.9% ± 4.6%, $P = 0.32$), anatomical cross-sectional area (PRO 16.2% ± 7.1% vs PLA 15.6% ± 4.4%, $P = 0.80$), 1-repetition maximum (PRO 41.8% ± 21.2% vs PLA 41.4% ± 19.9%, $P = 0.97$), and maximum voluntary force (PRO 12.0% ± 9.9% vs PLA 14.5% ± 8.3%, $P = 0.43$). **Conclusions**: In the context of this study, protein supplementation did not augment elbow flexor muscle strength and size changes that occurred after 12 wk of RT. **Key Words:** PROTEIN SUPPLEMENTATION, STRENGTH TRAINING, MUSCLE HYPERTROPHY, MUSCLE ARCHITECTURE, TRAINING RESPONSE

Both resistance exercise (4) and protein ingestion (31) are known to stimulate muscle protein synthesis (MPS), which is necessary for the accretion of skeletal muscle mass. Moreover, combining protein or amino acid ingestion with an acute bout of resistance exercise has been shown to further augment MPS (32). Based on these acute studies, it is surprising that the evidence for protein supplementation (PRO) enhancing the gains in muscle size and strength after longer-term resistance training (RT) programs in young men remains equivocal (17,24).

It has been suggested that the muscle strength (9,40) and size (1,17) responses to RT in young men may be amplified by PRO, although these effects are often marginal (20). In contrast, other studies in young men have shown no effect of PRO on gains in muscle size (8,24) or strength (1,24). Greater increases in muscle fiber area (1,17) and myofibrillar protein content (40) have been observed when RT was combined with PRO rather than CHO. However, none of these studies included detailed measurements of whole muscle size.

The apparent discrepancy between the acute studies of a single training bout and the longer-term RT studies may be compounded by a range of methodological issues with the latter. First, the individual response to longer-term RT is known to vary widely between individuals (13,19), yet numerous studies have used small participant groups (16,20,40) that may not have been powered to detect an influence of PRO. The effect of interindividual variability might be further reduced by greater experimental control of prior physical activity and habitual protein intake. Second, some studies have used crude measures of muscle hypertrophy, such as dual-energy x-ray absorptiometry to assess whole-body FFM (17) or muscle thickness determined with ultrasonography (7,35). Third, in the context of nutritional supplementation, no study has attempted to minimize or quantify the neural changes that occur with RT, which, together with muscle hypertrophy, are considered the major contributors to strength improvements (15). Thus, large and

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variable neural improvements could have confounded the potential influence of PRO on training-induced changes in muscle size and strength in previous studies. Finally, the resistance exercise model that has been most commonly researched has involved lower limb training (1,8,34) despite the upper limb musculature showing greater adaptive responses to RT (10,37). Therefore, an elbow flexor exercise model may offer a better chance of discriminating an influence of PRO on muscle hypertrophy and strength changes after RT.

Considering all these factors, we aimed to compare the training-induced changes in elbow flexor muscle size, architecture, and strength between two groups of previously untrained young men supplemented with either protein or placebo. We hypothesized that 20 g (26) of whey protein (31) ingested immediately before (33) and after (14) each training session would confer greater changes in muscle size and strength after 12 wk of RT compared with RT alone.

METHODS

Participants

Thirty-three healthy young men (mean ± SD: age = 23 ± 3 yr, height = 1.76 ± 0.06 m, body mass = 75.2 ± 10.7 kg) provided written informed consent before completing this 25-wk study, which complied with the Declaration of Helsinki and was approved by the Ethical Advisory Committee of Loughborough University. Volunteers were excluded from taking part in the study if they were vegans, had unusually high (>2 $g \cdot kg^{-1} \cdot d^{-1}$) or low (<0.8 $g \cdot kg^{-1} \cdot d^{-1}$) protein intake (see next paragraphs), reported use of potentially anabolic supplements in the previous 6 months or were taking any medication considered to influence muscle size or function, had a history of upper body exercise in the previous 12 months, or were <18 or >30 yr old.

Study Overview

The study was a single-center double-blind design. Participants completed 3-wk elbow flexor RT without nutritional supplementation, 6 wk of no training, and then 12-wk elbow flexor RT with nutritional supplementation (Fig. 1A). The 3-wk RT period was conducted to standardize training status before the 12-wk RT and to overcome neural adaptations that occur within the first few weeks of RT (27). The 6 wk of no training provided a clear break between the training periods to improve participant retention, and it has been shown to result in only a modest detraining effect (23). During the 6-wk no RT period, participants were pair-matched for their isometric strength response to the 3-wk RT and their normal protein intake (Table 1) and randomly assigned to protein (PRO, $n = 17$) or placebo (PLA, $n = 16$) supplementation groups. The groups had similar age, elbow flexor muscle strength and size, anthropometric, physical activity, and nutritional characteristics (Table 1). Participants then completed 12 wk of RT, during which they received PRO or PLA supplementation. Measurements of the dominant arm were performed before and 3–4 d after the 12-wk RT in the following order: muscle architecture (assessed with ultrasonography), dynamic and isometric strength (agonist and antagonist muscle activation was determined with sEMG), and muscle size were assessed with magnetic resonance imaging (MRI) at least 24 h after strength testing to ensure that measurements were not influenced by exercise-induced fluid shifts. All testing took place between 9:00 a.m. and 6:00 p.m., and for each participant, tests were performed at the same time of the day before and after training. Participants were instructed not to participate in strenuous physical activity, not to consume alcohol or excessive amounts of caffeine in the 24 h before measurement sessions, and to maintain their habitual diet and lifestyle throughout the study.

Resistance Training

During both RT periods, participants performed three training sessions per week (Monday, Wednesday, and Friday). Each session commenced with unilateral bicep curls using dumbbells and a modified preacher bench (Body Solid, Forest Park, IL), and sets were performed alternately with

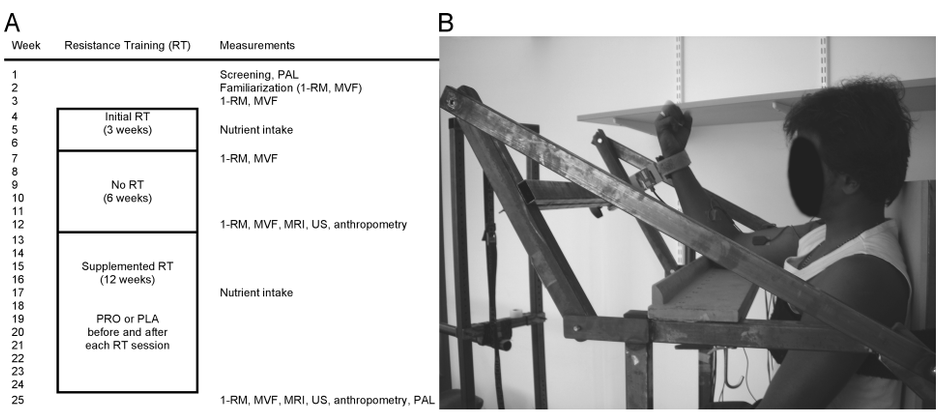

FIGURE 1—A. Overview of the study intervention periods and measurements: 1RM, 1-repetition maximum; MVF, isometric maximum voluntary force (including sEMG); MRI, magnetic resonance imaging to assess muscle size; US, ultrasound measurements of muscle architecture; anthropometry, skinfold measurements, body mass, and height; Nutrient intake, food and drink intake weighed and recorded over 3 d; PAL, physical activity level assessed via questionnaire. B. Isometric strength testing apparatus used to measure elbow flexion and extension MVF.

TABLE 1. Characteristics of the PRO and PLA groups before the 12-wk supplemented RT period.

Variable	PLA (n = 16)	PRO (n = 17)	t-test, P
Age (yr)	23.7 ± 2.9	23.1 ± 3.0	0.59
Body mass (kg)	75.6 ± 11.4	74.9 ± 10.3	0.87
Height (cm)	177.0 ± 6.5	175.9 ± 6.5	0.65
% body fat	22.1 ± 6.2	22.5 ± 5.1	0.85
Habitual physical activity rating	2.62 ± 0.44	2.50 ± 0.34	0.37
Energy intake ($kcal \cdot d^{-1}$)	2515 ± 706	2482 ± 581	0.89
Protein intake ($g \cdot d^{-1}$)	100.7 ± 21.4	95.3 ± 26.4	0.39
Protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)	1.36 ± 0.35	1.28 ± 0.37	0.41
Isometric MVF (N)	271.2 ± 43.0	253.8 ± 41.1	0.24
1RM (kg)	13.3 ± 3.2	12.3 ± 2.7	0.37
Isometric strength response to the initial 3-wk RT period (%)	+5.6 ± 7.5	+4.9 ± 12.9	0.85
Elbow flexor muscle volume (cm^3)	413.4 ± 73.1	388.3 ± 61.9	0.29
Elbow flexor muscle $\sum ACSA_{max}$ (cm^2)	28.5 ± 3.9	26.9 ± 4.3	0.29

Data are means ± SD, and independent t-test P values are displayed.

each arm. Subsequently, bilateral bicep curls were performed on an RT machine (Pro Club Line Bicep Curl; Body Solid). The loading for both exercises was an 8—10 reps maximum, and the load was increased when participants could lift 10 reps during the final set of an exercise. The 3-wk RT involved two sets of each exercise, i.e., two sets for each unilateral arm exercise; two sets bilateral, with a 2-min rest between each set. This was the same for weeks 1–2 of the 12-wk RT but increased to three sets (unilateral) and two sets (bilateral) during weeks 3–4 and three sets of both exercises for weeks 5–12. Apart from the supplementation provided, participants were instructed to consume only water in the 2 h before and 1.5 h after each training session, and to facilitate this, training sessions took place either midmorning (10:00 to 11:00) or midafternoon (2:30 to 4:30 p.m.). Furthermore, every participant completed all 36 training sessions.

Supplementation

Participants were given their supplementation in a double-blind manner in the form of an opaque drink bottle containing 250 mL of fluid, which was fully consumed immediately before, and another immediately after, each RT session. The PRO supplement comprised water mixed with 30 g of powder that contained 20 g of whey protein (~50% essential amino acids), and 6.7 g of lactose together with flavoring and sweeteners. Thus, the total protein supplementation on training days was 40 $g \cdot d^{-1}$, and when averaged across training and nontraining days, the PRO supplement increased protein intake by 17.1 $g \cdot d^{-1}$, CHO intake by 5.7 $g \cdot d^{-1}$, and energy intake by 91.2 $kcal \cdot d^{-1}$. Each PLA supplement contained 6.8 g of lactose and, when averaged during training and nontraining days, increased CHO intake by 5.8 $g \cdot d^{-1}$ and energy intake by 23.2 $kcal \cdot d^{-1}$.

Neuromuscular Measurements and Protocol

Muscle architecture. Fascicle pennation angle (Θ_p, the angle between the fascicular paths and their insertion into the deep aponeurosis) of the biceps brachii short head (BBS) and brachialis (BRACH) muscles was examined using B-mode ultrasonography (SSA-37OA Power Vision 6000; Toshiba, Otawara-Shi, Japan) with an 8-MHz linear-array transducer. Participants lay supine with the dominant elbow extended and the shoulder abducted by 90°. Strips of ultrasound-absorbent tape (2 mm wide; 3M, Neuss, Germany) were placed perpendicular to the long axis of the BBS at 50-mm intervals from the cubital crease to the shoulder, which formed markers on the sonographs and enabled Θ_p to be analyzed at the same location before and after RT. A midline between the medial and lateral boundaries of the BBS was marked along the length of the muscle. The probe (coated with gel) was applied to the cubital crease with minimal pressure and was carefully glided along this line to the proximal end of the BBS (in line with the direction of the muscle fascicles). Sonographs were analyzed offline using a public-domain software package (ImageJ; National Institutes of Health, Bethesda, MD). Fascicle Θ_p was determined in three BBS fascicles within 50 mm of its distal end and in three BRACH fascicles within 50 mm of its proximal end. The mean of the three measurements determined Θ_p for each muscle, and the test–retest coefficient of variation (CV) for this assessment was 2.9%.

Unilateral 1-repetition maximum. Unilateral 1-repetition maximum (1RM) bicep curl lifting strength was assessed with a series of incremental dumbbell lifts using the modified preacher bench that was used in training. The bench was customized with a horizontal rack at full elbow extension, which provided a consistent starting position for the 1RM lift. The height of the padded arm support was adjusted to arm length, ensuring the elbow was fully extended when the hand gripped the dumbbell on the rack. The warm-up comprised 10 reps at 40% of the previous 1RM, and after a 1-min rest, 3 reps were performed at 80% of the previous 1RM. Thereafter, a series of single lifts (concluding at full flexion, at which point the investigator removed the dumbbell from the participant) were performed interspersed with 1-min rest intervals, first at the previous 1RM and then at increments of +0.5 kg if the preceding lift was successful. The 1RM was generally determined within three to five attempts, although more attempts were completed if necessary. The test–retest CV for this assessment was 3.5%.

Isometric maximum voluntary force. Elbow flexor isometric strength was measured using a custom-built strength-testing chair (Fig. 1B) and the elbow joint angle fixed at 120° (180° = full elbow extension). The participant sat upright (hip joint angle of 90°) and was strapped at the hip and chest to the seat and back of the chair to prevent movement of the body. The shoulder joint was flexed to 90° with the upper arm placed on a horizontal board and externally rotated with the elbow position maintained by blocks anterior and lateral to the joint. The forearm was supinated, and the wrist was strapped to an S-Beam tension–compression load cell (Applied Measurements, Ltd., Aldermaston, United Kingdom), which was positioned perpendicular to the direction of forearm movement during isometric elbow flexion/extension. The force signal was interfaced with an analog-to-digital converter (CED micro 1401, Cambridge, United Kingdom), sampled at 2000 Hz with a PC using Spike 2 software (CED) and low-pass filtered (500-Hz edge frequency) with a second-order Butterworth digital filter. After a warm-up of four submaximal voluntary contractions, participants completed four elbow flexion isometric maximum voluntary contractions (MVC) separated by ≥30 s, in which they were instructed to flex the elbow as hard as possible for 3 s. Biofeedback and verbal encouragement were provided during and in between each MVC. Participants then completed four isometric elbow extension MVCs with an identical protocol to determine the maximum surface EMG ($sEMG_{max}$) amplitude of the musculus triceps brachii (TB; see details in the next paragraph). Maximum voluntary force (MVF) for elbow flexion and extension was the greatest instantaneous voluntary force achieved during that action, and the test–retest CV for this assessment was 3.4%.

sEMG. This was recorded from three agonist muscles (biceps brachii short head (BBS), biceps brachii long head (BBL), and brachioradialis (BR)) and one antagonist muscle (lateral head of TB (Bagnoli-4; Delsys, Boston, MA)). After preparing the skin (shaving, lightly abrading, and cleansing with 70% ethanol), double-differential surface electrodes (1-cm interelectrode distance, Model DE-3.1; Delsys) were attached over the belly of each muscle, parallel to the presumed orientation of the muscle fibers using adhesive interfaces, and a reference electrode placed on the clavicle. BBS and BBL electrodes were placed mid belly at 25% of the distance from the medial epicondyle of the humerus to the coracoid process, i.e., distal to the motor point region of each head (22). The BR electrode was placed over the proximal third of the muscle belly, identified during a submaximal isometric "hammer curl." Similarly, the TB electrode was placed over the distal third of the lateral head of the TB muscle, identified during a submaximal isometric elbow extension. Electrode locations were recorded for subsequent tests by measuring the distance from the center of the electrode to the cubital crease (BBS, BBL, and BR) or olecranon process (TB) with the elbow fully extended. Surface EMG signals were amplified (×100, differential amplifier = 20–450 Hz) and sampled at 2000 Hz with the same analog-to-digital converter and PC as the force signal before being band-pass filtered (6–500 Hz) using a fourth-order zero-lag Butterworth filter. The root mean square of the sEMG signal of a 500-ms epoch around MVF (±250 ms) was used to assess the activation of agonist and antagonist muscles. To minimize the variability in absolute sEMG (6), sEMG recorded at elbow flexion MVF was normalized to the evoked supramaximal M-wave (compound muscle action potential), or M_{max}, of the BBS and BBL (see below) and elbow extension TB $sEMG_{max}$. Normalization of the sEMG from the BR was not possible because it is not innervated purely by the musculocutaneous nerve, and thus, a reliable M_{max} cannot be evoked.

Neural stimulation and evoked M_{max}. A self-adhesive anode (5 × 5 cm; Verity Medical, Andover, United Kingdom) was attached to the skin over the TB muscle. The cathode (1 cm diameter; Electro Medical Supplies, Wantage, United Kingdom) was held to the skin over the musculocutaneous nerve, in between the BBS and BBL, at 50% of the distance between the medial epicondyle of the humerus and the coracoid process (the motor point of the BB muscle [22]). The precise location of the cathode was determined as the position that, on electrical stimulation (DS7AH; Digitimer Ltd., Welwyn Garden City, United Kingdom) with single square wave pulses (0.2 ms in duration), evoked the greatest M-wave response from BBS and BBL for a particular submaximal electrical current (typically three to five stimuli at 30–50 mA). M-waves were then evoked at 10- to 20-mA incremental current intensities until a plateau was achieved (typically between 80 and 140 mA). Thereafter, the electrical current was increased by 20%, and three supramaximal M-waves were evoked. M_{max} was defined as the mean peak-to-peak sEMG response to these three stimuli.

Muscle size. A MAGNETOM Symphony 1.5-T MRI scanner (Siemens AG, Erlangen, Germany) was used to perform three overlapping scans (each comprising ~25 contiguous axial "slices" perpendicular to the humerus/radius) from the acromion process to below the distal end of the radius of the dominant arm, which was secured in supination to minimize movement while the participant lay supine. The following parameters were used for each T1-weighted scan: time of repetition = 420 ms, time to echo = 1.2 s, matrix = 284 × 448 pixels, field of view = 181 × 200 mm, slice thickness = 10 mm, interslice gap = 0 mm. The scans were subsequently imported to a DICOM image viewer (OsiriX Foundation, Geneva, Switzerland), and using the lipid capsules that were placed on the skin midway along the humerus and radius and the anatomical markers (e.g., bone, blood vessel size), the relevant slice from the first scan was matched with the identical slice in the second scan, and so on. The anatomical cross-sectional area (ACSA) of each muscle of interest (BB, BRACH, and BR) was then manually outlined (excluding visible fat and connective tissue) and plotted against bone length (proximal end of the humerus to the distal end of the radius). A spline curve was fitted to the ACSA data

points of each muscle, and volume was calculated as the area under the curve (12); the sum of the three volumes provided total elbow flexor muscle volume. The maximum ACSA ($ACSA_{max}$) was recorded for BB, BRACH, and BR, and the sum of the three $ACSA_{max}$ provided $\sum ACSA_{max}$. The test–retest CV for the assessment of total volume and $\sum ACSA_{max}$ was 0.8% and 0.9%, respectively.

Nutrient Intake, Anthropometry, and Physical Activity

Participants used "Arc" electronic weighing scales (Salter, Tonbridge, United Kingdom) to weigh and record their nutrient intake for 3 d (Thursday to Saturday) during both the 3-wk and 12-wk RT periods (Fig. 1A). Records were scrutinized by a sports nutritionist and analyzed with Compeat v5.8 (Pro) software (Nutrition Systems, Grantham, United Kingdom). Participants who had an unusually high (>2 $g \cdot kg^{-1} \cdot d^{-1}$) or low (<0.8 $g \cdot kg^{-1} \cdot d^{-1}$) protein intake during the 3-wk RT were excluded from the supplemented 12-wk RT. Body mass and height were measured in conjunction with skinfold thickness, which was assessed in duplicate at four sites (biceps, triceps, subscapular, and iliac crest) using Harpenden skinfold calipers (Baty International, Burgess Hill, United Kingdom) because changes in the subcutaneous fat are likely to influence the sEMG signal and, therefore, the assessment of muscle activation. Further, percent body fat was calculated from the sum of these four skinfolds (11,30) and used to calculate FFM. The test–retest CV for the assessment of percent body fat was 0.8%. Habitual physical activity level (PAL) was assessed from a standard questionnaire (2) that was completed at the start and at the end of the whole study protocol (Fig. 1A).

Statistical Analysis

Analyses of raw data recordings were completed by the same investigator, who remained blinded to supplement group. Group data are expressed as mean ± SD. Between-group comparisons for pre-RT and post-RT absolute values were compared with two-way repeated-measures ANOVA (group: PLA vs PRO; time: pre vs post). Comparisons between groups before training or for percent change during the 12-wk supplemented RT period were performed with independent *t*-tests. Bivariate relationships were assessed with Pearson product–moment correlations, and statistical significance was defined as $P < 0.05$.

RESULTS

Muscle size and architecture. After 12 wk of supplemented RT, total elbow flexor muscle volume increased from 388.3 ± 61.9 to 454.8 ± 81.5 cm^3 (+17.0% ± 7.1%) for PRO and from 413.4 ± 73.1 to 474.1 ± 80.1 cm^3 (+14.9% ± 4.6%) for PLA, with no significance difference between the two groups (ANOVA: time, $P < 0.001$; group × time, $P = 0.52$; Table 2). The training-induced change in the volume of the individual elbow flexors also showed no effect of supplementation group (ANOVA: group × time: BB, $P = 0.86$; BRACH, $P = 0.68$; BR, $P = 0.77$; Fig. 2A and Table 2).

$\sum ACSA_{max}$ also displayed a clear effect of the training from 26.9 ± 4.3 to 31.3 ± 5.1 cm^2 (+16.2% ± 7.1%) for PRO and from 28.5 ± 3.9 to 32.9 ± 4.5 cm^2 (+15.6% ± 4.4%) for PLA but no difference between the groups (ANOVA: time, $P < 0.001$; group × time, $P = 0.87$; Table 2). Similarly, the changes in $ACSA_{max}$ for each of the individual elbow flexor muscles were not influenced by supplementation (ANOVA: group × time, $0.53 < P < 0.95$; Table 2).

Training increased muscle fascicle Θ_p of the BBS and BRACH in both the PRO (BBS, 18.5% ± 9.5%; BRACH, 15.7% ± 9.9%) and PLA (BBS, 15.8% ± 6.9%; BRACH, 14.6% ± 8.2%) supplementation groups, but these changes did not differ between the groups (ANOVA: group × time, $0.65 < P < 0.89$; Table 2).

Muscle strength. Isometric MVF of the elbow flexors increased after 12 wk of RT, but to a similar extent for both groups (PLA 271.2 ± 43.0 to 309.7 ± 48.8 N (+14.5% ± 8.3%); PRO 253.8 ± 41.1 to 283.8 ± 50.1 N (+12.0% ± 9.9%); ANOVA: time, $P < 0.001$; group × time, $P = 0.32$; Fig. 2B). After the 12-wk RT period, 1RM lifting strength increased, although there was no difference between groups

TABLE 2. Elbow flexor muscle volume, $ACSA_{max}$, and muscle fascicle pennation (Θ_p) angle before (Pre) and after (Post) the 12-wk RT period, with PLA or PRO supplementation before and after every training session.

	PLA (n = 16)		PRO (n = 17)		
	Pre	Post	Pre	Post	g × t, P
Muscle volume (cm^3)					
BB	184.6 ± 35.7	214.7 ± 39.4	172.1 ± 29.1	203.0 ± 37.9	0.86
BRACH	156.7 ± 28.5	176.4 ± 31.0	150.1 ± 28.4	174.3 ± 36.7	0.68
BR	71.6 ± 16.5	82.3 ± 17.5	65.6 ± 12.5	76.9 ± 15.5	0.77
Total	413.4 ± 73.1	474.1 ± 80.1	388.3 ± 61.9	454.8 ± 81.5	0.52
$ACSA_{max}$ (cm^2)					
BB	12.0 ± 2.3	14.0 ± 2.7	11.1 ± 1.9	13.0 ± 2.3	0.54
BRACH	12.3 ± 1.5	14.0 ± 1.6	11.8 ± 2.0	13.6 ± 2.5	0.80
BR	4.2 ± 0.8	4.9 ± 0.8	4.0 ± 0.8	4.7 ± 0.9	0.94
$\sum ACSA_{max}$	28.5 ± 3.9	32.9 ± 4.5	26.9 ± 4.3	31.3 ± 5.1	0.87
Θ_p (°)					
BB	15.1 ± 3.0	17.5 ± 3.8	13.7 ± 2.4	16.2 ± 2.9	0.89
BRACH	10.5 ± 1.6	12.0 1.8	11.0 ± 1.7	12.6 ± 1.5	0.65

Data are means ± SD, and ANOVA group × time P values are displayed.

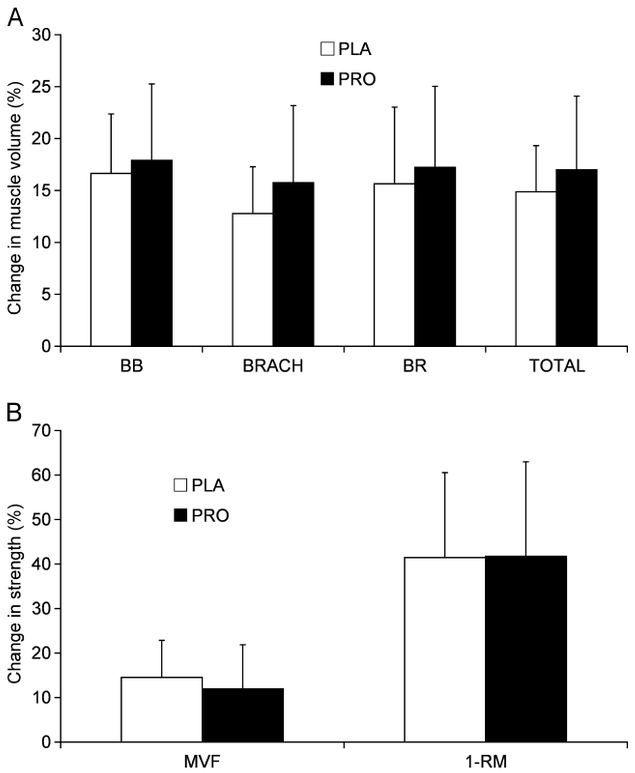

FIGURE 2—Relative training-induced changes in (A) elbow flexor muscle volume and (B) isometric MVF and 1RM after 12 wk of RT with PLA or PRO supplementation: BB, biceps brachii; BRACH, brachialis; BR, brachioradialis; TOTAL, all three elbow flexor muscles. Relative changes did not differ between PLA and PRO groups ($P > 0.05$). Values are means ± SD.

(PLA 13.3 ± 3.2 to 18.3 ± 3.7 kg (+41.4% ± 19.9%); PRO 12.3 ± 2.7 to 17.2 ± 3.7 kg (41.8% ± 21.2%); ANOVA: time, $P < 0.001$; group × time, $P = 0.90$; Fig. 2B).

Neurological changes during isometric elbow flexion. Two of the agonist muscles showed a drop in absolute sEMG at isometric MVF after training (ANOVA: time: BBL, $P = 0.045$; BBS, $P = 0.049$; BR, $P = 0.17$; Table 3), but there were similar changes in M_{max}, and hence, normalized sEMG was unchanged after training (ANOVA: time: BBL, $P = 0.173$; BBS, $P = 0.56$; Table 3). There was no interaction between supplementation group and RT for agonist sEMG (ANOVA: group × time: absolute values, $0.11 < P < 0.80$; normalized values, $0.60 < P < 0.95$; Table 3). The percentage change in sEMG at isometric MVF also showed no difference between groups for the individual agonist muscles (Table 3). When data were collapsed across the agonist muscles, there were no differences between the percent changes recorded for the supplementation groups (absolute values: PLA −9.5% ± 17.5% vs PRO −2.5% ± 27.6%, *t*-test $P = 0.41$; normalized values: PLA 6.1% ± 34.1% vs PRO 0.0% ± 34.9%, *t*-test $P = 0.63$). Antagonist sEMG at elbow flexion MVF was unchanged in both groups (ANOVA: group × time: absolute values, $P = 0.09$; normalized values, $P = 0.65$).

Nutrient intake, anthropometry, and physical activity. There were no differences between groups in their normal dietary intake of energy or macronutrients during the 3-wk (Table 1) or supplemented 12-wk RT (Table 4) and no changes over time, either in absolute terms or normalized to body mass (ANOVA: time, $P \geq 0.459$; group × time $P \geq 0.312$; Table 4). On training days during the 12-wk RT, protein intake was significantly higher in PRO compared with that in PLA, both in absolute terms (PLA 100 ± 21 $g \cdot d^{-1}$ vs PRO 140 ± 23 $g \cdot d^{-1}$; *t*-test, $P < 0.0005$) and when normalized to body mass (PLA 1.35 ± 0.47 $g \cdot kg^{-1} \cdot d^{-1}$ vs PRO 1.88 ± 0.37 $g \cdot kg^{-1} \cdot d^{-1}$; *t*-test, $P = 0.002$). However, when supplement intake was averaged across training and nontraining days, total protein intake was similar for both groups (absolute values: *t*-test, $P = 0.09$; normalized to body mass: *t*-test, $P = 0.12$; Table 4), and CHO, fat, and total energy intake remained similar (*t*-test, $P \geq 0.47$; Table 4).

Subcutaneous fat increased after the 12-wk RT period (ANOVA: time, $P = 0.03$), but there was no group interaction (ANOVA: group × time, $P = 0.09$). There was a significant effect of the supplemented RT period on body mass (ANOVA: time, $P = 0.005$) and percent body fat (ANOVA: time, $P = 0.022$) over the 12-wk supplemented RT period. However, the changes in body mass (ANOVA: group × time, $P = 0.17$; PLA 75.6 ± 11.4 to 77.1 ± 11.5 kg vs PRO 74.9 ± 10.3 to 75.5 ± 10.0 kg) and percent body fat

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TABLE 3. Surface EMG activity at isometric elbow flexion MVF before (Pre) and after (Post) the 12-wk RT period, with PLA or PRO supplementation.

Measure	PLA (n = 16)			PRO (n = 17)			ANOVA (g × t)	*t*-test (%Δ)
Muscle	**Pre**	**Post**	**%Δ**	**Pre**	**Post**	**%Δ**	***P***	***P***
Absolute values (mV)								
Agonists								
BR	0.75 ± 0.38	0.73 ± 0.38	−0.8 ± 26.4	0.67 ± 0.46	0.62 ± 0.33	5.6 ± 37.7	0.80	0.58
BBL	0.84 ± 0.54	0.68 ± 0.48	−11.3 ± 35.2	0.59 ± 0.38	0.48 ± 0.31	−10.6 ± 33.6	0.11	0.95
BBS	1.24 ± 0.81	0.94 ± 0.65	−15.6 ± 36.9	1.08 ± 0.71	0.88 ± 0.47	−6.2 ± 46.6	0.70	0.54
Antagonist								
TB	0.02 ± 0.03	0.02 ± 0.01	12.6 ± 53.1	0.02 ± 0.03	0.01 ± 0.01	−13.3 ± 44.0	0.09	0.14
Normalized values (%)								
Agonists								
BBL	8.8 ± 5.4	7.5 ± 3.1	−0.9 ± 46.7	9.6 ± 7.2	8.5 ± 4.5	−1.9 ± 38.6	0.91	0.95
BBS	10.0 ± 9.2	10.3 ± 8.1	13.1 ± 52.3	12.8 ± 9.6	10.7 ± 6.3	2.0 ± 61.3	0.46	0.60
Antagonist								
TB	14.7 ± 10.0	13.8 ± 9.0	2.6 ± 39.1	13.9 ± 6.7	11.9 ± 6.6	−3.1 ± 49.9	0.65	0.72

Data are means ± SD, and *P* values are shown for ANOVA group × time (g × t) interaction effect and independent *t*-tests for the percentage change (%Δ).
Data are expressed in absolute values and normalized to: M_{max} (agonist muscles: BBL and BBS) or maximum sEMG during elbow extension (antagonist muscle: triceps brachii (TB)).

TABLE 4. Energy and macronutrient intake during the initial 3-wk RT (no nutritional intervention) and 12-wk RT (PLA or PRO supplementation) periods.

Nutritional Measure (3- or 12-wk RT Period)	PLA (n = 16)	PRO (n = 17)	t-test, P
Energy intake			
3-wk RT (kcal·d^{-1})	2515 ± 706	2482 ± 581	0.89
12-wk RT – normal diet (kcal·d^{-1})	2522 ± 672	2480 ± 608	0.85
12-wk RT – total intake (kcal·d^{-1})	2545 ± 672	2572 ± 608	0.91
Protein intake			
3-wk RT (g·d^{-1})	101 ± 21	95 ± 26	0.39
3-wk RT (g·kg^{-1}·d^{-1})	1.36 ± 0.35	1.28 ± 0.37	0.41
12-wk RT – normal diet (g·d^{-1})	101 ± 28	100 ± 23	0.85
12-wk RT – normal diet (g·kg^{-1}·d^{-1})	1.35 ± 0.47	1.33 ± 0.32	0.88
12-wk RT – total intake (g·d^{-1})	101 ± 28	117 ± 23	0.09
12-wk RT – total intake (g·kg^{-1}·d^{-1})	1.35 ± 0.47	1.56 ± 0.33	0.12
CHO intake			
3-wk RT (g·d^{-1})	325 ± 106	343 ± 101	0.62
3-wk RT (g·kg^{-1}·d^{-1})	4.41 ± 1.79	4.59 ± 1.38	0.75
12-wk RT – normal diet (g·d^{-1})	316 ± 89	341 ± 104	0.47
12-wk RT – normal diet (g·kg^{-1}·d^{-1})	4.23 ± 1.34	4.56 ± 1.50	0.49
12-wk RT – total intake (g·d^{-1})	322 ± 89	347 ± 104	0.47
12-wk RT – total intake (g·kg^{-1}·d^{-1})	4.31 ± 1.35	4.64 ± 1.50	0.50
Fat intake			
3-wk RT (g·d^{-1})	81 ± 26	86 ± 32	0.60
3-wk RT (g·kg^{-1}·d^{-1})	1.10 ± 0.39	1.16 ± 0.39	0.57
12-wk RT – normal diet (g·d^{-1})	92 ± 37	84 ± 34	0.52
12-wk RT – normal diet (g·kg^{-1}·d^{-1})	1.22 ± 0.56	1.14 ± 0.53	0.61

Data are mean ± SD, and P values are displayed for independent t-tests.
During the 12-wk supplemented RT period, data include training and nontraining days.

(ANOVA: group × time, P = 0.09; PLA 21.5% ± 6.1% to 23.1% ± 6.3% vs PRO 22.5% ± 5.1% to 22.7% ± 4.9%) were similar for the two groups. There were no changes in FFM during the supplemented RT (ANOVA: time, P = 0.58; group × time, P = 0.55; PLA 60.2 ± 10.2 to 60.2 ± 9.6 kg vs PRO 57.9 ± 7.5 to 58.2 ± 7.1 kg).

Before performing the 3-wk RT, the whole cohort had a PAL of 2.6 ± 0.4, indicating that they were "recreationally active," and it remained stable over the course of the study (after the 12-wk RT = 2.6 ± 0.4). There was no difference in PAL between PLA and PRO (ANOVA: time, P = 0.36; group × time, P = 0.48), and the mean PAL (pre and post) was unrelated to any of the training responses after the 12-wk RT ($R^2 \leq 0.06$, $P \geq 0.17$).

DISCUSSION

The aim of this study was to determine the effect of protein supplementation (PRO) on changes in elbow flexor muscle size, architecture, and strength after 12 wk of RT. Using sensitive state-of-the-art techniques, we found that PRO did not influence any of these training-induced adaptations.

There are several factors in our study that we thought would accentuate an effect of PRO on the training-induced changes in muscle size and strength: 1) it featured larger cohorts than have been used in most previous studies of this kind; 2) an initial preintervention RT period was included to overcome neural changes, standardize preintervention training status, and familiarize participants with all training and measurement procedures; 3) participants included only young, healthy male participants who might be more responsive to RT (37) and PRO (36) than older individuals; 4) we used an upper limb RT program to maximize the muscle hypertrophic response. The measurements incorporated 1.5-T MRI for documenting changes in muscle size, ultrasonographic assessments of muscle architecture, as well as careful functional measurements. In addition, possible confounding factors were considered prospectively by pair matching participants according to their normal nutrient intake and isometric strength response to the initial 3 wk of RT before their random allocation to supplementation groups, and retrospectively by assessing normal dietary behavior and neural drive.

The ~16% changes in elbow flexor muscle volume and maximum ACSA observed in this study were similar to the 14% to 23% changes reported in previous upper limb RT studies (10,19,37). Despite these substantial changes, there was no difference in muscle hypertrophy between PRO and PLA groups. Accepting the methodological differences between studies, this finding is broadly similar to previous investigations in young men (8,20), postmenopausal women (18), and older people (34), which used MRI (8,18,20) or computed tomography (34) to quantify changes in lower limb muscle ACSA after RT supplemented with PRO or PLA. The lower limb nature of those previous strength training studies resulted in markedly smaller changes in muscle size (5%–10%) compared with those reported in our study and thus may have restricted their capacity to determine any hypertrophic benefit from PRO. However, despite using a more responsive muscle group, our results suggest that PRO does not amplify the hypertrophic adaptation to elbow flexor RT.

In contrast, some previous studies have used other measures of muscularity, such as dual-energy x-ray absorptiometry to assess lean body mass (17) and ultrasonography to measure muscle thickness (7), and have found a positive effect of PRO after a period of RT. However, these methods are widely considered to have lower precision and reliability than

MRI (3). Furthermore, other RT studies have reported no difference between PRO and PLA regarding changes in either lean body mass (34) or muscle thickness (35). By measuring muscle fiber CSA (*f*CSA), the effect of PRO on RT-induced muscle hypertrophy has been determined at the cellular level but with contradictory findings (1,17,18,34). The lack of concurrence may reflect the large variability in the histological measurement of *f*CSA from biopsy samples (25) that may not mirror training-induced changes in whole muscle size measured with MRI (28).

We observed significant increases in biceps brachii and brachialis muscle fascicle pennation angle (Θ_p) in both PRO and PLA groups, but no difference between groups. These findings are in accord with the relative changes in whole muscle volume and $ACSA_{max}$ observed in both training groups but are in contrast to those of a previous RT study that reported an increase in gastrocnemius medialis Θ_p in participants supplemented with essential amino acids and no change in PLA (35). This is surprising, especially because RT is known to increase muscle Θ_p even when no nutritional supplementation is provided (13).

Together with muscle hypertrophy, neural changes are considered to be major contributors to strength improvements after RT (15), and the current study was the first to quantify the neural adaptations to RT in the context of PRO. Voluntary muscle activation and antagonist muscle coactivation, assessed with normalized sEMG, did not change after the 12-wk elbow flexion RT in either group. This finding suggests that elbow flexor muscle activation was very high before the 12-wk RT period and that neural adaptations did not confound any potential effect of PRO on the strength gains found in this study.

After the 12-wk supplemented RT, elbow flexor 1RM increased by ~41% and isometric strength increased by ~13%, changes that are in accord with previous RT studies on the elbow flexor muscle group (10). However, given that there were no differences in neural adaptations or muscle morphology between PRO and PLA groups, it was not surprising that RT-induced strength gains did not differ between the two groups. This finding is similar to that reported in previous RT studies of the lower limb (1,17,34) but different from those reporting a significant effect of PRO on RT-induced increases in 1RM (8,9). Although the reasons for these discrepancies are not clear, changes in 1RM are probably influenced by neural adaptations and the involvement of stabilizer muscles (29), which were not accounted for in these studies and may have confounded their results.

Normal dietary behavior, as assessed from two 3-d records of weighed nutrient intake, was similar for the PRO and PLA groups both before and during the 12-wk supplemented RT period, and there was no change over time. Therefore, it seems unlikely that this could have confounded our findings. However, we acknowledge that a longer recording period and/or additional assessments of weighed nutrient intake might have provided a more comprehensive account of habitual diet and further reinforced our results. On the basis of the available evidence, we considered that whey protein, as opposed to soy or casein (31), supplemented immediately before and after each session (14,33), as well as a dosage of ≥20 g (26), would promote MPS and maximize the hypertrophic response. The conventional PRO supplementation approach of this study, i.e., targeted at the time of training, did influence protein intake on training days, but did not affect total protein intake averaged over training and nontraining days. It is possible, therefore, that influencing total protein intake could be an important factor in any benefits of PRO, although several previous studies that also found no influence of training-targeted PRO on total protein intake (17,20) did find positive effects of PRO on indicators of muscle hypertrophy (17) or strength responses (20).

While the timing of PRO with respect to a resistance exercise bout has previously been shown to influence the augmentation of MPS after a single bout of resistance exercise (33), some recent work indicates that the sensitivity of MPS to PRO persists for up to 24 h after a bout of resistance exercise (5). Therefore, it is feasible that prolonged sensitivity to MPS after each training session in our study may have enabled the protein content within the regular meals of the PLA group to stimulate similar net protein synthesis to the PRO group. Furthermore, although PRO has been found to elevate the anabolic hormone response to multiple-limb resistance exercise (21), higher levels of circulating anabolic hormones after multiple-limb versus solely upper limb RT do not seem to affect acute MPS (39) or chronic muscle hypertrophy (38). While the focus of the current study was on the functional and hypertrophic changes with chronic RT, the acute MPS and hormonal responses may have helped inform these effects, and future work should consider acute MPS and hormonal changes alongside chronic adaptations. Considering the relatively small muscle mass trained, and the equivalent training responses of the two groups, the habitual protein intake of both groups may have been sufficient to maintain optimal net protein synthesis, thus facilitating similar accretion of muscle mass. In this case, the available free amino acids within the muscle and the blood of the PLA group may have been adequate to satisfy the protein requirements for increased synthesis after each RT bout. It is feasible, therefore, that RT incorporating a much greater muscle mass, such as whole body RT, could have a higher protein requirement and might benefit from the provision of supplementary protein. However, the few studies that have used MRI to assess muscle hypertrophy after RT of multiple muscle groups have demonstrated that PRO either was of marginal (20) or no benefit (18).

In conclusion, protein supplementation did not augment the adaptations of muscle strength and size that occurred after 12 wk of elbow flexor RT in previously untrained young men. We suggest that future studies should use similar sensitive measures of muscle size and strength to investigate whether the adaptations to RT in different circumstances may be more responsive to protein supplementation, such as whole body RT.

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