The Effects of Resistance Training With or Without Peanut Protein Supplementation on Skeletal Muscle and Strength Adaptations in Older Individuals

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Abstract

Several studies suggest resistance training (RT) with protein supplementation has positive effects on strength and muscle mass in older individuals. However, to date, no study has examined the effects of RT with a high-protein, defatted peanut powder (PP) supplement on these markers. Herein, 39 older, untrained individuals (n=17 female, n=22 male; age=58.6±8.0 years; body mass index =28.7±5.8) completed a 6-week (n=22) or 10-week (n=17) RT program, where full-body training was implemented twice weekly (ClinicalTrials.gov trial registration NCT04015479; registered July 11, 2019). Participants in each program were randomly assigned to consume either a PP supplement once per day (35 g protein, 315 kcal; n=20) or no supplement (CTL; n=19). Right leg vastus lateralis (VL) muscle biopsies were obtained prior to and 24 hours following the first training bout in all participants to assess the change in myofibrillar protein synthetic rates (MyoPS) as measured via the deuterium-oxide (D_2O) tracer method. Pre- and Post-intervention testing in all participants was conducted using dual energy x-ray absorptiometry (DXA), VL ultrasound imaging, a peripheral quantitative computed tomography (pQCT) scan at the mid-thigh, and right leg isokinetic dynamometer assessments. Integrated MyoPS rates over a 24-hour period were not significantly different ($p<0.05$) between supplement groups following the first training bout. Regarding chronic changes, there were no significant supplement-by-time interactions in DXA-derived fat mass, lean soft tissue mass or percent body fat between supplementation groups. There was, however, a significant increase in VL thickness in PP versus CTL participants when the 6- and 10-week cohorts were pooled (interaction $p=0.041$). There was also a significant increase in knee flexion torque in the 10-week PP group versus the CTL group (interaction $p=0.032$). In conclusion, a high-protein, defatted peanut powder supplement in combination with RT positively affects select markers of muscle hypertrophy and strength in an untrained, older adult population.

Introduction

The size and number of muscle fibers remain relatively stable until the fifth decade of life, at which point an appreciable decrease in both total muscle fibers and size occurs (1, 2). The gradual age-related decrease in muscle mass and strength, termed sarcopenia, culminates in a reduction of nearly 40% of an individual's total muscle mass by the eighth decade of life (3–5). Sarcopenia coincides with cellular, neuromuscular and metabolic perturbations (6). Not only does sarcopenia directly contribute to increased frailty and fragility (7, 8), but it indirectly contributes to more serious health consequences such as decreased quality of life and premature death (9). Therefore, interventions targeting the retention of muscle mass with aging have garnered much attention.

A plethora of research has demonstrated that 8–16 weeks of resistance training (RT) can increase muscle mass and strength in older individuals (reviewed in (10, 11)). Given that protein feeding stimulates an anabolic response in skeletal muscle (12), it stands to reason that combining protein supplementation with RT likely optimizes increases in muscle mass. Animal-based protein sources possess the full complement of essential amino acids needed to stimulate the muscle-building process at the molecular level (i.e., increases in post-meal myofibrillar protein synthesis or MyoPS rates) (13).

Moreover, it has been well documented that dairy-derived protein supplements (e.g., milk or whey protein concentrates or isolates) can enhance increases in muscle mass with RT relative to other protein sources (14). However, there has been a growing interest in the health benefits of plant-based foods as well as concerns related to the sustainability of procuring animal-based proteins (15). In this regard, data from the National Health and Nutrition Examination Survey indicate that intakes of plant proteins increased significantly from 1999 to 2010 (16), and there is sentiment that consumers will continue to increase plant protein intake for the foreseeable future (17).

Protein isolates from several plant-based foods (i.e. soy, pea, rice, and hemp) are currently sold to consumers with the intent of supporting the rigorous demands of exercise training. There has also been a recent growth in the popularity and availability of peanut flour and defatted peanut powder. With the exception of containing low methionine and threonine levels, peanut protein possesses a full complement of essential and non-essential amino acids (18). Relative to other plant-based proteins (e.g., wheat or legumes), peanut protein possesses a relatively high protein digestibility corrected amino acid score (0.70/1.00) (18). In fact, it has been posited that peanut protein can be used as an ingredient for protein fortification in low-protein food sources (19). Despite these positive statistics surrounding peanut protein, no study to date has examined if a peanut protein supplement combined with RT can enhance training adaptations. Therefore, the purpose of this study was two-fold. First, we sought to determine if post-exercise PP supplementation could enhance the MyoPS response to one resistance exercise bout in older participants with no prior formal resistance training experience. Second, we sought to determine if PP supplementation with 10 weeks of RT could enhance muscle quality, body composition and strength in these same participants.

Methods

Ethical Approval and Participant Screening

Prior to any data collection, this study was approved by the Auburn University Institutional Review Board (IRB) (Protocol # 19–249 MR 1907), conformed to standards set by the latest revision of the Declaration of Helsinki, and was registered as a clinical trial (NCT04015479). Men and women aged 50–80 years with minimal RT experience, defined here as not having performed structured RT for at least three months prior, were recruited for this study. Participants were recruited via flyer, email inquiry and newspaper advertisement. Interested participants were informed of the study and testing procedures either over the phone or face-to-face at the Auburn University School of Kinesiology. Eligibility criteria indicated that potential participants had to: 1) be between the ages of 50–80 years old, 2) not actively be participating in structured RT for at least 3 months prior, 3) be free of metal implants, and 4) possess blood pressure readings within normal ranges, with or without medication (i.e. <140/90 SBP/DBP). Exclusion criteria included: 1) individuals having a known peanut allergy, 2) individuals having a body mass index $\geq$ 35 kg/m^2, 3) individuals being exposed to medically-necessary radiation in the last 6 months, or 4) individuals having a medical condition contradicting participation in a RT program, giving blood or donating a skeletal muscle biopsy (i.e. blood clotting disorders or taking blood thinning medications).

Participants deemed eligible based on the aforementioned criteria provided written and verbal consent to participate. A medical history questionnaire was obtained at the time of consenting and participants were scheduled to return to the Auburn University School of Kinesiology to complete study procedures described below.

Study Design

Our original intent was to recruit two separate ten-week cohorts. Due to the SARS-CoV-2 pandemic, we voluntarily decided to end the second cohort after only six weeks of training. As such, the primary difference between cohorts was the length of the intervention. The study design for the 10-week and 6-week cohorts is presented in Fig. 1 below.

INSERT FIGURE 1 HERE

Briefly, participants in the 10-week cohort reported to the Auburn University School of Kinesiology on 24 separate occasions, whereas participants in the 6-week cohort reported on 16 separate occasions. Visit one (V1) included screening to determine eligibility, gathering consent and obtaining a health history. Visit two (V2; PRE) occurred at least three days prior to visit 3 (V3) and included a battery of assessments comprised of urine specific gravity (USG), height and body mass, ultrasound of the right leg vastus lateralis (VL), full body dual energy x-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) scan at the mid-thigh of the right leg and right leg strength assessment using an isokinetic dynamometer. Following the battery of assessments, participants were provided with deuterium oxide (D2O)-enriched water, a three-day food log, and three separate salivettes to measure D2O enrichment. The food log was returned prior to V3 at each participant's convenience.

V3 included the participant's first muscle tissue sample collection, randomization to either the peanut protein supplement group (PP) or wait-list control (CTL), the participant's first resistance exercise bout, and immediate post-exercise PP supplementation or no supplementation. A complete nutritional breakdown for the PP supplement is presented in Table 1. V4 included the participant's second muscle tissue sample collection and salivette return. Visit five (V5) through visit twenty-three (V23) for the 10-week cohort and V5 through visit fifteen (V15) for the 6-week cohort included a single RT session. During V23 for the 10-week cohort and V15 for the 6-week cohort participants were provided with their second set of food logs. Visit twenty-four (V24; POST) for the 10-week cohort and visit sixteen (V16; POST) for the 6-week cohort occurred roughly 72 hours following V23 and V15, respectively, and included a repeat of the V2 testing battery. Specific testing methodologies are detailed below.

Table 1
Baseline Participant Characteristics

Variable (units)	Mean ± SD		p-value
Gender	PP	12 M / 8 F	N/A
	CTL	10 M / 9 F	
Age (years)	PP	60 ± 9	p = 0.61
	CTL	58 ± 7	
Height (cm)	PP	171.7 ± 8.3	p = 0.41
	CTL	172.0 ± 9.3	
Weight (kg)	PP	84.9 ± 17.6	p = 0.52
	CTL	88.8 ± 20.5	
BMI (kg/m^2)	PP	27.8 ± 5.5	p = 0.23
	CTL	29.7 ± 6.2	
DXA % body fat (%)	PP	36.0 ± 7.1	p = 0.92
	CTL	36.2 ± 7.6	

Legend: Baseline participant characteristics are presented as means ± standard deviation values. Abbreviations: PP, peanut protein supplemented participants (n = 20); CTL, non-supplemented participants (n = 19); DXA, dual x-ray absorptiometry; BMI, body mass index; cm, centimeters; kg, kilograms; kg/m^2, kilograms per meter squared.

INSERT Table 1 HERE

Pre- and Post-intervention Testing Battery

The testing sessions described below occurred during morning hours (05:00–09:00) following an overnight fast for all but 7 participants who reported to the laboratory after working hours at 17:00–18:30 following a ~ 4–5 hour fast.

Body Composition Assessments. During V2 and V24 (10-week participants) or V2 and V16 (6-week participants), participants reported to the Auburn University School of Kinesiology wearing casual sports attire (i.e. athletic shirt and shorts, tennis shoes). Participants submitted a urine sample (~ 5 mL) to assess USG levels using a handheld refractometer (ATAGO; Bellevue, WA, USA). Notably, all participants possessed USG values less than 1.020 indicating that they were well hydrated. Height and body mass were assessed using a digital column scale (Seca 769; Hanover, MD, USA) with mass and height being collected to the nearest 0.1 kg and 0.5 cm, respectively. Thereafter, right leg VL images were captured in the transverse plane using real-time B-mode ultrasonography (LOGIQ S7 Expert, GE Healthcare, USA) utilizing a multi-frequency linear-array transducer (3–12 MHz, GE Healthcare, USA) and subsequently analyzed for VL thickness. Participants were instructed to stand and displace bodyweight to the left leg to

ensure the right leg was relaxed. Measurements were standardized by placing the transducer at the midway point between the inguinal crease and proximal border of the patella. All images were captured and analyzed by the same investigator (S.C.O.) with a 24-hr test-retest reliability using intraclass correlation coefficient ($ICC_{3,1}$), standard error of the measure (SEM), and minimal difference (MD) to be considered real of 0.991, 0.06, and 0.16 cm, respectively. Participants then underwent a full body dual-energy x-ray absorptiometry (DXA) scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA) for determination of total lean soft tissue mass (LSTM) and fat mass (FM). Quality assurance testing and calibration were performed the morning of data-collection days to ensure the scanner was operating to manufacturer specification. Scans were analyzed by the same technician using the manufacturer's standardized software. Test-retest reliability using $ICC_{3,1}$, SEM, and MD were previously determined for LSTM (0.99, 0.36, and 0.99 kg, respectively) and FM (0.99. 0.43, and 1.19 kg). Following the DXA scan, a cross-sectional image of the right thigh at 50% of the femur length was acquired using a pQCT scanner (Stratec XCT 3000, Stratec Medical, Pforzheim, Germany). Scans were acquired using a single 2.4 mm slice thickness, a voxel size of 0.4 mm and scanning speed of 20 mm/sec. All images were analyzed for total muscle cross-sectional area (mCSA, cm^2) and density (mg/cm^3) using the pQCT BoneJ plugin freely available through ImageJ analysis software (NIH, Bethesda, MD). All scans were performed and analyzed by the same investigator (K.C.Y.). Test-retest reliability using $ICC_{3,1}$, SEM, and MD was previously determined for mCSA (0.99, 0.84, and 2.32 cm^2, respectively).

Right Leg Isokinetic Strength Assessment. Participants performed maximal isokinetic right leg extensions on an isokinetic dynamometer (System 4 Pro, BioDex Medical Systems, Shirley, NY, USA). Participants were fastened to the dynamometer so that the right knee was aligned with the axis of the dynamometer. Seat height was adjusted to ensure the hip angle was approximately 90°. Prior to peak torque assessment, each participant performed a warmup consisting of submaximal to maximal isokinetic knee extensions. Participants then completed five maximal voluntary isokinetic knee extension actions at 60°/sec and 120°/sec. Sets were separated by 60 sec of rest. Participants were provided verbal encouragement during each set. The isokinetic extension resulting in the greatest peak torque value was used for analyses. Right leg extensor peak torque testing occurred ~ 1–3 days prior to the muscle biopsy at the PRE (V2) time point in both the 10-week and 6-week cohorts, whereas this test occurred approximately 10 minutes following the biopsy at the POST time point for the 10-week cohort only (V24). This difference in methodology between time points was due to logistical constraints. However, we have unpublished data suggesting peak torque values are not affected by muscle biopsies when isokinetic testing occurs within a 10-minute post-biopsy window (20).

Supplement Randomization and Resistance Training

During V3, immediately following collection of the first muscle sample, participants were randomized to either consume PP during the intervention (n = 20) or after the intervention (n = 19). The PP supplement (PBfit; BetterBody Foods, Lindon, UT, USA) provided the following per daily serving: 315 kcal, 35 g protein, 10.7 g essential amino acids (where 2.44 g was L-leucine), 9.0 g fat and 22.5 g carbohydrate (with 14.8 g fiber and 7.7 g sugars).

Randomization was stratified by gender in blocks of four, hence the slight differences in allocation to study arms. Afterwards, participants were escorted to the Auburn University School of Kinesiology Fitness and Performance Optimization Laboratory for their first resistance exercise session. Participants were provided detailed instructions on proper posture, technique, range-of-motion, body positioning and breathing to ensure safety. Participants completed supervised RT twice weekly for either ten weeks or six weeks. All RT sessions were separated by at least 48 hours to allow for a period of recovery. Each RT session consisted of five exercises including seated leg press, leg extensions, lying leg curls, barbell bench press and cable pull-downs. For each exercise, participants performed three sets of 10–12 repetitions with 1 minute of rest between sets. At the end of each set, participants were asked to rate the level of difficulty where 0 = easy, 5 = moderate difficulty and 10 = hard. If values were below 7, weight was modestly added to increase exertion on the subsequent set. If values were 10, or the participant could not complete the set, weight was removed prior to the next set. Participants were encouraged to be as truthful as possible when assessing difficulty and were provided verbal encouragement and feedback during and following each set. The intent of this training method was to consistently challenge participants so that perceived exertion after each set of 10–12 repetitions was at a 7–9 rating. Training data for each participant were logged, allowing us to ensure that training effort was maximized within each training session, and participants were successfully implementing progressive overload in an individualized fashion.

Notably, study personnel supervised all training throughout the study. Participants in the PP group were instructed to consume one daily serving of the PP supplement. On workout days, PP supplements were provided to participants in the PP group immediately following exercise, and supplementation compliance was supervised. On non-workout days, participants were instructed to consume one serving between meals. Product bottles were returned to the study coordinator to ensure compliance to the supplementation protocol.

Muscle Sample Collection and Integrated Myofibrillar Protein Synthesis Rate Determination using Deuterium Oxide

MyoPS rates were determined after the first bout of training with or without PP supplementation using the integrated D_2O technique. Briefly, participants consumed a total 4.5 $mL \bullet kg^{-1}$ of lean body mass (LBM) of D_2O-enriched water (70 atom percent; Sigma-Aldrich, St. Louis, MO) over the course of four separate days beginning 2 days prior to V2 through V3. Participants were provided with six individual servings of D_2O. Three of these servings contained 1 $mL \bullet kg^{-1}$ LBM D_2O and were consumed in a single day as a loading phase, and three of these servings contained 0.5 $mL \bullet kg^{-1}$ LBM D_2O and were consumed over the next three consecutive days.

Skeletal muscle biopsies at V3 and V4 were obtained from the right thigh (i.e VL; in the same plane as ultrasound and pQCT assessments) midway between the patella and iliac crest using a 5-gauge needle

with suction and sterile laboratory procedures. Briefly, upon arrival to the laboratory, participants were instructed to lie in a supine position on an athletic training table. Approximately 5 minutes afterwards, 1.5 mL of 1% lidocaine was injected subcutaneously above the skeletal muscle fascia a small pilot incision was made for needle insertion using a sterile Surgical Blade No. 11 (AD Surgical; Sunnyvale, CA, USA). After 5 minutes of allowing the anesthetic to take effect, the biopsy needle was inserted into the pilot incision just beyond the fascia and approximately 50–100 mg of skeletal muscle was removed using a double chop method and applied suction (21). Following biopsies, tissue was rapidly teased of blood and connective tissue and subsequently stored at -80 °C until the isolation of myofibrils. The day of myofibril isolation, all samples were batch-processed using the recently published MIST method; for further details refer to our recent publication (22). Thereafter, isolated myofibrils and saliva (collected from salivettes) were shipped on dry ice to Metabolic Solutions (Nashua, NH, USA) for tracer analyses.

MyoPS rates over the 24-hour period following the first training bout were calculated similar to Bell et al. (23) (see equation below).

$$FSR\left(\%day^{-1}\right) = \left[\frac{(E_{Ala2} - E_{Ala1})}{E_{BW} \times t}\right] \times 3.7 \times 100$$

Briefly, E_{Ala1} and E_{Ala2} represent ^{2}H enrichment in the first and second muscle biopsies, respectively (in atom percent excess). E_{BW} is the average ^{2}H enrichment (in atom percent excess) of total body water from the second and third salivettes after subtracting background values from the baseline salivette. *t* is time in the number of days D_2O was ingested (which equals 1). The 3.7 coefficient adjusts for average ^{2}H atoms that can be bound to alanine, and final values were expressed as % synthesis per day by multiplying values by 100.

Food log analysis

Participants were instructed to self-report their habitual food intake for three consecutive days and return these food logs at V3 and V24 or V16 (10- and 6-week cohort, respectively). Participants were asked not to change their diet in any way, with the exception of PP participants who were instructed to consume the supplement as described above. Study staff entered each food log into the Automated Self-Administered 24-Hour Dietary Assessment tool (ASA24), which uses the United States Department of Agriculture Food and Nutrient Database for Dietary Studies to provide values for 195 nutrients, nutrient ratios and other food components (24).

Statistical analysis

All statistical analyses were performed using SPSS v26.0 (IBM Corp, Armonk, NY, USA). For MyoPS and training volume comparisons between the PP and CTL groups independent samples t-tests were used. For all dependent variables over time, repeated measures two-way (group × time; GxT) ANOVAs were performed. When a significant interaction occurred, LSD post hocs were performed between and within groups to determine the level of significance. With the exception of MyoPS data, all data in figures are

presented to show the 6-week cohort individually, 10-week cohort individually, and pooled cohorts (6-week and 10-week) collectively. Group, time and GxT p-values are provided for each cohort individually and when pooled. Statistical significance was established as $p < 0.05$, and relevant p-values are depicted in-text or within figures.

Results

Study CONSORT Diagram

Figure 2 provides a detailed CONSORT diagram of the study. Briefly, 120 potential participants contacted the study coordinator. Of these, 41 were eligible and agreed to participate in the study, and n = 22 were randomized to the PP group whereas n = 19 were randomized to the CTL group. Two participants in the PP group had to discontinue the study due to injury from weightlifting (n = 1) or health reasons outside of the study (n = 1), whereas none of the CTL participants discontinued the study. Thus, n = 20 PP participants and n = 19 CTL participants were included in most analyses unless stated otherwise in the results, figures, or tables.

INSERT FIGURE 2 HERE

Baseline Participant Characteristics

Baseline participant characteristics between the PP and CTL cohorts are presented in Table 1. Notably, there were no differences between cohorts regarding age or body composition metrics.

INSERT Table 1 HERE

MyoPS response to the first bout of training with or without PP supplementation

There were no significant differences in total leg extension or leg press volumes for the first bout of training between supplementation groups (Fig. 3a and 3b). Twenty-seven participants (n = 15 PP, N = 12 CTL) yielded salivettes with viable enrichment values for tracer analysis (Fig. 3c). There was an enrichment effect over time fromV2 to V3 ($p < 0.001$), but no further differences in enrichment from V3 to V4. There was no difference in the 24-hour MyoPS rates between supplement groups following the first bout of RT (Fig. 3d).

INSERT FIGURE 3 HERE

Food log data over the duration of the study

Data from the pre- and post-training food logs between the PP and CTL cohorts are presented in Table 2. Thirty-one participants (n = 16 PP, n = 15 CTL) returned completed food logs suitable for analyses, and data from the PP group includes one serving of PP per day. Notably, there was a significant interaction for protein consumption in the pooled participants ($p = 0.008$). While protein consumption decreased slightly

in the CTL group (Pre = 71 ± 30 g versus Post = 68 ± 23 g, $p > 0.05$), protein consumption significantly increased in the PP group (Pre = 95 ± 24 g versus Post = 119 ± 22 g, $p = 0.007$). Additionally, fiber consumption significantly increased in both the 10-week ($p = 0.013$) and pooled cohorts ($p = 0.003$). Despite a slight decrease in fiber consumption for the 10-week CTL cohort (Pre = 13 ± 4 g versus Post = 12 ± 7 g, $p > 0.05$), there was a significant increase in fiber consumption for the 10-week PP cohort (Pre = 19 ± 15 g versus Post = 26 ± 9 g, $p = 0.032$). There was also a difference in fiber consumption of the pooled cohorts. While there was no increase in fiber consumption for the CTL cohort (Pre = 14 ± 4 g versus 15 ± 6 g, $p > 0.05$), there was a significant increase in fiber consumption for the PP cohort (Pre = 19 ± 10 g versus Post = 27 ± 6 g, $p < 0.001$).

Table 2
Pre- and post-intervention food recall data

Variable	Pooled CTL (n = 15)		Pooled PP (n = 16)	
	PRE	POST	PRE	POST
Energy (kcal)	1799 ± 647	1683 ± 569	2106 ± 383	2181 ± 412
Pro (g)	71 ± 30	68 ± 23	95 ± 24	119 ± 22*#
Fat (g)	74 ± 31	66 ± 27	89 ± 18	91 ± 21
CHO (g)	210 ± 82	206 ± 71	216 ± 75	209 ± 49
Sugar (g)	105 ± 54	92 ± 54	124 ± 63	98 ± 44
Fiber (g)	14 ± 4	15 ± 6	19 ± 10	27 ± 6*#
	10-week CTL (n = 7)		10-week PP (n = 6)	
Energy (kcal)	1682 ± 737	1523 ± 578	2130 ± 336	2150 ± 456
Pro (g)	72 ± 36	62 ± 19	92 ± 20	111 ± 14#
Fat (g)	74 ± 37	60 ± 25	83 ± 16	85 ± 22
CHO (g)	178 ± 70	180 ± 79	214 ± 75	208 ± 49
Sugar (g)	83 ± 32	85 ± 67	90 ± 55	87 ± 29
Fiber (g)	13 ± 4	12 ± 7	19 ± 15	26 ± 9*#
	6-week CTL (n = 8)		6-week PP (n = 10)	
Energy (kcal)	1901 ± 588	1823 ± 560	2092 ± 426	2200 ± 408
Pro (g)	71 ± 27	73 ± 27	96 ± 27	123 ± 26
Fat (g)	74 ± 29	71 ± 29	93 ± 19	94 ± 21
CHO (g)	239 ± 84	229 ± 58	218 ± 79	209 ± 51
Sugar (g)	124 ± 63	98 ± 44	87 ± 39	79 ± 31
Fiber (g)	15 ± 5	17 ± 3	19 ± 6	28 ± 5

Legend: These data are from the 3-day food recalls averaged to intakes per one day. All data are presented as means ± standard deviation values. Abbreviations: PP, peanut protein supplemented participants; CTL, non-supplemented participants; kcal, kilocalorie; g, gram; Pro, protein; CHO, carbohydrate. Symbols: *, significant increase within PP from Pre to Post ($p < 0.05$); #, PP > CTL at Post ($p < 0.05$).

Figure captions and legends

INSERT Table 2 HERE

Differences in Resistance Training Volumes after 6- or 10-weeks of Training

There were no between-group differences in total volume lifted in the 6-week cohort, 10-week cohort, and pooled cohorts for bench press, cable pull-down, leg press, leg extension or leg curl exercises (Fig. 4a-e).

INSERT FIGURE 4 HERE

Changes in DXA Fat Mass, LSTM and Percent Body Fat

There was no significant GxT interaction for fat mass in any of the cohorts. Similarly, there was no significant main effect of group in any of the cohorts. There was, however, a significant main effect of time in the 6-week cohort ($p = 0.023$, Fig. 5a). There were no significant group or interaction effects for LSTM in any cohort (Fig. 5b). However, there was a significant main effect of time for LSTM in the 6-week ($p < 0.001$), 10-week ($p = 0.044$) and pooled cohorts ($p = 0.001$). There was no significant GxT interaction for body fat percentage for any cohort (Fig. 5c). However, there was a significant main effect of time in body fat percentage in both the 6-week ($p = 0.001$) and pooled ($p = 0.036$) cohorts, but not the 10-week cohort. There was a significant main effect of group for body fat percentage in the 10-week cohort ($p = 0.007$), but not the 6-week or pooled cohorts.

INSERT FIGURE 5 HERE

Changes in Mid-thigh VL Thickness, mCSA and Muscle Density

There was no GxT interaction for VL thickness in the 6- or 10-week cohorts. However, when pooled, there was a significant GxT interaction for VL thickness ($p = 0.041$). After decomposing the data, post-hoc analyses revealed that VL thickness significantly increased in the PP group (Pre = 1.8 ± 0.4 cm versus Post = 2.0 ± 0.4 cm, $p < 0.001$) (Fig. 6a) but did not change in the CTL (Pre = 2.1 ± 0.5 cm versus Post = 2.1 ± 0.5 cm, $p > 0.05$). Importantly, the change in VL thickness observed in the PP group was greater than the minimal difference to be considered real (0.16 cm). There was no significant GxT interaction for mCSA in any cohort (Fig. 6b). However, it is noteworthy that the interaction effect approached significance in both the 6-week ($p = 0.068$) and pooled cohorts ($p = 0.088$). There was a significant main effect of time for mCSA in the 10-week ($p = 0.001$) and pooled ($p = 0.001$) cohorts, but not the 6-week cohort ($p = 0.064$). Finally, there was no main effect of group for any cohort. After decomposing the mCSA data for the pooled cohort, post-hoc analysis revealed that mid-thigh mCSA significantly increased in the PP group (Pre = 109.6 ± 32.8 cm^2 versus Post = 115.8 ± 32.8 cm^2, $p = 0.01$) but not the CTL (Pre = 117.1 ± 22.9 cm^2 versus Post = 120.0 ± 22.5 cm^2, $p = 0.07$). There were no significant interaction or main effects for group in the 6-week, 10-week or pooled cohorts for muscle density (Fig. 6c). However, there was a main effect of time for this variable in the pooled cohorts ($p = 0.044$).

INSERT FIGURE 6 HERE

Right Leg Isokinetic Peak Torque

There was no difference in knee extensor torque at 60°/sec between groups and no GxT interaction. There was, however, a significant effect of time in the 6-week ($p = 0.004$) and pooled ($p = 0.011$) cohorts (Fig. 7a). When testing knee flexor torque at 60°/sec, there was no difference between groups in any cohort. However, there was a significant effect of time in the 6-week ($p = 0.020$), 10-week ($p = 0.014$) and pooled ($p = 0.001$) cohorts. While there was no interaction in the 6-week and pooled cohorts, there was a significant GxT interaction in the 10-week cohort ($p = 0.032$). The PP group significantly increased knee flexor torque (Pre = 49.5 ± 26.5 N × m versus Post = 75.0 ± 41.9 N × m, $p = 0.007$), whereas the CTL did not (Fig. 7b).

INSERT FIGURE 7 HERE

Discussion

There is good evidence to suggest that protein supplementation with RT enhances various training adaptations (reviewed in (25)). Notwithstanding, the majority of these studies have examined the effects of animal-based or soy protein supplements, and no studies to date have evaluated the efficacy of RT with PP supplementation on measures of muscle mass, function and strength.

There are several noteworthy findings herein. First, PP supplementation significantly increased knee flexion peak torque in the 10-week cohort relative to the CTL group. Additionally, when the 6- and 10-week cohorts were pooled, PP participants experienced significant increases in VL thickness compared to CTL participants. A similar trend was also observed regarding mid-thigh mCSA; specifically, the interaction trended ($p = 0.088$) and forced post hoc tests indicated that this metric increased in the PP participants from pre- to post-training ($p < 0.05$), whereas there was not a significant change in CTL participants. Our dynamometry data align with various studies that have reported RT with protein supplementation enhances lower-body leg strength relative to placebo supplementation (26, 27). Likewise, our muscle imaging data agree with various studies demonstrating protein supplementation with RT enhances muscle mass relative to placebo supplementation (27–30). However, there are also data showing that protein supplementation with RT does not affect variables related to muscle hypertrophy or strength in older individuals (31–33). Discrepancies between studies are likely due to various factors including the type of protein administered as well as the duration and type of RT. There is strong evidence to suggest protein needs (specifically, the intake of more essential amino acids) increase with age due to decreases in gastrointestinal function (34, 35). As mentioned prior, PP contains a full complement of amino acids. It is also notable that several of the studies cited above have examined the effects of whey protein with RT, and positive findings from these studies are likely due to the high leucine and essential amino acid content as well as the high protein digestibility corrected amino acid score of whey (36). One serving of PP in the current study (i.e., ~ 35 g protein) provided approximately 10 g of essential amino acids as well as 2 g of leucine. In comparison, 35 g of a whey protein isolate provides roughly 17 g of essential amino acids and ~ 4 g of leucine (36). Whether PP supplementation would be equally as good as or less

effective in enhancing training adaptations relative to whey protein supplementation remains to be determined. Notwithstanding, the current findings with PP supplementation are promising, and warrant future studies that are longer in duration and with different study populations.

Contrary to the data above, PP supplementation after one bout of resistance exercise did not enhance integrated MyoPS rates within a 24 h period following the first training bout. This finding is difficult to reconcile given that PP supplementation enhanced various training adaptations as described above. However, it is notable that post-exercise increases in MyoPS rates hours following an exercise bout have been shown to demonstrate poor agreement with long-term hypertrophic outcomes (reviewed in (37)). RT studies examining integrated MyoPS rates using D_2O over days or weeks into training have yielded better associations with hypertrophic outcomes (23, 38, 39). However, again, these correlations are modest at best. Therefore, we posit that the current MyoPS data continue to suggest that tracer data should be viewed independently of chronic training outcomes. Moreover, had we used an acute tracer infusion protocol with a phenylalanine stable isotope, we may have observed enhanced post-exercise MyoPS rates compared to no supplementation within a more acute time frame (i.e., 3–6 hours). Thus, given the paucity of data in this area, future research is needed to examine how the ingestion of different PP doses acutely affect MyoPS rates relative to a placebo supplement or other protein supplements.

A unique aspect of this study is the implementation of the pQCT to ascertain muscle quality. While no interactions existed for the metrics provided, it is interesting that muscle density increased over the duration of training, regardless of supplementation. This metric is not commonly reported in the exercise physiology literature given that pQCT and CT scanners are not readily available. However, our data agree with a study by Claassen et al. (40) where the authors used a CT scanner to report that six weeks of RT increased Hounsfield units of the mid-thigh by ~ 4–5% in college-aged men. In explaining their findings, the authors speculated that the observed increase in muscle density was due to either an increase in connective tissue density and/or an increase in contractile protein density. Thus, we interpret our data to suggest that RT increases muscle density through an increase in contractile and/or connective tissue density. Notably, this is an important finding given that a higher muscle density has been shown to be associated with physical function in overweight/obese older participants (41).

What should finally be noted is the lack of agreement between some of our body composition metrics. Although PP supplementation was found to increase vastus lateralis hypertrophy, no interactions between supplement groups were observed for whole-body DXA LSTM. Additionally, although a significant interaction was not observed for pQCT-derived mid-thigh mCSA changes, the interaction trended ($p = 0.088$) as discussed above. While these data are difficult to reconcile, we have noted in the past that the agreement between methods used to assess skeletal muscle hypertrophy poorly agree with one another (42). Thus, our data re-iterate the notion that various measures of muscle mass determination do not exhibit good agreement, and these findings continue to warrant future research in this area.

Experimental considerations

A notable limitation of the current study is the duration of the intervention of the second cohort. Given the unforeseen consequences of the SARS-CoV-2 pandemic, we voluntarily decided to prematurely end the second cohort 4 weeks early rather than jeopardize the health and safety of our participants. These data are also limited in that, while males and females completed the intervention, a lack of statistical power precluded the determination of gender interactions with PP supplementation. In spite of this limitation, it is notable that other studies have shown that males and females exhibit similar strength and hypertrophic responses to RT (43), as well as protein supplementation (25). Nevertheless, future studies are needed to determine if gender plays a role in the response to PP supplementation.

Conclusions

While preliminary, the results of the current study indicate that PP supplementation with 6–10 weeks of RT enhance certain aspects of muscle hypertrophy and strength in older adults, compared to a RT program alone in the elderly population. Future studies are needed to determine whether PP supplementation would be equally or less efficacious in affecting RT variables relative to other protein sources.

Abbreviations

CTL, control

DXA, dual-energy x-ray absorptiometry

FM, fat mass

GxT, group-by-time interaction

LSTM, lean soft tissue mass

mCSA, muscle cross sectional area

MyoPS, myofibrillar protein synthesis

PP, peanut protein

pQCT, peripheral quantitative computed tomography

RT, resistance training

VL, vastus lateralis

Declarations

Ethics approval and consent to participate

All procedures described herein were approved by the Auburn University IRB (protocol #19-249 MR 1907).

Consent for publication

Not applicable

Availability of data and material

All raw data can be obtained by emailing the corresponding author (mdr0024@auburn.edu).

Competing interests

None of the authors has competing interests to declare.

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Authors' contributions

This experiment was performed at Auburn University's School of Kinesiology in the Molecular and Applied Sciences Laboratory. A.D.F., M.D.R., K.W.H., and K.C.Y were responsible for the conception and design of the experiment. D.A.L. primarily drafted the manuscript. All authors were involved in different aspects of data collection. All authors read and approved the final manuscript.

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Figures

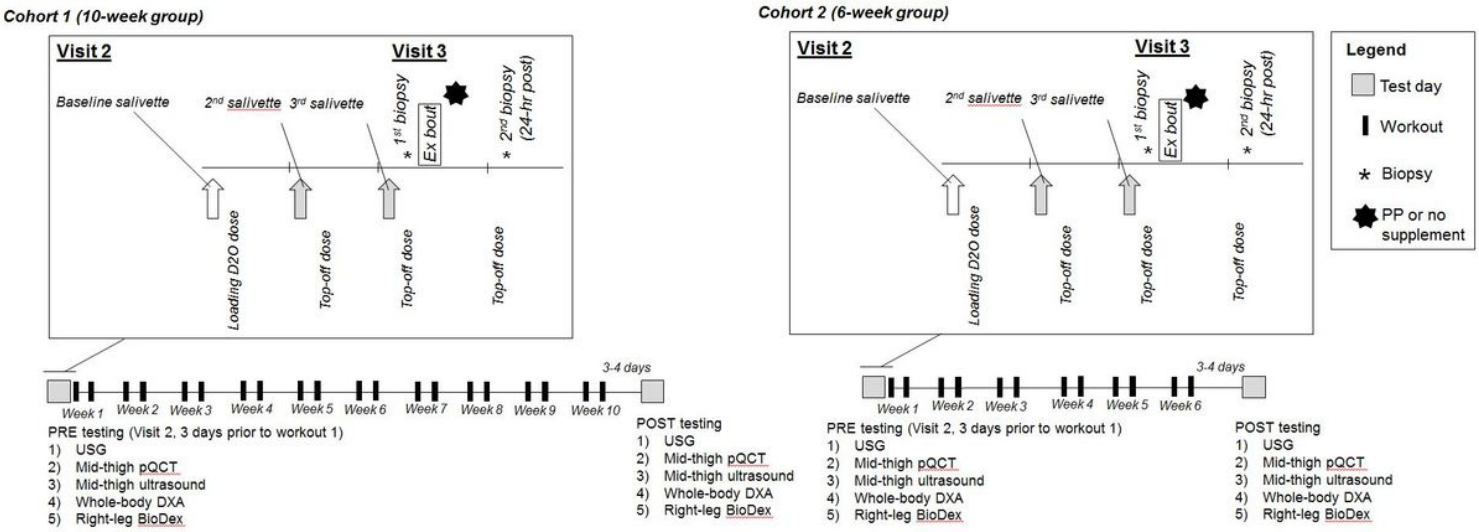

Figure 1

Study Design. The figure above outlines the study design for the 10- and 6-week cohorts.

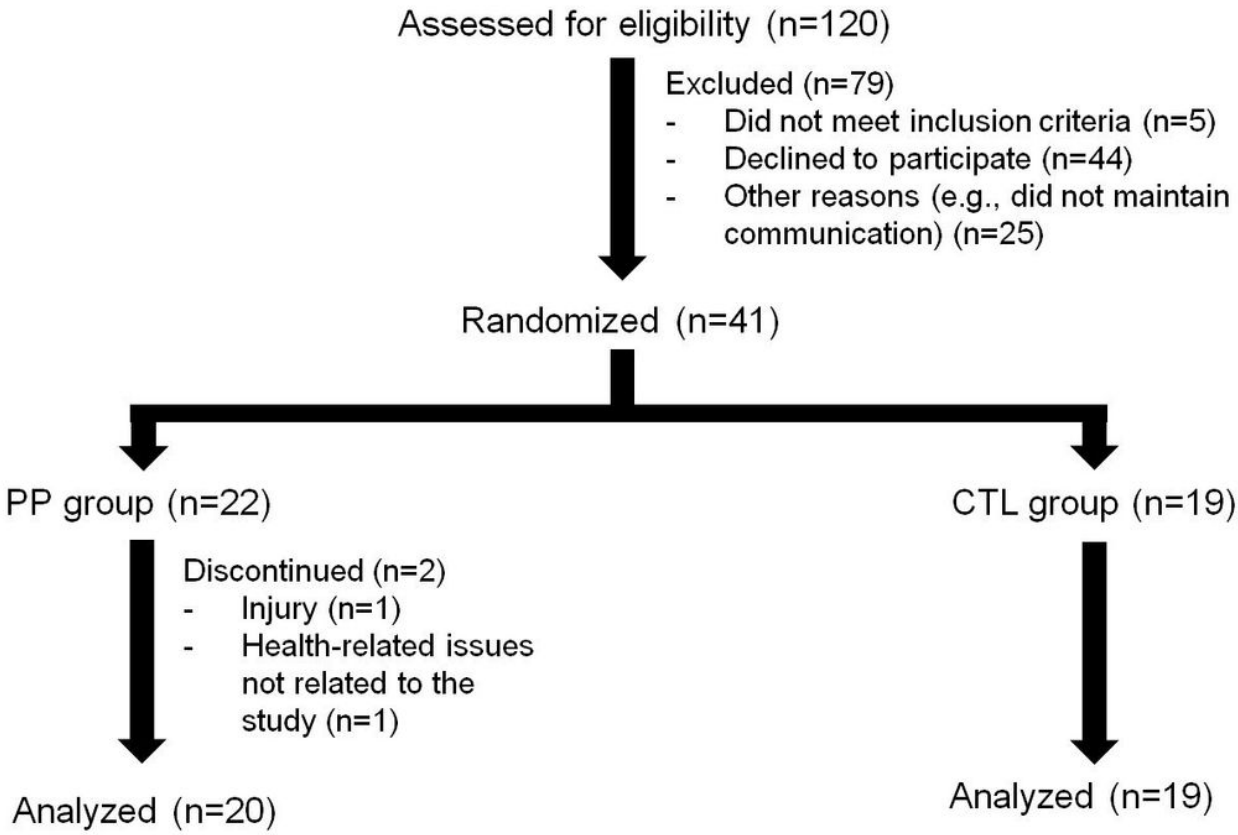

Figure 2

CONSORT Diagram The diagram indicates how many individuals were screened and completed the intervention.

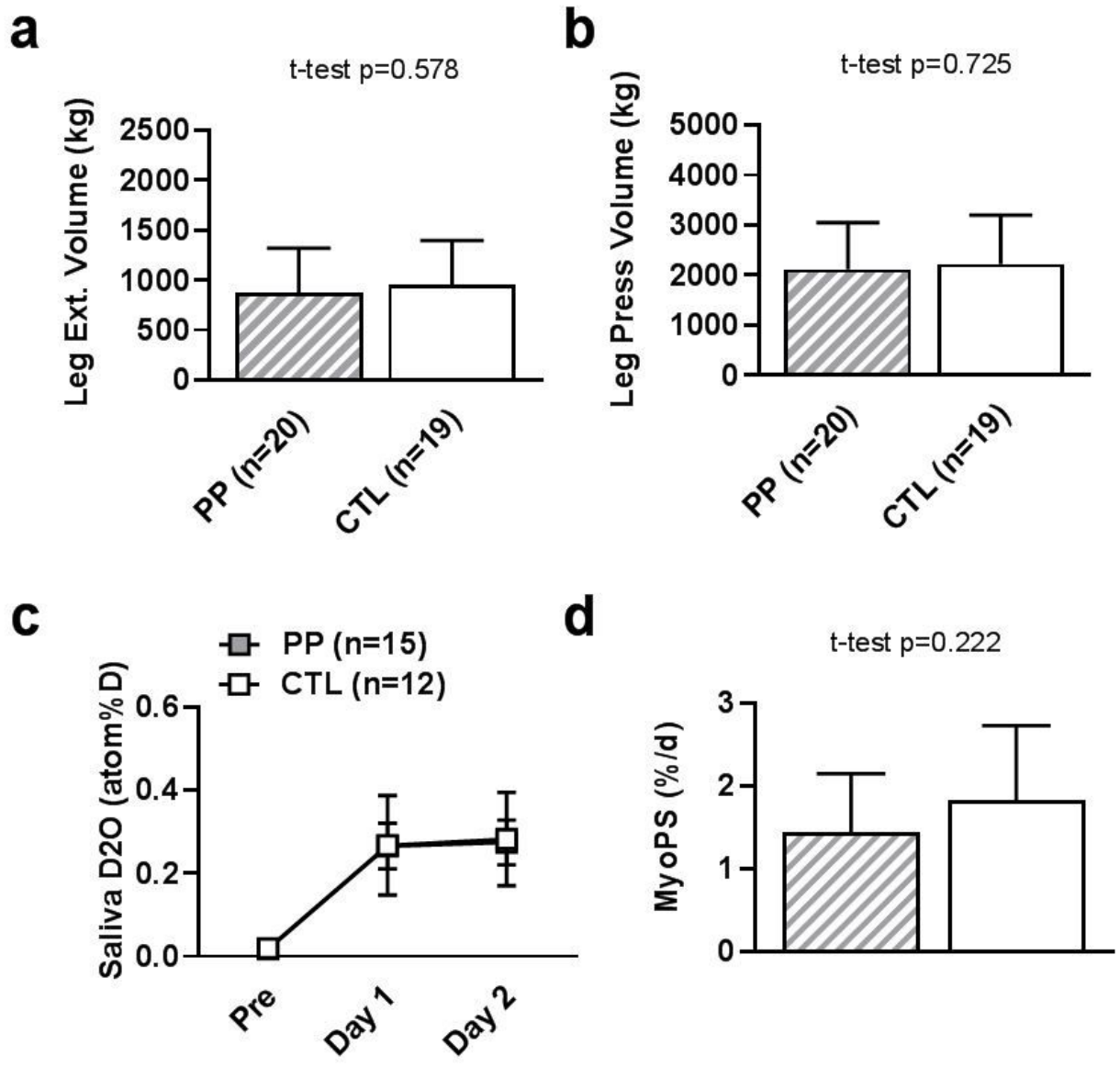

Figure 3

Myofibrillar protein synthesis rates following the first bout of training with or without PP supplementation No differences between conditions existed for the leg extensor (panel a) or leg press (panel b) training volume during the first training bout. Saliva D2O enrichment increased from baseline V2 to V3 and V4 regardless of supplementation (panel c). Myofibrillar protein synthesis rates 24 hours following the first exercise bout did not differ between PP and CTL participants (panel d). All data are presented as mean ± standard deviation values. Abbreviations: PP, peanut protein supplemented participants; CTL, non-supplemented participants.

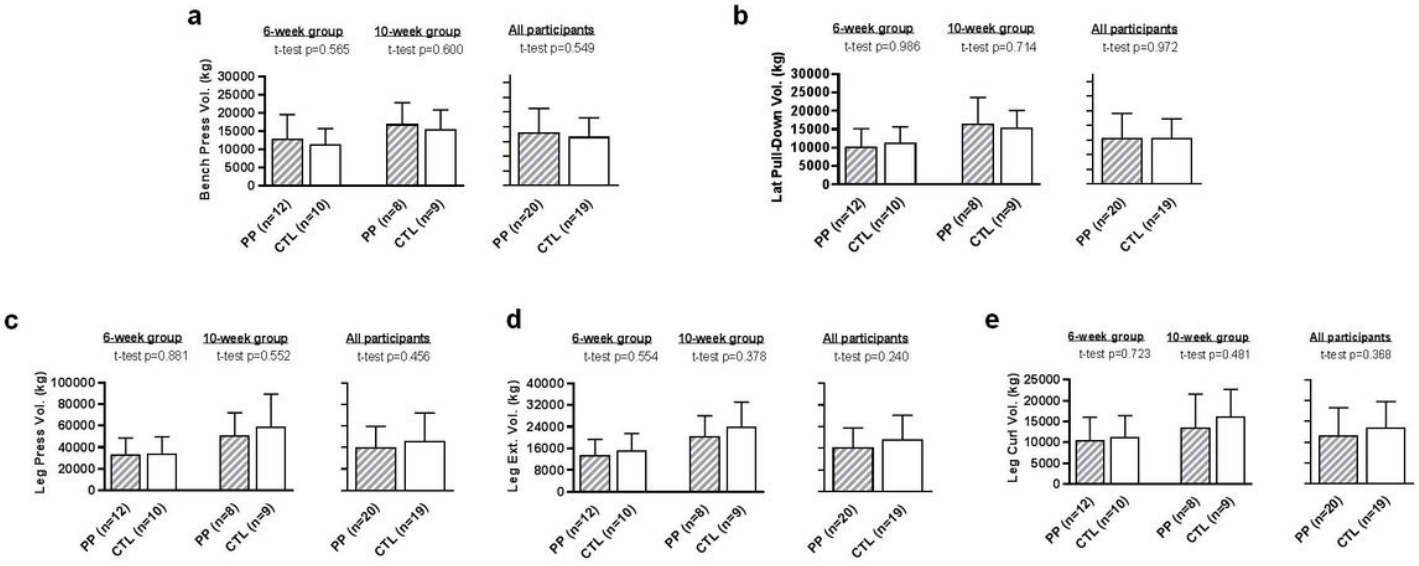

Figure 4

Differences in Exercise Volumes over the Duration of Training Data in this figure indicate that bench press volume (panel a), lat pulldown volume (panel b), leg press volume (panel c), leg extension volume (panel d), and leg curl volume (panel e) did not differ between supplementation groups in the 6-week, 10-week or pooled cohorts. All data are presented as mean ± standard deviation values. Abbreviations: PP, peanut protein supplemented participants; CTL, non-supplemented participants.

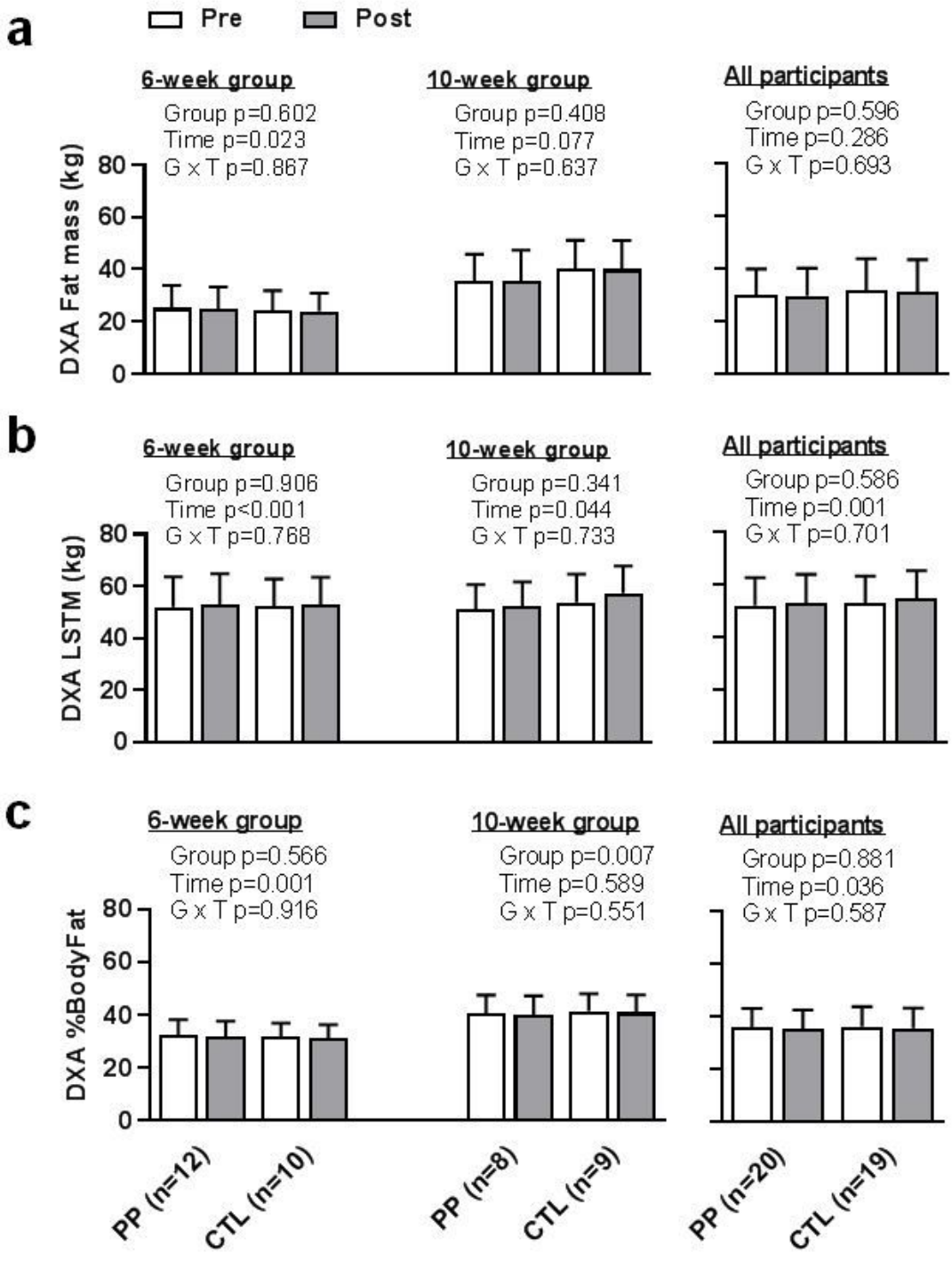

Figure 5

Changes in DXA Fat Mass, LSTM and Percent Body Fat Data in this figure indicate that changes in DXA-derived fat mass (panel a), DXA-derived lean soft tissue mass (panel b), or DXA-derived percent body fat (panel c) did not differ between supplementation groups. All data are presented as mean ± standard deviation values. Abbreviations: PP, peanut protein supplemented participants; CTL, non-supplemented participants.

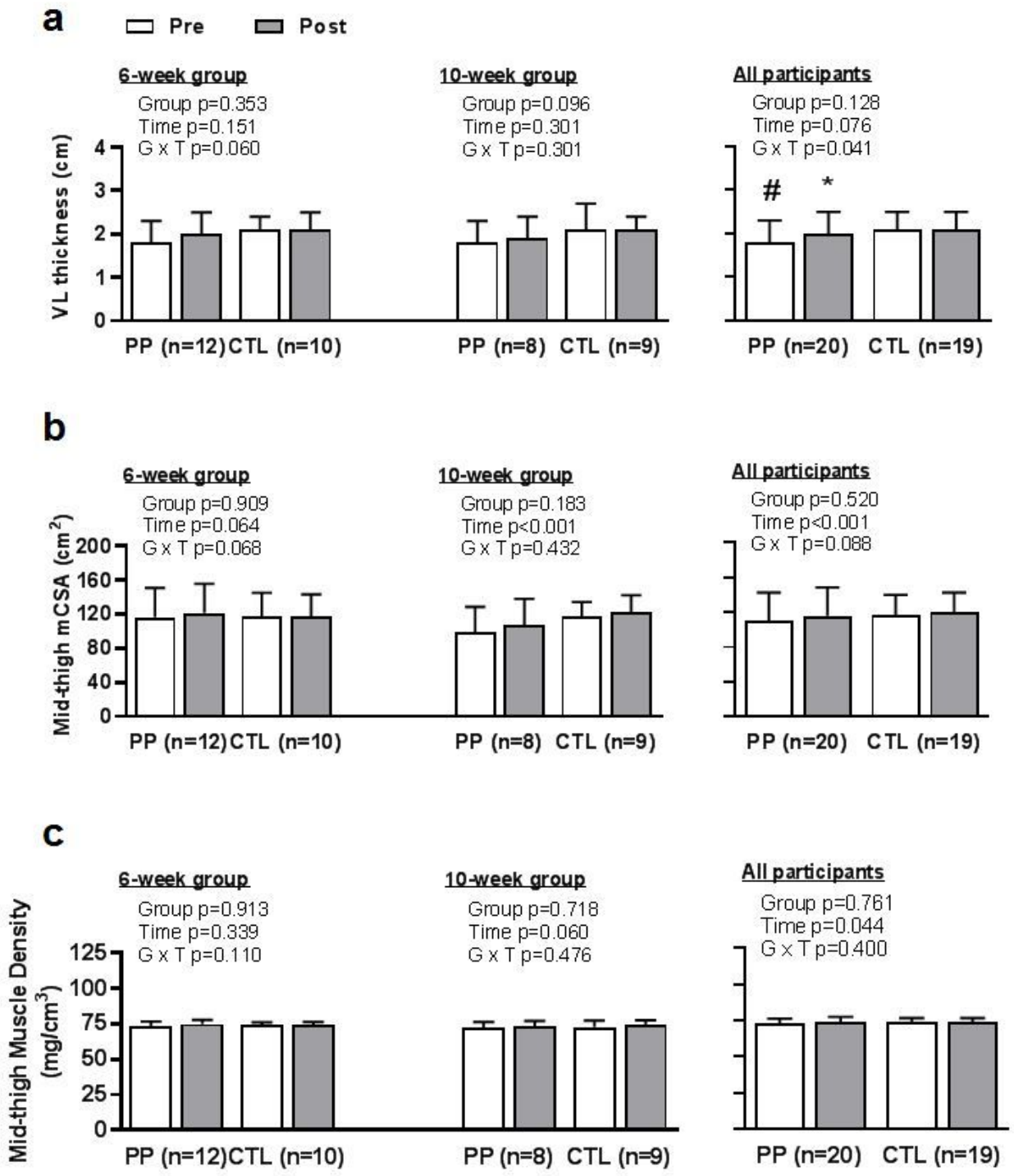

Figure 6

Changes in Mid-thigh Muscle Hypertrophy Measurements Data in this figure indicate that vastus lateralis (VL) muscle thickness increased in PP participants when the 6- and 10-week cohorts were pooled, whereas this did not occur in CTL participants (panel a). However, a significant interaction was not observed in pQCT-derived mid-thigh lean muscle cross sectional area values (panel b) or mid-thigh pQCT-derived muscle density (panel c). All data are presented as mean ± standard deviation values.

Abbreviations: PP, peanut protein supplemented participants; CTL, non-supplemented participants. Symbols: *, significant increase within PP from Pre to Post ($p<0.05$); #, PP < CTL at Pre ($p<0.05$).

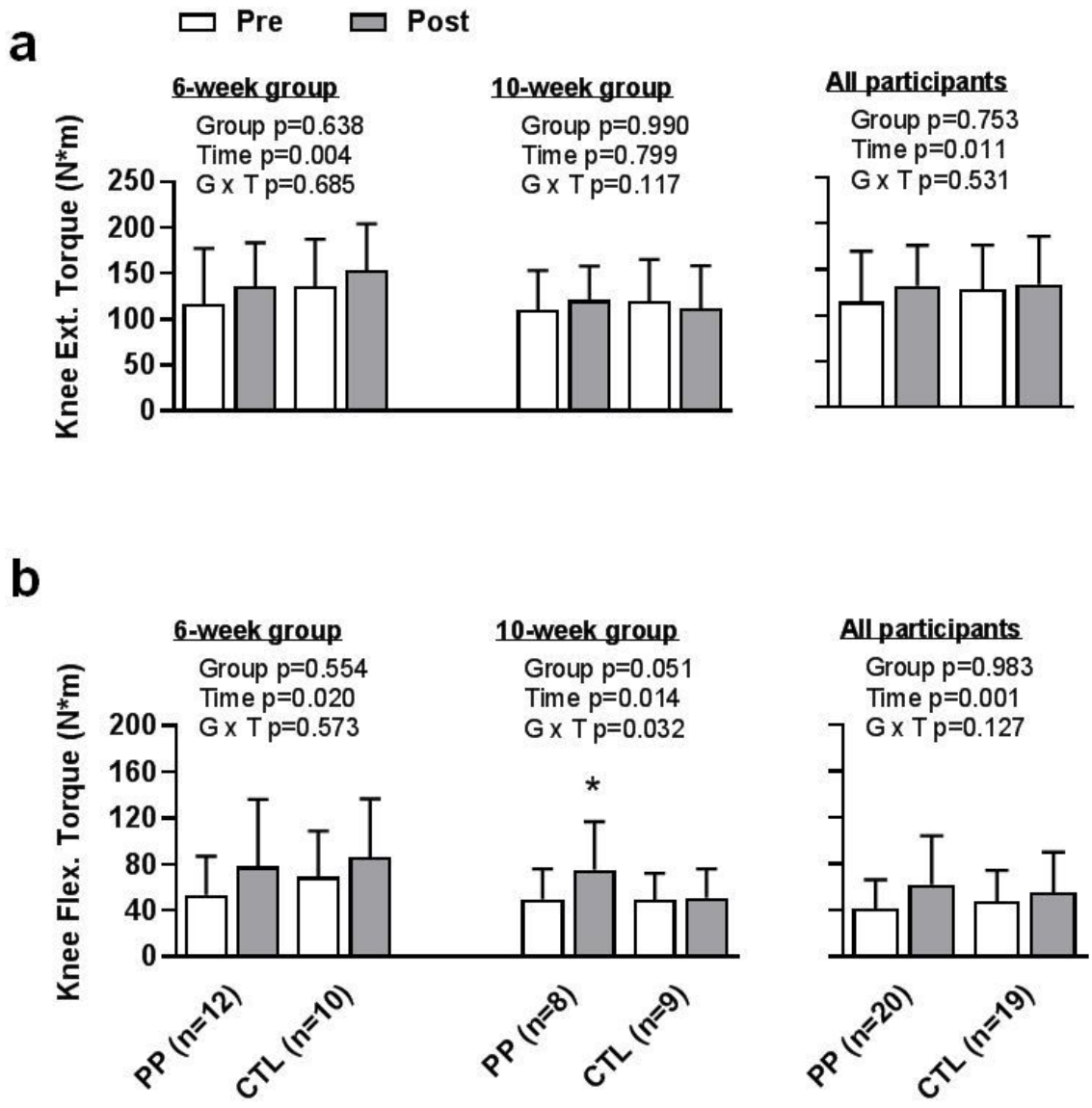

Figure 7

Right Leg Knee Extensor and Flexion Peak Torque Data in this figure indicate that knee extensor peak torque increased with training, regardless of supplementation (panel a). The same was observed with knee flexion peak torque (panel b); however, PP supplementation increased this metric in the 10-week cohort, whereas this metric did not increase in CTL participants. All data are presented as mean ± standard deviation values. Abbreviations: PP, peanut protein supplemented participants; CTL, non-supplemented participants. Symbols: *, significant increase within PP from Pre to Post ($p<0.05$).