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ORIGINAL ARTICLE

Darren G. Candow · Philip D. Chilibeck · Marina Facci
Saman Abeysekara · Gordon A. Zello

Protein supplementation before and after resistance training in older men

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Abstract We determined the effects of protein supplementation immediately before (PRO-B) and after (PRO-A) resistance training (RT; 12 weeks) in older men (59–76 years), and whether this reduces deficits in muscle mass and strength compared to younger men (18–40 years). Older men were randomized to PRO-B (0.3 g/kg protein before RT + placebo after RT, $n=9$), PRO-A (placebo before + protein after RT, $n=10$), or PLA (placebo before and after RT, $n=10$). Lean tissue mass, muscle thickness of the elbow, knee, and ankle flexors and extensors, and leg and bench press strength were measured before and after RT and compared to databases of younger subjects ($n=22$–60). Myofibrillar protein degradation (3-methylhistidine) and bone resorption (cross-linked N-telopeptides) were also measured before and after RT. Lean tissue mass, muscle thickness (except ankle dorsi flexors), and strength increased with training ($P<0.05$), with little difference between groups. There were no changes in 3-methylhistidine or cross-linked N-telopeptides. Before RT, all measures were lower in the older compared to younger groups ($P<0.05$), except for elbow extensor muscle thickness. Following training, muscle thickness of the elbow flexors and ankle dorsi flexors and leg press strength were no longer different than the young, and elbow extensor muscle thickness was greater in the old men ($P<0.05$). Supplementation with protein before or after training has no effect on muscle mass and strength in older men. RT was sufficient to overcome deficits in muscle size of the elbow flexors and ankle dorsi flexors and leg press strength in older compared to younger men.

Keywords Age · Muscle · Strength · Catabolism · Bone

D. G. Candow
School of Human Kinetics, Laurentian University, Sudbury, ON, Canada

P. D. Chilibeck (✉)
College of Kinesiology, University of Saskatchewan, 87 Campus Drive, S7N 5B2 Saskatoon, SK, Canada
E-mail: phil.chilibeck@usask.ca
Fax: +1-306-9666464

M. Facci
Department of Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, SK, Canada

S. Abeysekara · G. A. Zello
College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada

Introduction

Aging is associated with a loss of muscle mass which has a negative effect on strength. While resistance training (RT) increases muscle mass in healthy older individuals (Chrusch et al. 2001), it is unclear if nutritional supplementation with protein adds any benefit.

Resistance training results in significant muscle protein turnover and the rate of muscle protein synthesis following exercise is elevated with oral consumption of amino acids (Tipton et al. 2001). Given these findings, one would expect that an increase in amino acid availability through additional dietary protein during RT would increase muscle mass and strength. However, amino acid ingestion during 12 weeks of RT did not increase muscle mass and strength to a greater extent than training alone in older men (Godard et al. 2002). Furthermore, high-protein meals (~28% of energy intake/meal) did not enhance the increase in myofibrillar protein synthesis induced by RT in sedentary older men and women (Welle and Thornton 1998). Increasing the quantity of dietary protein intake during RT may not be effective for increasing muscle mass and strength in older individuals.

Recent evidence suggests that the timing of protein intake during RT is important for increasing muscle mass (Andersen et al. 2005; Esmarck et al. 2001). Protein supplementation before and after RT sessions for 14 weeks resulted in a significant muscle fiber hypertrophy in young males compared to placebo (Andersen et al. 2005), and older males who ingested protein (~10 g)

immediately after RT sessions for 10 weeks had significant increases in muscle size and strength compared to when protein was ingested 2 h post-exercise (Esmarck et al. 2001).

Recently Tipton et al. (2001) showed that ingesting an amino acid solution immediately before resistance exercise was more beneficial for stimulating amino acid uptake and protein synthesis than when the solution was consumed immediately after exercise. The elevated blood flow during exercise resulted in greater net amino acid uptake and protein synthesis when the amino acid solution was consumed before the exercise bout compared to amino acids consumed after the exercise bout. Muscle protein synthesis is depressed (Bylund-Fellenius et al. 1984) or unchanged (Carraro et al. 1990) during an exercise bout and protein breakdown is elevated (Rennie et al. 1981). Increasing amino acid availability during exercise may counter this net loss of muscle protein. Based on the findings of Tipton et al. (2001) that protein synthesis is elevated to a greater degree when amino acids are consumed before, rather than after an acute bout of resistance exercise, we hypothesized that consuming a protein supplement before resistance exercise training sessions over 12 weeks would result in greater increases in muscle mass and strength compared to consuming a protein supplement immediately after exercise. The primary purpose of this investigation was to examine the effect of protein supplementation before and after RT on muscle mass and strength in older men.

We previously assessed deficits in lean tissue mass, strength, and muscle size (thickness) of older compared to younger men. Older men had lower lean tissue mass, strength, and muscle thickness for the elbow flexors, and knee and ankle flexors and extensors compared to younger men (Candow and Chilibeck 2005). An additional purpose of the current study was to determine if RT could eliminate deficits between young and older men.

Secondary measures in the current study included markers of muscle protein turnover (myofibrillar protein degradation, by 3-methylhistidine) and bone resorption (cross-linked N-telopeptides of type I collagen). Protein or amino acid supplementation increases the ratio of protein synthesis to degradation post-exercise (Biolo et al. 1997) and protein supplementation in older adults may have a favorable effect on bone by increasing anabolic hormone production and decreasing bone resorption (Dawson-Hughes et al. 2004). We therefore hypothesized that protein supplementation would decrease myofibrillar protein degradation and bone resorption.

Methods

Subjects

A group of 38 men (59–76 years) who were not engaged in RT volunteered for the study. All subjects were instructed not to change their diets before or during the RT program. Subjects less than 70 years of age were required to fill out a Physical Activity Readiness Questionnaire, which screens for health problems that may present a risk with performance of physical activity (Thomas et al. 1992). Subjects that indicated a risk and all subjects over 69 years of age were required to have medical approval before participating in the study. The study was approved by the University Ethics Review Board for Research in Human Subjects. The subjects were informed of the risks and purposes of the study before their written consents were obtained.

Study design

The study used a double-blind repeated measures design in which every subject participated in RT three times per week and were randomized to one of two protein conditions (either supplementing with protein before or after training sessions) or placebo for 12 weeks. Prior to the first visit to the laboratory for initial testing and data collection, all subjects were instructed to refrain from physical activity for 48 h. The dependent variables measured before and after the 12 weeks of supplementation and training were (1) lean tissue mass, (2) muscle thickness of flexors and extensors of the elbow, knee, and ankle, (3) strength (leg press and bench press one repetition maximum; 1-RM), (4) urinary 3-methylhistidine excretion (an index of myofibrillar protein degradation), and (5) urinary cross-linked N-telopeptides of type I collagen (an index of bone resorption). In addition, subjects completed dietary records for 3 days during the first and final week of RT and supplementation to assess nutrient differences between groups. At the end of the study, subjects were asked whether they perceived they were on the protein supplement or placebo.

In addition to measuring changes in lean tissue mass, muscle thickness, and strength before and after supplementation and training, these pre- and post-training measures from the older subjects were compared to three reference groups of moderately active young males: One for comparison of lean tissue mass ($n=57$; age $=24.5\pm0.6$ years; mass $=81.6\pm1.8$ kg; height $=178\pm1$ cm), one for comparison of muscle thickness ($n=22$; age $=22.0\pm0.7$ years; mass $=80.8\pm3.0$ kg; height $=179\pm1$ cm; Candow and Chilibeck 2005); and one for comparisons of bench press and leg press strength ($n=60$; age $=26.6\pm0.8$ years; mass $=80.9\pm1.9$ kg; height $=178\pm1$ cm) to determine if the deficits in lean tissue mass, muscle thickness, and strength of the older men could be overcome with the intervention.

Randomization and supplementation

An individual who was not involved in the study was responsible for randomizing the subjects and coding the supplements to ensure all subjects and investigators remained blinded throughout the study. Each subject was randomly assigned to supplement orally with either

protein [0.3 g protein/kg body mass (in 0.54 g/kg of Myoplex®, Experimental and Applied Sciences, Inc., Golden, CO, USA), and 0.09 g/kg chocolate cocoa; see Table 1 for Myoplex ingredients] immediately before and placebo (PLA, maltodextrin/sucrose/chocolate cocoa, 0.63 g/kg body mass) immediately after RT (PRO-B); placebo immediately before and protein immediately after RT (PRO-A); or placebo immediately before and after RT (PLA). We used this combination of Myoplex and chocolate cocoa for the protein supplement and maltodextrin/sucrose/chocolate cocoa for the placebo because we found this was effective for matching the protein and placebo for energy content, taste, texture, color, and appearance. The Myoplex supplement provided approximately 2.1 kcal/kg/day. The protein blend in Myoplex includes whey protein from whey protein concentrate and whey protein isolate, calcium caseinate, milk protein isolate, sodium caseinate, and egg albumin. The protein dose of 0.3 g/kg body mass was chosen because it is an approximate amount shown to increase muscle mass during RT (Burke et al. 2001; Esmarck et al. 2001). Subjects were instructed to arrive for their training sessions in a fasted state (at least 3 h). The supplements were mixed in cold water and provided to each subject immediately before and immediately after each RT session. The supplement was consumed within 5 min.

Resistance training

Prior to the start of the study, each subject familiarized themselves with the RT equipment by participating in three supervised RT sessions three times a week for 2 weeks. Familiarization with the RT equipment helped decrease the amount of learning (i.e., rapid improvement in the ability to perform a training exercise) which may contribute to the increase in strength during the initial stages of RT (Chilibeck et al. 1998). Subjects subsequently participated in 12 weeks of RT combined with the protein or placebo supplements. Prior to training sessions, but after the supplement drink was consumed, each subject warmed up for 10 min on a stationary Monarch (Ergomedic 818E; Stockholm, Sweden) cycle ergometer and completed light stretching. Training sessions were supervised as previous research has demonstrated greater gains compared to unsupervised training (Mazzetti et al. 2000). Training sessions were completed at the convenience of each subject and were approximately 60 min in duration. There was no prescribed order to completing their weekly sessions, however subjects were encouraged to take at least 1 day rest between subsequent training days to reduce the chance of injury and minimize fatigue. Subjects trained 3 days/week for 3 sets of 10 repetitions with 2-min rest between sets for each exercise at an intensity corresponding to approximately 70% 1-RM for the leg press and bench press and a weight corresponding to their 10 repetition maximum for other exercises. We have previously used a similar RT program successfully to increase muscle mass and strength in older men (Chrusch et al. 2001). Resistance exercises included leg press, leg (knee) extension, leg curl (knee flexion), and calf press using Hammer Strength equipment (Life Fitness, Franklin Park, IL, USA), and bench press, shoulder press, lat pull down, and biceps curl using Lever equipment (Pulse Fitness Systems, Winnipeg, MB, Canada), and triceps extension using Paramount Fitness equipment (Apple Fitness; Edmonton, AB, Canada). Subjects maintained daily training logs where average training volume per session (weight × sets × repetitions) was determined for each subject. Resistance was increased by 2–5 kg once a subject completed 3 sets of 10 repetitions for an exercise with good technique.

Table 1 Ingredients in Myoplex supplement (g/kg/day)

Protein	3.0×10^{-1}
Carbohydrates	1.7×10^{-1}
Fat	1.8×10^{-2}
Cholesterol	1.1×10^{-4}
Sodium	2.9×10^{-3}
Calcium	3.6×10^{-3}
Iron	1.0×10^{-5}
Vitamin A	2.1×10^{-6}

Body composition

Body composition was assessed by air-displacement plethysmography (Bod Pod S/L, Life Measurement Inc., Concord, CA, USA). Prior to measurements all subjects were instructed to refrain from physical activity for 24 h and food and drink for 3 h. Subjects were also instructed to remove all jewelry and shave all excess hair that is part of their normal routine. Subjects were weighed and measured (height) wearing Lycra shorts and a swim cap after voiding their bladder. Subjects were then seated in the Bod Pod chamber and sealed so that measurements of whole-body volume could be made. Subjects were instructed to relax, breathe normally, and sit still during the 20-s measurement. Repeated trials were completed until consistent results were achieved as determined by the Bod Pod software (Life Measurement Instruments, Software Version 1.69, Concord, CA, USA). Once the test was complete (2–5 min), density was calculated by dividing the individual's mass by body volume, corrected for estimated lung volume. Percent body fat (% fat) was derived using the equation: % fat = 495/density - 450 (Siri 1966). Lean tissue mass was then determined by: total body mass - (% fat × total body mass). Reproducibility was assessed by testing 16 subjects 1 week apart. The coefficient of variation [i.e., the square root of the between-test variance (standard deviation), divided by the combined (marginal) mean of the test results for days 1 and 2, multiplied by 100 (to produce a percentage)] for lean tissue mass was 0.87%. The validity of our Bod Pod was checked by measuring 15 of the subjects on the Bod Pod and by dual-energy X-ray absorptiometry (DXA; Hologic QDR 2000, Waltham, MA, USA). The correlation coefficient between Bod Pod and DXA measurements was 0.96 ($P < 0.01$).

Muscle thickness

Muscle thickness of the flexors and extensors of the elbow, knee, and ankle was measured using B-Mode ultrasound (Aloka SSD-500, Tokyo, Japan) as we have described in detail recently (Candow and Chilibeck 2005). Reproducibility of muscle thickness measurements was determined by testing 16 subjects 1 week apart. The coefficients of variation for muscle thickness measurements were: elbow flexors (2.6%), elbow extensors (2.1%), knee flexors (2.3%), knee extensors (2.1%), ankle plantar flexors (3.1%), and ankle dorsi flexors (4.0%).

Muscular strength

Leg press and bench press strength was assessed using a 1-repetition maximum standard testing procedure (Chrusch et al. 2001) prior to and following supplementation and RT. These two exercises were chosen as an index of muscular strength because they involve the major muscle groups in the lower and upper body. Reproducibility of the strength measures was assessed on ten subjects, 1 week apart. The leg press and bench press strength measures had coefficients of variation of 3.8 and 3.1%, respectively.

Myofibrillar degradation and bone resorption

For the measurement of 3-methylhistidine, an index of myofibrillar protein degradation, and cross-linked N-telopeptides of type I collagen, an index of bone resorption, urine was collected during the last 24-h of a 72-h meat-free diet immediately before and immediately after the study. A meat-free diet was implemented because meat consumption increases urinary 3-methylhistidine values and may falsely represent an increase in myofibrillar protein turnover (Lukaski et al. 1981). Three days of a meat-free diet are required to return 3-methylhistidine levels to baseline (Lukaski et al. 1981). The designated urine collection procedure was to discard the first urination upon waking in the morning and then collect all urine samples for 24 h, including the first urination upon waking the following morning. Urine samples were brought to the researcher where the subject's urine volume was recorded. Aliquots of each urine sample were drawn off from the 24-h collection and stored at −20°C until analyzed. The concentration of 3-methylhistidine was determined by high-performance liquid chromatography (3-mm Chromsep ODS-2 column, Varian Inc., Mississauga, ON, Canada; flow rate 1.0 ml/min) and 2475 multi-wavelength fluorescence detection (Waters, Mississauga, ON, Canada) using the methods of Wassner et al. (1980) with modification for sample volumes. Derivatization was completed by placing 200 µl of diluted (ten times with 0.9% NaCl) urine samples or 3-methylhistidine standards (Pfaltz and Bauer, Waterbury, CT, USA), 1 ml of borate buffer (0.25 M boric acid, adjusted to pH 9.5 with NaOH), 1 ml of fluorescamine reagent (acetonitrile containing 1 mg fluorescamine per ml) in glass autosampler vials, which were then mixed and allowed to stand at room temperature for 5 min. Two hundred microliters of 70% perchloric acid was added to the vials, which were capped with teflon-lined seals and heated at 80°C for 1 h. After cooling to room temperature, samples were filtered using Acrodisc® 13-mm syringe filters with 0.2 Supor® membrane (Pall Corporation, MI, USA). Filtered samples were injected (20 µl) with an autosampler (715 Ultra WISP autoinjector, Waters, Mississauga, ON, Canada). The mobile phase was 23% acetonitrile and 77% 20 mM Na_2HPO_4 adjusted to pH 7.2 with NaOH. Peaks were monitored at 365 nm (excitation) and 460 nm (emission) and integrated with chromatography software (Millenium chromatography manager Millenium^{32}, version 4, Waters, Mississauga, ON, Canada). The intra-assay coefficient of variation from duplicate samples was 5.1%. The daily amount of 3-methylhistidine excreted by each subject was determined by multiplying the concentration by the 24-h urine volume.

The concentration of cross-linked N-telopeptides of type I collagen was determined using a competitive-inhibition enzyme-linked immunosorbent assay according to procedures from commercially available kits (Osteomark NTx test, Ostex International, Inc., Seattle, WA, USA). Samples were analyzed in triplicate within a single assay. The intra-assay coefficient of variation was 7.3%. The daily amount of cross-linked N-telopeptides of type I collagen excreted by each subject was determined by multiplying the concentration by the 24-h urine volume.

Dietary intake

Dietary intake was recorded for 3 days early in the first week and during the final week of supplementation and RT to assess whether there were differences in total energy and macronutrient composition between the protein and placebo treatment conditions. Dietary intake was not recorded immediately before and immediately following the study because habitual dietary intake immediately before and immediately following the study would be altered because of the 72-h meat-free diet required for determination of urinary 3-methylhistidine. Subjects used a 3-day food booklet to record what they ate for 2 weekdays and 1 weekend day. Subjects were instructed to record all food items, including portion sizes consumed for the 3 designated days. The Interactive Healthy Eating Index (Center for Nutrition Policy and Promotion, USDA) was used to analyze 3-day food records. Each food item was entered and the program provided total energy consumption on average over the 3 days as well as energy from carbohydrates, fats, and proteins individually.

Statistical analyses

A 3 (PRO-B vs. PRO-A vs. PLA group) × 2 (pre- and post-test periods) ANOVA with repeated measures on

the second factor was used to determine whether there were any differences between the protein and placebo groups over time for the dependent variables of lean tissue mass, muscle thickness, strength, 3-methylhistidine, cross-linked N-telopeptides of type I collagen, and diet (energy and macronutrient contents). To clarify presentation of the results, a one-factor ANOVA was used to evaluate change scores for the dependent variables and to determine differences between the protein groups combined and the placebo group. Change scores were determined by subtracting baseline measurements from week 12 measurements. A one-factor ANOVA was used to determine differences in average training volume (kg × sets × reps) per session between the protein and placebo groups and to determine whether there were differences in baseline measurements between groups. An LSD post-hoc test was used to identify differences between means when interactions were found. Separate one-factor ANOVAs were used to compare pre- and post-training values for lean tissue mass, muscle thicknesses, and strength of the old group to our young reference groups. All results are expressed as means (SEM). Statistical analyses were carried out using SPSS version 11.5 for Windows XP (SPSS Inc., Chicago, IL, USA). Significance was set at $P<0.05$.

Results

Of the original 38 subjects, 29 completed the study. Of the original subjects, 1 subject in the PLA group withdrew because of cataract surgery. Two subjects (1 from the PRO-B and 1 from the PRO-A) withdrew because of shoulder and knee pain. There were 6 subjects (2 from each group) who withdrew due to time constraints. There were 27 subjects (8 in PRO-B group, 10 in PRO-A group, and 9 in the PLA group) who were able to provide urine samples for analysis of 3-methylhistidine and N-telopeptides. Twenty-eight subjects (9 in PRO-B group, 10 in PRO-A group, and 9 in the PLA group) were able to provide 3-day food records during the first and final week of training. Five subjects (1 in PRO-B group, 2 in PRO-A group, and 2 in the PLA group) were correct in perceiving they were on the protein supplement or placebo, with the remaining subjects not knowing whether they were on the protein supplement or placebo. Baseline characteristics of subjects who completed the study are shown in Table 2. There were no differences between the protein and placebo groups for any of the baseline measurements.

There was a significant time main effect for lean tissue mass ($P<0.05$) but no differences between groups (Fig. 1). The relative increases in lean tissue mass for the PRO-B, PRO-A, and PLA groups were 1.2±0.7, 1.7±1.0, and 1.0±1.3%, respectively. Lean tissue mass of the combined groups of older men before (58.3±1.3 kg) and after (59.0±1.2 kg) training was lower than younger men (64.3±1.0 kg) from our database ($P<0.05$).

Table 2 Mean (SEM) subject characteristics at baseline for protein before (PRO-B), protein after (PRO-A), and placebo (PLA) groups

Group	Age (year)	Mass (kg)	Height (cm)
PRO-B ($n=9$)	63.3 (1.1)	87.5 (6.4)	176 (2)
PRO-A ($n=10$)	66.5 (1.7)	85.3 (3.6)	173 (2)
PLA ($n=10$)	64.6 (1.3)	87.2 (5.8)	173 (1)

Note: There was no difference between groups at baseline

There was a significant time main effect for leg press and bench press strength with training (Fig. 2a, b; $P<0.05$). The relative increases in leg press 1-RM for the PRO-B, PRO-A, and the PLA groups were 31±5, 25±5, and 22±5%, respectively. The relative increases for bench press 1-RM for the PRO-B, PRO-A, and PLA groups were 28±9, 23±7, and 28±7%, respectively. There were no differences between groups for changes in strength with training. Bench press strength of the combined groups of older men before (77±4 kg) and after training (95±4 kg) were lower than younger men (121±4 kg) from our database ($P<0.05$). Leg press strength of the combined groups of older men before training (169±7 kg) was lower than the younger men (231±7 kg, $P<0.05$). After training, leg press strength of the older men (210±8 kg) did not differ from the younger men.

A significant increase in muscle thickness for all muscle groups ($P<0.05$), except the ankle dorsi flexors, was observed with training. There was a greater increase in muscle thickness of the knee extensors ($P<0.05$) for the PRO-B group vs. PLA group, with no other differences between groups (Table 3). The increase in muscle thickness for the six muscle groups combined was 3.3±0.3 cm for the PRO-B group, 2.4±0.4 cm for the PRO-A group, and 2.6±0.4 cm for the PLA group. Before the training program, muscle thickness of the old group was significantly less than the young group for all sites, except the elbow extensors (Table 4). Following 12 weeks of training in the older men, muscle thickness of the elbow flexors and ankle dorsi flexors was no longer different compared to the young, and elbow extensor muscle thickness was greater in the old vs. young men (Table 4, $P<0.05$).

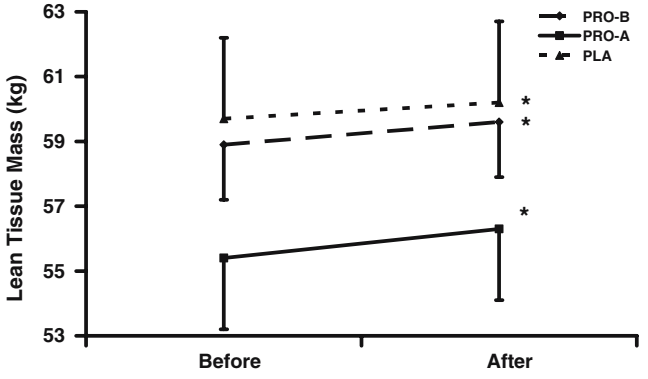

Fig. 1 Lean tissue mass before and after 12 weeks of supplementation and resistance training for PRO-B, PRO-A, and PLA groups. Values are means ± standard error. *Time main effect ($P<0.05$), with no differences between groups

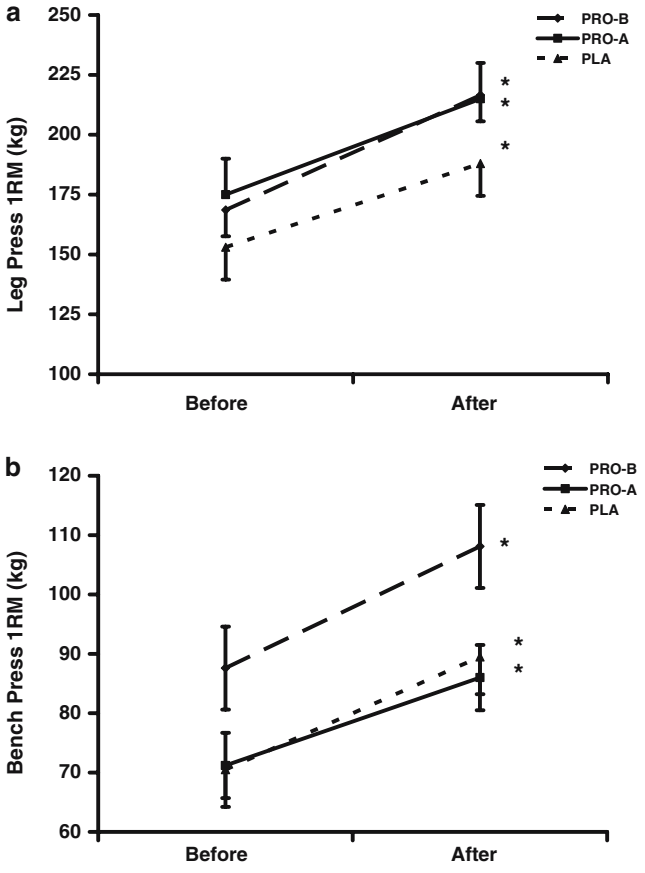

Fig. 2 **a** Leg press and **b** bench press strength (1-RM) before and after 12 weeks of supplementation and resistance training for PRO-B, PRO-A, and PLA groups. Values are means ± standard error. *Time main effect ($P < 0.05$), with no differences between groups

There were no differences in urinary 3-methylhistidine levels or cross-linked N-telopeptides of type I collagen over time in any groups. For 3-methylhistidine, the PRO-B group had values of 4.4±0.9 and 4.4±0.6 μmol/kg lean tissue mass, the PRO-A group had values of 5.8±0.9 and 5.4±1.0 μmol/kg lean tissue mass, and the PLA group had values of 2.7±0.3 and 3.2±0.5 μmol/kg lean tissue mass before and after training, respectively. For cross-linked N-telopeptides of type I collagen, the PRO-B group had values of 6.7±1.5 and 9.4±1.8 nmol/kg lean tissue mass, the PRO-A group had values of 10.7±1.9 and 10.7±1.9 nmol/kg lean tissue mass, and the PLA group had values of 7.6±0.5 and 7.2±1.8 nmol/kg lean tissue mass, before and after training, respectively.

There were no differences in average training volume per session between the PRO-B, PRO-A, and the PLA groups. The PRO-B group had a mean average volume of 14,897±1,205 kg per training session, the PRO-A group had an average volume of 14,278±1,185 kg per training session, while the PLA group had an average volume of 13,310±1,022 kg per training session. From analysis of 3-day dietary intakes, there was a time main effect for energy intake, with energy intake increasing from the beginning to end of the training program across groups ($P < 0.05$, Table 5). There was a significant group × time interaction for dietary protein intake ($P < 0.05$). Post-hoc analysis indicated that the PRO-B group consumed significantly more dietary protein before training compared to the PRO-A group ($P < 0.05$), but the difference between groups was not significant at the end of training. There were no differences between the protein groups and the placebo group.

Discussion

To our knowledge, this is the first study to investigate the effects of protein supplementation when taken before and after RT in healthy older men. We hypothesized that

Table 3 Muscle thickness measurements (cm) for the flexor and extensor muscles surrounding the elbow, knee, and ankle in older men before and after 12 weeks of supplementation and resistance training

Muscle group	PRO-B			PRO-A			PLA		
	Pre	Post	%	Pre	Post	%	Pre	Post	%
Elbow flexors	2.9 (0.2)	3.5 (0.1)*	22.3 (5.1)	2.8 (0.2)	3.3 (0.1)*	20.4 (3.9)	2.7 (0.1)	3.1 (0.1)*	16.3 (3.9)
Elbow extensors	4.1 (0.2)	5.1 (0.2)*	24.7 (4.0)	3.9 (0.2)	4.6 (0.3)*	16.1 (3.8)	3.9 (0.2)	4.7 (0.2)*	25.0 (5.0)
Knee flexors	4.4 (0.3)	5 (0.2)*	16.7 (8.7)	4.4 (0.2)	4.7 (0.2)*	8.2 (4.8)	4.5 (0.4)	5 (0.4)*	14.9 (4.5)
Knee extensors	3.1 (0.2)	3.7 (0.2)*	21 (6.1)**	3.4 (0.1)	3.8 (0.2)*	11.9 (4.3)	3.6 (0.3)	3.8 (0.4)*	4.9 (2.3)
Ankle plantar flexors	3.1 (0.2)	3.5 (0.2)*	12.1 (4.3)	3.5 (0.4)	4 (0.4)*	18.1 (8.3)	3.0 (0.2)	3.3 (0.2)*	14.6 (6.7)
Ankle dorsi flexors	2.2 (0.2)	2.4 (0.1)	11.6 (7.9)	2.4 (0.1)	2.5 (0.2)	3.5 (4.4)	2.2 (0.1)	2.5 (0.2)	12 (8.7)
Ave. total change	18.2 (5.3)			13 (4.6)			15 (4.1)		

Note: Values are means (SEM)
% = percent change over time
*Significant time main effect ($P < 0.05$)
**PRO-B had greater gains in knee extensor muscle thickness vs. PLA ($P < 0.05$)

Table 4 Muscle thickness measurements (cm) for the flexor and extensor muscles surrounding the elbow, knee, and ankle in older men before and after 12 weeks of supplementation and resistance training compared to untrained young men

Muscle group	Old ($n=22$)		Young ($n=22$)
	Before	After	
Elbow flexors	2.8 (0.1)*	3.3 (0.1)	3.2 (0.1)
Elbow extensors	4.1 (0.1)	4.8 (0.1)**	4.0 (0.1)
Knee flexors	4.5 (0.1)*	4.9 (0.1)*	5.5 (0.1)
Knee extensors	3.5 (0.2)*	3.8 (0.2)*	4.2 (0.2)
Ankle plantar flexors	3.3 (0.2)*	3.6 (0.2)*	4.4 (0.3)
Ankle dorsi flexors	2.3 (0.1)*	2.5 (0.1)	2.7 (0.1)

Note: Values are means (SEM)
*Values for older men are less than for young men ($P<0.05$)
**Older men had greater muscle thickness after training compared to young men ($P<0.05$)

protein supplementation immediately before resistance exercise would increase muscle mass and strength over protein ingestion immediately following RT. Our results do not support our hypotheses. Results showed that protein supplementation either before or after training had minimal beneficial effects. Despite the lack of benefit from protein supplementation, a unique and important finding of this study was that only 12 weeks of RT in these older men was sufficient for eliminating deficits in muscle size of the elbow flexors and ankle dorsi flexors compared to young men. However, at the conclusion of RT, the older group still had significantly lower muscle thickness of the knee extensors and flexors and ankle plantar flexors compared to untrained young men. The greatest deficits for muscle size in older compared to young individuals are for lower body muscle groups (Janssen et al. 2000). These lower body muscle group deficits may be too large to overcome with 12 weeks of RT. Despite these deficits in lower body muscle groups, the older group was able to achieve a leg press strength after training that was comparable to the young reference group. This suggests that the improvement in leg press strength with training may be caused by neural, rather than muscular adaptations (Chilibeck et al. 1998). Complex exercises, such as those involving movement at one or more joint (i.e., leg press), may involve a longer initial neural adaptation compared to single-joint exercises (i.e., arm curl), resulting in delayed muscle hypertrophy (Chilibeck et al. 1998). Therefore, longer training periods (i.e., >12 weeks) than the one used in the present study may be required to increase muscle hypertrophy in these lower body muscle groups.

Our results of no effect from protein supplementation on muscle mass and strength are in agreement with Godard et al. (2002) who found no effect from amino acid supplementation during RT on muscle mass and strength in healthy older men. On the other hand, in examining the effects of protein (~30 g/day; 10 days) supplementation on body composition and whole-body protein kinetics in 17 malnourished elderly subjects, Bos et al. (2000) found that protein supplementation significantly increased muscle protein synthesis and fat-free mass. Malnourished older individuals may have a greater loss of muscle protein as a result of a hypercatabolic state (Beaumont et al. 1989). Therefore, a greater protein requirement for these unhealthy individuals may have accounted for the differences between studies.

The majority of research on the timing of protein ingestion has examined its effect in the post-exercise period. Results from the present study do not support an ergogenic effect from protein supplementation (~28 g) consumed immediately after RT in older men. These results are in agreement with Godard et al. (2002) who found no effect from essential amino acid supplementation (12 g/day) during 12 weeks of RT on muscle size and strength in healthy older men. However, Esmarck et al. (2001) showed that protein (10 g) consumed immediately following RT for 12 weeks was effective for increasing muscle mass in older individuals. Differences between these studies may be related to methodological differences. Our study used a whole-body RT program;

Table 5 Mean (SEM) dietary variables for PRO-B, PRO-A, and PLA groups for 3 days during the first and final week of supplementation and training

	PRO-B		PRO-A		PLA	
	Week 1	Week 12	Week 1	Week 12	Week 1	Week 12
Kilocalories per day*	2,310 (162)	2,350 (215)	2,150 (176)	2,756 (396)	2,387 (225)	2,769 (293)
Carbohydrates (g/d)	237 (16)	242 (17)	258 (27)	323 (54)	260 (21)	297 (48)
Fat (g/d)	92 (9)	99 (14)	85 (9)	105 (19)	91 (14)	114 (12)
Protein (g/d)	132 (17)**	113 (15)	98 (9)	117 (15)	112 (15)	128 (12)

Note: Values do not include the protein supplement
*Time main effect ($P<0.05$)
**PRO-B consumed more dietary protein before training compared to PRO-A ($P<0.05$)

whereas the one by Esmarck et al. (2001) focused mainly on the knee extensors. We used muscle thickness to assess for changes in muscle size; whereas Esmarck et al. (2001) used magnetic resonance imaging and muscle biopsies to assess muscle hypertrophy of the quadriceps femoris.

3-Methylhistidine is an amino acid located primarily in skeletal muscle from the post-translation modification of specific histidine residues in myofibrillar proteins (Lukaski et al. 1981). During muscle protein catabolism, the released 3-methylhistidine is neither re-utilized for protein synthesis nor metabolized oxidatively but, instead, is quantitatively excreted in the urine (Lukaski et al. 1981); therefore, it serves as a useful indicator of myofibrillar protein degradation (Pivarnik et al. 1989). Our results of no significant effect from protein supplementation on urinary 3-methylhistidine excretion is in agreement with Campbell et al. (1995) who also found no effect from protein supplementation (0.8–1.6 g/kg/day) during 12 weeks of RT on urinary 3-methylhistidine levels in older adults. However, the normal rise in muscle protein degradation following RT was reduced with amino acid supplementation in young men (Biolo et al. 1997). Disparity in these results may be attributed to the unknown contribution of bodily tissues (i.e., gut, skin) to whole-body protein degradation, which contain significant amounts of actin (Phillips 2004; Rennie and Millward 1983) and therefore may contribute to the release of 3-methylhistidine. Recent evidence however has shown that 3-methylhistidine release into the interstitum after RT is not increased (Trappe et al. 2004); therefore, most of the increase in 3-methylhistidine after RT most likely arises from skeletal muscle.

One observation from the current experiment was that 3-methylhistidine levels were not significantly elevated with RT. A rise in protein degradation following repeated training would be expected, especially in previously sedentary older individuals. One reason for a lack of increase in 3-methylhistidine could be a blunted protein turnover in older subjects. Welle et al. (1995) found that 3 months of RT (similar to the duration of our study) resulted in a 20% elevation in 3-methylhistidine in a younger group of subjects (22–31 years) compared to virtually no change in an older group of subjects (62–72 years). Although neither group in this study had a statistically significant increase in 3-methylhistidine, measurements in our lab indicate that the increase in 3-methylhistidine may be blunted with training in older individuals. Using the same methodology as the current study we recently found that 7 weeks of the same RT program resulted in a significant 33% increase in 3-methylhistidine level in a group of young individuals (~25 years) (Pinkoski et al. 2006). We therefore feel that the increase in 3-methylhistidine in response to RT is indeed blunted in older compared to younger individuals.

A limitation of our study was that we assessed dietary intake in our subjects during the first week of supplementation and therefore may not have accurately measured completely their usual dietary intake. This was done to avoid the period before training during which they were required to be on a meat-free diet while collecting urine samples for 3-methylhistidine analysis. The assessment of dietary intake was done early in the first week of supplementation and training; therefore, most of our subjects would have been exposed to only 1 day of training and supplementation during the time the 3-day food diary was recorded. We therefore expect that their 3-day dietary record was close to their normal dietary intake.

A second limitation of our study was the lack of hormonal measurements. It is well known that anabolic hormones decrease with age and the anabolic hormone response to RT is blunted compared to young subjects (Kraemer et al. 1999). Protein supplementation before or after RT sessions enhances the anabolic hormone response to training (Kraemer et al. 1998, 2006). Future studies should therefore assess hormone responses to training and protein supplementation in older men to see if it can improve the anabolic hormone response.

It has been observed that protein elevates urinary calcium excretion (Pannemans et al. 1997). The acid load produced as a consequence of protein metabolism obligates urinary calcium losses (Barzel 1995) and may have a negative effect on bone. In young individuals who increased dietary protein consumption for 4 days, Kerstetter et al. (1999) observed higher levels of urinary N-telopeptides, an index of bone resorption. However, others suggest an overall benefit of increased protein intake on bone health. Recently, Dawson-Hughes et al. (2004) found that protein supplementation in older subjects decreased bone resorption in association with an increase in release of anabolic hormone (IGF-1) secretion. Our results showed no change in urinary N-telopeptide levels from protein supplementation and therefore do not support either a positive or negative effect of protein supplementation on bone.

In summary, supplementing with protein immediately before and immediately after RT did not enhance the gains in muscle mass and strength from exercise alone. Twelve weeks of RT was effective for overcoming deficits in leg press strength and muscle size of the elbow flexors and ankle dorsi flexors between young and older men.

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