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The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Protein Supplementation after Exercise and before Sleep Does Not Further Augment Muscle Mass and Strength Gains during Resistance Exercise Training in Active Older Men

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Abstract

Background: The proposed benefits of protein supplementation on the skeletal muscle adaptive response to resistance exercise training in older adults remain unclear.

Objective: The present study assessed whether protein supplementation after exercise and before sleep augments muscle mass and strength gains during resistance exercise training in older individuals.

Methods: Forty-one older men [mean ± SEM age: 70 ± 1 y; body mass index (kg/m^2): 25.3 ± 0.4] completed 12 wk of whole-body resistance exercise training (3 sessions/wk) and were randomly assigned to ingest either protein (21 g protein, 3 g total leucine, 9 g carbohydrate, 3 g fat; $n = 21$) or an energy-matched placebo (0 g protein, 25 g carbohydrate, 6 g fat; $n = 20$) after exercise and each night before sleep. Maximal strength was assessed by 1-repetition-maximum (1RM) strength testing, and muscle hypertrophy was assessed at the whole-body (dual-energy X-ray absorptiometry), upper leg (computed tomography scan), and muscle fiber (biopsy) levels. Muscle protein synthesis rates were assessed during week 12 of training with the use of deuterated water (^2H_2O) administration.

Results: Leg-extension 1RM increased in both groups (placebo: 88 ± 3 to 104 ± 4 kg; protein: 85 ± 3 to 102 ± 4 kg; $P < 0.001$), with no differences between groups. Quadriceps cross-sectional area (placebo: 67.8 ± 1.7 to 73.5 ± 2.0 cm^2; protein: 68.4 ± 1.4 to 72.3 ± 1.4 cm^2; $P < 0.001$) increased in both groups, with no differences between groups. Muscle fiber hypertrophy occurred in type II muscle fibers (placebo: 5486 ± 418 to 6492 ± 429 μm^2; protein: 5367 ± 301 to 6259 ± 391 μm^2; $P < 0.001$), with no differences between groups. Muscle protein synthesis rates were 1.62% ± 0.06% and 1.57% ± 0.05%/d in the placebo and protein groups, respectively, with no differences between groups.

Conclusion: Protein supplementation after exercise and before sleep does not further augment skeletal muscle mass or strength gains during resistance exercise training in active older men. This study was registered at the Netherlands Trial Registry (www.trialregister.nl) as NTR5082. *J Nutr* 2018;148:1723–1732.

Keywords: aging, exercise, dietary protein, muscle mass, muscle protein synthesis

Introduction

The age-related decline in skeletal muscle mass and strength, termed sarcopenia, is accompanied by impairments in functional capacity and an increased risk of developing chronic metabolic diseases (1, 2). Physical activity, and resistance exercise in particular, may be the only effective strategy to increase muscle mass and strength. Meta-analyses have shown that protein supplementation may further augment the skeletal muscle adaptive response to resistance exercise training (3, 4). However, although some studies have shown greater increases in muscle mass, strength, or both after protein supplementation during resistance exercise training (5–8), most individual studies have been unable to show such benefits of protein supplementation (9–18). The apparent discrepancy between studies may be attributed to the differences in study design, including the population studied, the exercise training program, and characteristics of the protein supplementation regimen.

Over the past decade, the application of stable isotope tracer methodology has taught us that the postprandial stimulation of muscle protein synthesis is modulated by the type (19–24), amount (23, 25–28), and timing (29–31) of dietary protein

Manuscript received May 15, 2018. Initial review completed June 6, 2018. Revision accepted July 3, 2018.
First published online September 24, 2018; doi: https://doi.org/10.1093/jn/nxy169.

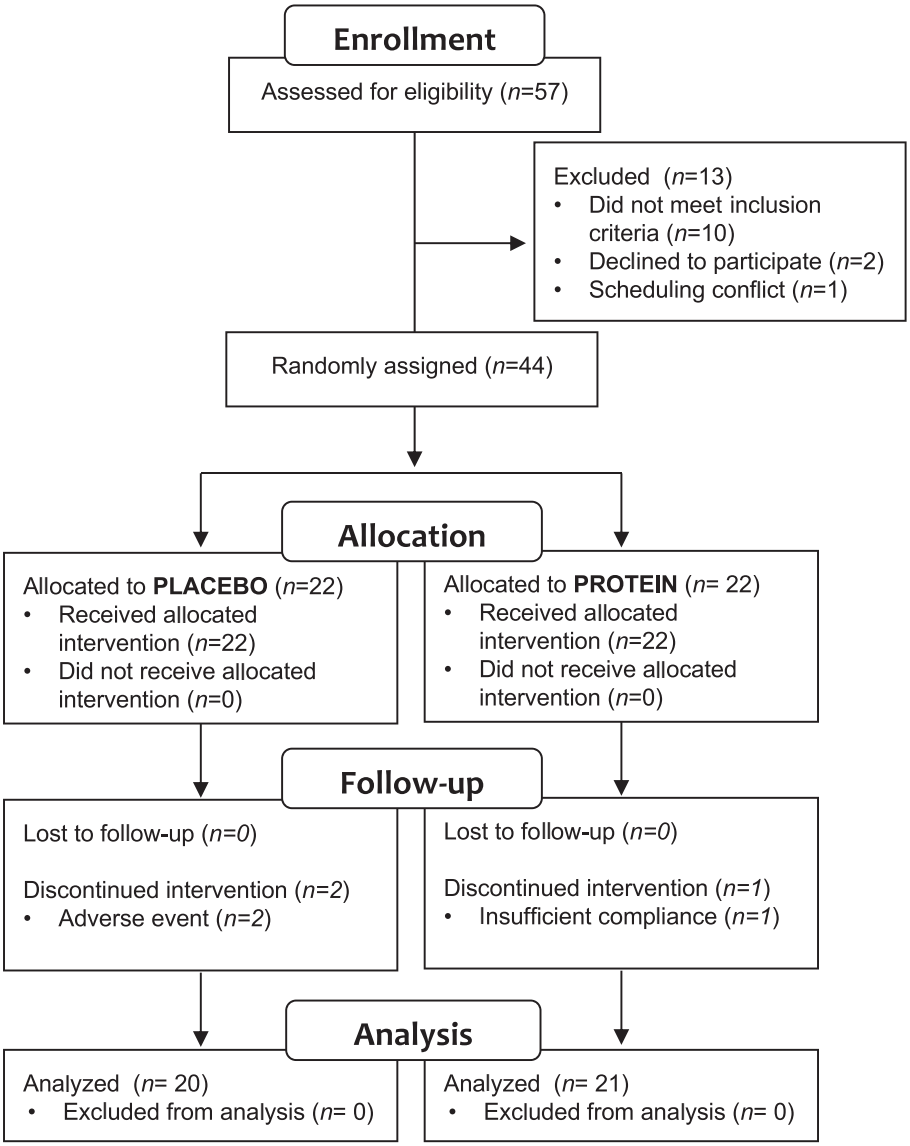

FIGURE 1 CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials.

ingestion. The ingestion of a rapidly digestible protein source (e.g., whey) has been shown to augment the muscle protein synthetic response when compared with more slowly digestible protein sources (20, 23, 24). Furthermore, coingestion of free leucine has been shown to further augment the muscle protein synthetic response to protein ingestion in older individuals (32–35). In addition to enhancing the muscle protein synthetic response to ingestion of the main meals, our laboratory has shown efficacy for dietary protein ingestion before sleep to increase overnight muscle protein synthesis rates (36, 37) and support recovery from resistance exercise (38–40). We followed up on this work by showing that dietary protein ingestion before sleep augments muscle mass and strength gains during 12 wk of resistance exercise training in healthy, younger men (41). Therefore, we hypothesized that leucine-enriched whey protein supplementation, provided immediately after training and before sleep, would maximize the muscle protein synthetic response to exercise and, as such, further augment muscle mass and strength gains after more prolonged resistance exercise training in older individuals.

Methods

Subjects. A total of 44 healthy, normoglycemic older men volunteered to complete 12 wk of whole-body resistance exercise training (3 sessions/wk) and ingested either protein or an energy-matched placebo after exercise and each night before sleep (**Figure 1**). Subjects' characteristics are presented in **Table 1**. All of the subjects were

Supported by the Top Institute Food and Nutrition. Study products were provided by Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, Netherlands.
Author disclosures: AMH, MO, KJMP, JSJS, JvK, EMPB, APG, and JPBG, no conflicts of interest. LBV received speaker's fees from Friesland Campina and Nutricia Research. LJCvL has received research grants, consulting fees, speaking honoraria, or a combination of these, from Friesland Campina, Nutricia Research, and PepsiCo. The project is funded by Top Institute Food and Nutrition, a public-private partnership on precompetitive research in food and nutrition. The researchers were responsible for the study design, data collection and analysis, decision to publish, and preparation of the manuscript. The industrial partners contributed to the project through regular discussion.
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Abbreviations used: CSA, cross-sectional area; IGF-I, insulin-like growth factor I; OGTT, oral-glucose-tolerance test; SHBG, sex hormone–binding globulin; SPPB, short physical performance battery; 1RM, 1-repetition maximum.

TABLE 1 Subjects' characteristics[1]

	Placebo group ($n = 20$)	Protein-supplemented group ($n = 21$)	P
Age, y	71 ± 1	69 ± 1	0.10
Body mass, kg	78.7 ± 1.8	77.4 ± 1.9	0.60
Height, m	1.77 ± 0.02	1.74 ± 0.01	0.11
BMI, kg/m^2	25.1 ± 0.5	25.5 ± 0.6	0.56
SMI, kg/m^2	8.5 ± 0.2	8.6 ± 0.1	0.67
Leg volume, L	8.6 ± 0.2	8.3 ± 0.1	0.42
Fasting plasma glucose, mmol/L	5.7 ± 0.1	5.7 ± 0.1	0.89
Fasting plasma insulin, mU/L	7.7 ± 0.8	9.8 ± 1.3	0.21
HbA1c, %	5.6 ± 0.1	5.4 ± 0.1	0.13
HOMA-IR	2.0 ± 0.2	2.5 ± 0.4	0.27
Systolic blood pressure, mm Hg	142 ± 4	142 ± 3	0.97
Diastolic blood pressure, mm Hg	75 ± 2	80 ± 2	0.07
Resting heart rate, beats/min	64 ± 3	61 ± 1	0.32

[1] Values are means ± SEMs. Data were analyzed by using a Student's unpaired t test. No significant differences were detected between treatment groups. HbA1c, glycated hemoglobin; SMI, skeletal muscle mass index; 1RM, 1-repetition maximum.

screened for medical issues and excluded if any gastrointestinal, cardiovascular, neurological, or renal diseases were present. Fasting and rested blood glucose and glycated hemoglobin were also assessed to screen for the presence of type 2 diabetes. Subjects were cleared to perform resistance exercise by a cardiologist who examined electrocardiograms measured at rest and during submaximal cycling (performed at 70% of age-predicted heart rate maximum). All of the subjects were living independently and had not been participating in any structured, progressive resistance exercise training program within the past 3 y. All of the subjects were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. The study was approved by the Medical Ethical Committee of the Maastricht University Medical Center+, Netherlands (METC 15-3-003) and conformed to standards for the use of human subjects in research as outlined in the most recent version of the Helsinki Declaration. This study was registered at the Netherlands Trial Registry (www.trialregister.nl) as NTR5082.

Study design. After inclusion in the study, subjects were randomly allocated in a double-blinded fashion to either a protein or a placebo group. Before and after the exercise training intervention, 2 separate experimental test days were performed within the same week. On the first experimental test day, anthropometric measurements (height, body mass, and leg volume), DXA, and computed tomography scans were performed and a muscle biopsy sample was collected. The second experimental test day was performed 4 d later and included an oral-glucose-tolerance test (OGTT), short physical performance battery (SPPB) testing, and maximal strength assessments [1-repetition maximum (1RM) on leg press and leg extension]. During week 12 of the training, deuterated water (^2H_2O) was provided to assess myofibrillar protein synthesis rates.

Diet and physical activity. All of the subjects were instructed to refrain from any exhaustive physical activity and to keep their diet as consistent as possible 72 h before the experimental test days. On all test days, the subjects arrived at the laboratory by car or public transportation in a fasting and rested state. Before the onset of training and during week 12 of training, subjects recorded 3-d dietary intake records to assess potential changes in daily food intake that might have occurred during the intervention period. Food intake records were evaluated by a dietitian and analyzed online with the use of a publicly available nutrition database (NEVO 2016).

Exercise intervention program. Supervised whole-body resistance exercise training was performed 3 times/wk (Monday, Wednesday, and Friday) during the 12-wk exercise training intervention. All sessions were performed between 0800 and 1100. Training consisted of a 5-min warm-up on a cycle ergometer, followed by a warm-up and 4 sets on both the leg press and leg extension machines. Upper body exercises were paired (chest press with lateral pulldown and shoulder press with horizontal row) and were performed in an alternating manner between training sessions, with 2 sets of each exercise performed. After training, subjects performed 5 min of cool-down on the cycle ergometer. During the first 4 wk of training, the workload was increased from 70% 1RM (8 repetitions in each set) to 80% 1RM (10 repetitions). Resting periods of 2 and 3 min were allowed between sets and exercises, respectively. The workload was adjusted under the guidance of the investigators, which was primarily based on the 1RM estimations (weeks 0, 4, and 8). In addition, the workload was increased when >10 repetitions could be performed. Compliance with the resistance exercise training program was 95% ± 1%.

Protein supplementation. Subjects were randomly assigned to ingest either 21 g leucine-enriched whey protein (3 g total leucine) or an energy-matched placebo after exercise and each night before sleep, including rest days. The nutritional composition of the test beverages is shown in Table 2. The intervention was performed in a double-blinded fashion whereby treatment randomization and beverage preparation and labeling were performed independent of the study team. Subjects received labeled packages containing dried contents of their randomly assigned beverages, which were dissolved in 250 mL water and ingested by the subjects. All of the beverages were flavored (vanilla or strawberry) to mask their contents. On training days, no food or drinks were allowed between breakfast and lunch other than the experimental beverages. The subjects were allowed to drink water before, during, and after each exercise session. Compliance to the supplementation was assessed by supplementation logs and was confirmed by counting returned supplement packages. Compliance for protein and placebo supplementation was 99.6% ± 0.1%.

TABLE 2 Nutritional composition of the test beverages ingested in a double-blind, randomized fashion after each resistance exercise training session and each night before sleep during 12 wk of resistance exercise training in active older men[1]

Component	Protein[2]	Placebo[3]
Energy, kJ	628	628
Energy, kcal	150	150
Protein, g	20.7	—
EAAs, g	10.6	—
Leucine, g	2.8	—
Carbohydrate, g	9.4	24.5
Fat, g	3	5.8
Fiber, g	1.3	—
Cholecalciferol, μg	20	—

[1] Dried beverage contents were dissolved in 250 mL water. EAA, essential amino acid (Leu, Ile, Val, Phe, Met, His, Trp, Thr, and Lys).
[2] The protein drink also contained other micronutrients: sodium (150 mg), potassium (279 mg), chloride (70 mg), calcium (500 mg), phosphorus (250 mg), magnesium (37 mg), iron (2.4 mg), zinc (2.2 mg), copper (270 μg), manganese (0.5 mg), fluoride (0.15 mg), molybdenum (15 μg), selenium (15 μg), chromium (7.5 μm), iodine (20 μg), vitamin A (152 μg), vitamin E (7.5 mg), phylloquinone (12 μg), thiamin (0.23 mg), riboflavin (0.25 mg), nicotinic acid (8.8 mg), pantothenic acid (0.81 mg), pyridoxine (0.76 mg), folic acid (203 μg), cyanocobalamin (3 μg), biotin (6.1 μg), ascorbic acid (32 mg), provitamin A carotenoids (0.3 mg), and choline (56 mg).
[3] The placebo drink also contained other micronutrients: sodium (123 mg), potassium (154 mg), chloride (329 mg), calcium (0.71 mg), phosphorus (0.3 mg), and iodine (0.3 μg).

Body composition. Body composition was measured at the whole-body and regional level using DXA (Discovery A; Hologic). Anthropometric measurements were assessed using standardized procedures, body mass by a digital scale to within 100 g, and height by a stadiometer to within 0.5 cm. Anatomic cross-sectional area (CSA) of the quadriceps muscle was assessed by computed tomography scanning (Philips Brilliance 64; Philips Medical Systems) before and after the 12-wk exercise training intervention, as described previously (42).

Muscle biopsy sampling. Muscle biopsy samples were obtained from the middle region of the *vastus lateralis*, 15 cm above the patella and 4 cm below entry through the fascia, using the percutaneous needle biopsy technique (43). Muscle samples were prepared for analyses as previously described (41).

Immunohistochemistry. Muscle biopsy specimens were sliced into 5-mm-thick cryosections and stained for muscle fiber type, and type I and II muscle fiber CSA and composition were determined as described in detail previously (13, 18). No differences in fiber circularity were observed in response to training or between groups. The mean fiber numbers included in analyses were 206 ± 16 and 161 ± 11 from the muscle biopsy samples collected before and after the 12-wk training intervention, respectively.

Myofibrillar fractional synthesis rates. Deuterated water (^2H_2O) was provided throughout week 12 of exercise training to assess average daily myofibrillar protein synthesis rates as described previously (44). In short, subjects ingested 400 mL of 70% deuterated water spread evenly over a 12-h period on the Friday of week 11 of the training intervention. Subjects ingested 50 mL of 70% deuterated water in the morning of each of the 10 remaining days of the intervention period to maintain the body water deuterium enrichment. A muscle biopsy sample was collected in the morning before the Monday training of week 12. Myofibrillar proteins were isolated from this muscle biopsy sample and from the posttraining muscle biopsy sample collected the following Monday, and the increase in ^2H-alanine enrichment was assessed by using GC-pyrolysis-isotope-ratio mass spectrometer (44). Samples were measured in quadruplicate along with a series of known standards every 12 injections. Myofibrillar protein synthesis rates were calculated as previously described (44).

Muscle strength and physical performance. Maximal strength was assessed by 1RM strength tests on leg press and leg extension machines (Technogym) before and after the training intervention. During the subject intake visit, proper lifting technique was demonstrated and practiced and maximal strength was estimated by using the multiple repetitions testing procedure (45). On the second experimental test day, each subject's 1RM was determined as described previously (46). To ensure progressive loading during training, 1RM was estimated at the end of weeks 4 and 8. Posttraining maximal strength was assessed 7 d after the last training session. Physical performance was assessed by the SPPB, which consists of 3 components: balance, gait speed, and chair-rise ability (47).

Glucose tolerance and plasma hormone concentrations. A standard OGTT was performed before and after the training intervention, as described previously (18). Testosterone, free testosterone, growth hormone, insulin-like growth factor I (IGF-I), and sex hormone–binding globulin (SHBG) were assessed in serum samples collected in a resting and fasting state before and after the training intervention. Serum testosterone was measured by using an electrochemiluminescent immunoassay (Cobas 8000 instrument; Roche Diagnostics). Growth hormone and IGF-I were measured by using a chemiluminescent immunometric assay (IDS iSYS instrument; Immunodiagnostic Systems). SHBG was determined in serum by using a chemiluminescent immunometric assay (XPi instrument; Siemens Medical Solutions Diagnostics). Free testosterone was calculated according to Vermeulen et al. (48).

Statistical analysis. All of the data are expressed as means ± SEMs. Baseline characteristics between groups were compared by using a Student's unpaired *t* test. A 2-factor repeated-measures ANOVA (time × treatment) with time (pre-post) as a within-subjects factor and treatment group (placebo-protein) as a between-subjects factor was performed for the analysis of dietary intake, appendicular lean body mass, quadriceps CSA, muscle fiber CSA, muscle strength and performance measures, serum hormone concentrations, and the AUC of glucose and insulin

TABLE 3 Energy intake and macronutrient composition of the diet before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation[1]

	Placebo ($n = 20$)		Protein ($n = 21$)		*P*		
	0 wk	12 wk	0 wk	12 wk	Treatment × time interaction	Treatment effect	Time effect
Energy intake, MJ/d							
Diet	9.7 ± 0.5	10.3 ± 0.5	9.4 ± 0.4	9.1 ± 0.3	0.062	0.151	0.525
Total	9.7 ± 0.5	11.2 ± 0.5*	9.4 ± 0.4	10.0 ± 0.3*	0.062	0.151	<0.001
Protein intake, g/d							
Diet	93 ± 4	94 ± 4	87 ± 3	81 ± 3	0.153	0.052	0.299
Total	93 ± 4	94 ± 4	87 ± 3	111 ± 3*	<0.001	0.204	<0.001
Protein intake, g/kg body mass/d							
Diet	1.19 ± 0.06	1.17 ± 0.06	1.14 ± 0.05	1.04 ± 0.04	0.164	0.191	0.061
Total	1.19 ± 0.06	1.17 ± 0.06	1.14 ± 0.05	1.43 ± 0.04*	<0.001	0.138	<0.001
Protein intake, % of energy							
Diet	16 ± 1	14 ± 1	16 ± 1	15 ± 1	0.664	0.771	0.044
Total	16 ± 1	14 ± 1	16 ± 1	19 ± 1	<0.001	0.005	0.308
Total fat intake, g/d	89 ± 6	98 ± 7	86 ± 4	80 ± 5	0.045	0.168	0.701
Total fat intake, % of energy	34 ± 1	32 ± 1	34 ± 1	30 ± 1	0.336	0.331	0.005
Total carbohydrate intake, g/d	242 ± 16	303 ± 16*	235 ± 13	253 ± 11*	0.022	0.11	<0.001
Total carbohydrate intake, % of energy	42 ± 1	45 ± 2	42 ± 1	43 ± 1	0.137	0.531	0.093
Vitamin D intake, μg/d							
Diet	3.7 ± 0.5	3.8 ± 0.7	3.5 ± 0.4	3.0 ± 0.03	0.531	0.314	0.624
Total	3.7 ± 0.5	3.8 ± 0.7	3.5 ± 0.4	31.6 ± 0.3*	<0.001	<0.001	<0.001

[1]Values are means ± SEMs. "Diet" indicates only dietary intake data. "Total" indicates dietary intake plus protein or placebo supplementation. *Different from 0 wk, $P < 0.05$.

TABLE 4 Body composition before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation[1]

	Placebo ($n = 20$)			Protein ($n = 21$)			P		
	0 wk	12 wk	Difference	0 wk	12 wk	Difference	Treatment × time interaction	Treatment effect	Time effect
Body mass, kg	78.7 ± 1.8	80.5 ± 1.8*	+1.8 ± 0.3	77.4 ± 1.9	78.1 ± 1.8*	+0.8 ± 0.2	0.007	0.463	<0.001
Height, m	1.77 ± 0.02	1.77 ± 0.02	0 ± 0	1.74 ± 0.01	1.74 ± 0.01	0 ± 0		0.11	
BMI, kg/m^2	25.1 ± 0.5	25.6 ± 0.5*	+0.6 ± 0.1	25.5 ± 0.6	25.8 ± 0.6*	+0.3 ± 0.1	0.008	0.695	<0.001
Lean body mass, kg	60.8 ± 1.3	62.4 ± 1.3	+1.6 ± 0.3	59.2 ± 1.1	60.2 ± 1.2	+1.1 ± 0.2	0.183	0.278	<0.001
Appendicular lean body mass, kg	26.7 ± 0.7	27.7 ± 0.7	+1.0 ± 0.2	26.0 ± 0.5	26.6 ± 0.5	+0.6 ± 0.1	0.126	0.303	<0.001
Leg lean mass, kg	13.4 ± 0.4	13.9 ± 0.4	+0.7 ± 0.1	13.0 ± 0.2	13.4 ± 0.2	+0.4 ± 0.1	0.146	0.316	<0.001
Fat mass, kg	16.7 ± 1.0	16.3 ± 0.9	−0.5 ± 0.2	16.8 ± 0.9	16.2 ± 0.8	−0.6 ± 0.2	0.690	0.999	<0.001
Body fat, %	20.7 ± 0.9	19.8 ± 0.9	−0.9 ± 0.3	21.1 ± 0.7	20.3 ± 0.7	−0.8 ± 0.2	0.880	0.679	<0.001
Bone mineral content, g	2925 ± 69	2918 ± 69	−8 ± 14	2727 ± 72	2698 ± 69	−30 ± 14	0.279	0.040	0.069
Bone mineral density, g/cm	1.235 ± 0.025	1.232 ± 0.026	−0.003 ± 0.007	1.179 ± 0.024	1.175 ± 0.022	−0.003 ± 0.006	0.921	0.100	0.527

[1] Values are means ± SEMs. *Different from 0 wk, $P < 0.05$.

concentrations during the OGTT. In case of a significant interaction, Student's paired t tests were performed to determine time effects within groups. Myofibrillar protein synthesis rates were compared between groups by using a Student's unpaired t test. Significance was set at $P < 0.05$. All calculations were performed with the use of SPSS (version 21.0; IBM Corporation).

Results

Subjects. Subjects' characteristics are presented in Table 1. In total, 44 subjects were included in the experimental protocol. Three subjects were excluded from analyses; one was excluded due to insufficient exercise compliance (>6 missed sessions), one dropped out due to knee aggravation, and one dropped out due to an adverse event that occurred during a training session. Therefore, analysis was performed in 41 subjects, 20 in the placebo group and 21 in the protein group. Training volume load throughout the entire training intervention did not differ between the placebo and protein groups for any of the exercises performed. At baseline, no differences in age, body mass, height, BMI, skeletal muscle mass index, or leg volume were observed between the placebo and protein groups (Table 1). We observed a significant increase in body mass in response to 12 wk of resistance exercise training ($P < 0.001$), with a greater increase observed in the placebo group ($P = 0.007$). We observed a significant increase in BMI in response to 12 wk of resistance exercise training ($P < 0.001$), with a greater increase observed in the placebo group ($P = 0.008$).

Dietary intake. Dietary intake data are shown in Table 3. Analysis of the 3-d dietary intake records collected before and during week 12 of the experimental protocol showed no baseline differences in total daily energy intake between groups but an increase during the intervention ($P < 0.001$). Dietary macronutrient composition did not differ at baseline between groups and did not change during the experimental protocol (Table 3). In particular, daily protein intake from the diet averaged 1.2 ± 0.1 and 1.1 ± 0.1 g/kg body mass/d at baseline in the placebo and protein groups, respectively, and did not change during the training intervention. Supplementation caused daily protein intake relative to body mass to significantly increase in the protein group when compared with the placebo group (treatment × time interaction, $P < 0.001$). Supplementation also resulted in greater daily vitamin D intake in the protein group than in the placebo group (treatment × time interaction, $P < 0.001$).

Body composition. At baseline, no significant differences in body-composition measurements were observed between the placebo and protein groups (Table 4). Whole-body lean mass significantly increased in response to 12 wk of resistance exercise training in the placebo and the protein groups ($P < 0.001$), with no differences between groups. In accordance, appendicular lean mass and leg lean mass significantly increased in response to 12 wk of resistance exercise training in the placebo and protein groups ($P < 0.001$), with no differences between groups. Whole-body fat mass and body fat percentage declined in response to 12 wk of resistance exercise training in the placebo and protein groups ($P < 0.001$), with no differences between groups.

Skeletal muscle hypertrophy. At baseline, no significant differences in quadriceps muscle CSA were observed between the placebo and protein groups (Figure 2). Quadriceps muscle CSA increased in response to 12 wk of resistance exercise training (Figure 2; $P < 0.001$), with no differences between groups. Before training, no significant differences were observed

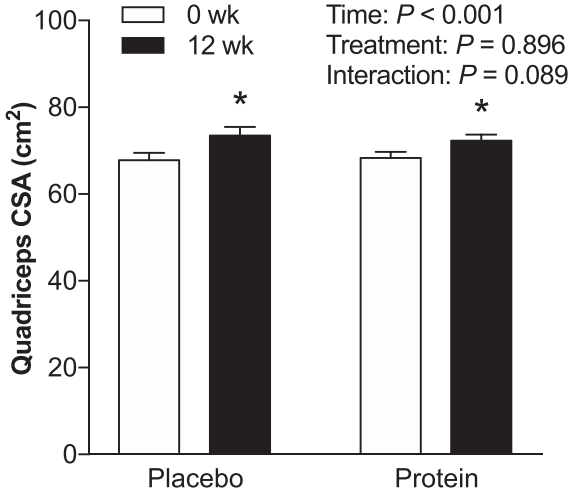

FIGURE 2 Quadriceps muscle CSA before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation. Values are means ± SEMs, $n = 20$ (placebo) or 21 (protein). Data were analyzed with the use of 2-factor ANOVA. *Different from values before exercise training, $P < 0.05$. CSA, cross-sectional area.

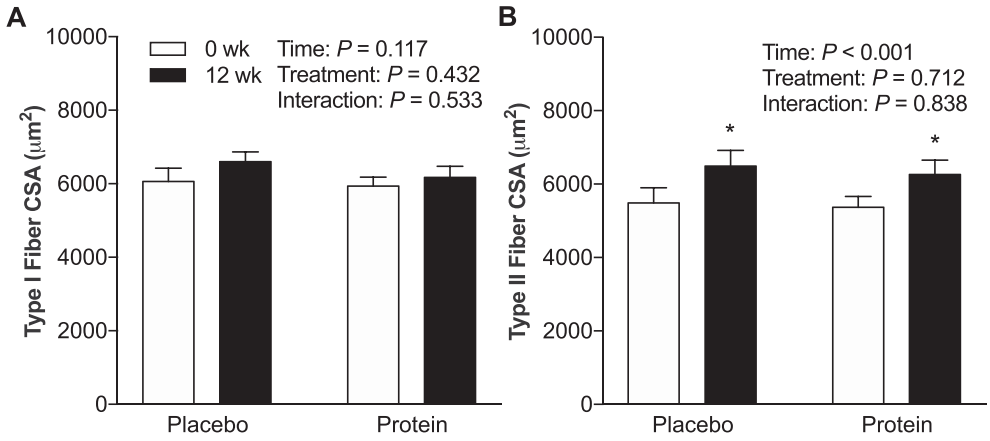

FIGURE 3 Type I (A) and type II (B) muscle fiber CSA before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation. Values are means ± SEMs, $n = 20$ (placebo) or 20 (protein). Data were analyzed with the use of 2-factor ANOVA. *Different from values before exercise training, $P < 0.05$. CSA, cross-sectional area.

in type I and type II muscle fiber size between placebo and protein groups. Type I muscle fiber CSA (Figure 3A) did not change in response to 12 wk of resistance exercise training in both the placebo and protein groups. Type II muscle fiber CSA (Figure 3B) increased in response to 12 wk of resistance exercise training in both groups ($P < 0.001$), with no differences between groups.

Muscle fiber–type composition. At baseline, no significant differences in the percentage of type I and type II muscle fibers and percentage of CSA occupied by type I and II muscle fibers were observed between the placebo and protein groups (Table 5). The percentage of type I and type II muscle fibers and the percentage of CSA occupied by type I and II muscle fibers did not change in response to 12 wk of resistance exercise training in either group.

Myofibrillar protein synthesis rates. Myofibrillar protein synthesis rates during week 12 of the training protocol were assessed by administration of deuterated water and by measuring myofibrillar protein–bound ^{2}H-alanine enrichments in muscle biopsy samples collected on Monday of week 12 and the following Monday (posttraining muscle biopsy sample). Body water deuterium enrichment over the 7-d assessment period averaged 0.75% ± 0.01% in both groups, with no differences between groups. The increase in myofibrillar protein–bound ^{2}H-alanine enrichments averaged 0.315 ± 0.015 and 0.308 ± 0.016 mole percent excess in the placebo group and protein group, respectively. Myofibrillar protein synthesis rates (Figure 4) were not different between groups.

Muscle strength and physical performance. At baseline, no significant differences in leg extension or leg press 1RM (Table 6) were observed between the placebo and protein groups. Leg press and leg extension 1RM significantly increased in both groups ($P < 0.001$) in response to 12 wk of resistance exercise training, with no differences detected between groups. Likewise, physical performance (SPPB) scores (Table 6; $P < 0.01$) and sit-to-stand time decreased in response to 12 wk of resistance exercise training (Table 5; $P < 0.001$), with no differences between groups.

Glucose tolerance. Glucose and insulin concentrations during the OGTT are shown in Figure 5A. Glucose tolerance improved in response to 12 wk of resistance exercise training, as shown by a significant reduction in plasma glucose AUC during the OGTT (Figure 5B; $P = 0.027$), with no differences for plasma insulin AUC and no differences observed between groups.

Plasma hormone concentrations. Serum testosterone, free testosterone, growth hormone, IGF-I, and SHBG concentrations are shown in Table 7. Plasma hormone concentrations did not differ between groups and did not change in response to 12 wk of resistance exercise training.

Discussion

The present study shows that 12 wk of resistance exercise training increased muscle mass and strength in active older men. Protein supplementation after each exercise session (3 times/wk) and every night before sleep did not further

TABLE 5 Muscle fiber–type composition before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation[1]

	Placebo ($n = 20$)		Protein ($n = 20$)		P		
	0 wk	12 wk	0 wk	12 wk	Treatment × time interaction	Treatment effect	Time effect
Type I fibers, %	50 ± 4	48 ± 3	55 ± 4	53 ± 3	0.737	0.252	0.432
Type II fibers, %	50 ± 4	52 ± 3	45 ± 4	47 ± 3	0.737	0.252	0.432
Type I CSA, %	52 ± 4	49 ± 4	58 ± 4	52 ± 3	0.662	0.326	0.161
Type II CSA, %	48 ± 4	51 ± 4	42 ± 4	48 ± 3	0.662	0.326	0.161

[1] Values are means ± SEMs. CSA, cross-sectional area.

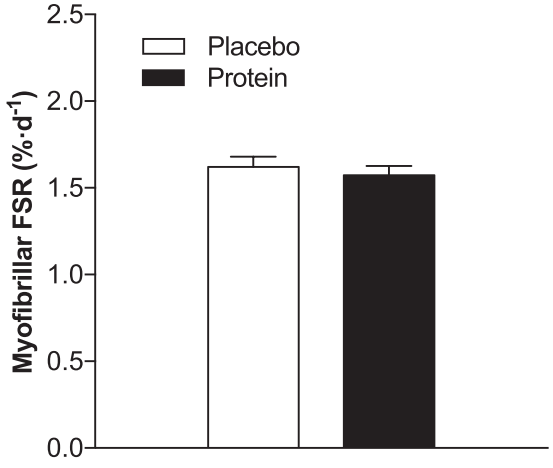

FIGURE 4 Myofibrillar protein FSRs assessed throughout week 12 of the resistance exercise training intervention in active older men who did or did not receive protein supplementation. Values are means ± SEMs, $n = 20$ (placebo) or 21 (protein). Data were analyzed with the use of Student's unpaired t test. FSR, fractional synthesis rate.

enhance the training-induced gains in skeletal muscle mass or strength in older men.

Twelve weeks of progressive whole-body resistance exercise training resulted in a combined average gain of 1.3 ± 0.2 kg lean body mass and a concomitant decrease in whole-body fat mass. These results are in line with previous studies that showed that 12 wk of resistance exercise training results in a gain of 1.0 kg lean body mass (4, 10, 13, 18). The increase in lean body mass primarily occurred in the lower extremities, with quadriceps CSA being increased by 7% ± 1%. This increase in muscle mass was accompanied by 15% ± 1% and 21% ± 3% increases in leg press and leg extension strength, respectively. As expected, our findings are in line with previous studies that showed strength increases of ≥25% after a 12-wk resistance exercise training program (13, 18, 49, 50). The observed increase in muscle strength and shift in body composition also contributed to improvements in functional capacity (Table 5) and oral-glucose tolerance (Figure 5).

In the present study, we tested the hypothesis that supplementation with leucine-enriched whey protein ingested after each exercise session and every night before sleep would further augment gains in muscle mass and strength during resistance exercise training in active older men. Despite the significant gains in lean body mass observed over the course of the training period, we observed no differences between the protein-supplemented and placebo groups (1.1 ± 0.2 and 1.6 ± 0.3 kg, respectively; $P = 0.18$). These findings correspond with more direct assessments of skeletal muscle hypertrophy, because we observed no differences between groups in quadriceps CSA (Figure 2; $P = 0.09$) or type I (Figure 3A; $P = 0.53$) and type II (Figure 3B; $P = 0.84$) muscle fiber CSA. We also observed no differences in leg press 1RM (Table 6; $P = 0.77$), leg extension 1RM (Table 6; $P = 0.80$), or sit-to-stand time (Table 6; $P = 0.74$) between groups.

In addition to our assessments of skeletal muscle mass and strength, we also provided subjects with deuterated water (^2H_2O) to assess the muscle protein synthetic response to protein supplementation throughout the last week of the training intervention. With the use of this approach, we and others have shown that resistance exercise stimulates a robust increase in muscle protein synthesis rates over days or weeks (44, 51, 52). In line with the present findings on muscle mass, we observed no differences in myofibrillar protein synthesis rates between the placebo and protein groups. Daily fractional muscle protein synthesis rates averaged 1.57% ± 0.05% and 1.62% ± 0.06%/d in the protein and placebo groups, respectively (Figure 4; $P = 0.54$). Our findings seem to be in contrast with observations of higher muscle protein synthesis rates during recovery from a single bout of exercise when whey protein (20, 23, 24), leucine (34, 35, 53, 54), or leucine-enriched protein (55) are ingested. The deuterated water approach applied in this study allowed the assessment of muscle protein synthesis rates during 7 d of the exercise training intervention. During this week, other factors such as regular food intake, habitual physical activity, stress, and sleep may have modulated muscle protein synthesis rates. The present findings indicate that protein supplementation does not further augment muscle protein accretion during the latter stages (week 12) of a 12-wk exercise training program in active older men.

Dietary protein intake during the intervention period was 1.2 ± 0.1 g/kg body mass/d in the placebo group and 1.4 ± 0.1 g · kg body mass^{-1} · d^{-1} in the protein-supplemented group. Instead of merely increasing dietary protein intake, the present study focused on timed protein supplementation, with supplements being provided immediately after exercise and before sleep. The ingestion of dietary protein in close temporal proximity to exercise has been shown to further improve postexercise net muscle protein balance (56–59). In addition, we have previously shown that the ingestion of 40 g casein before sleep increases overnight muscle protein synthesis rates (36, 37, 40) and could therefore serve to promote skeletal muscle mass gains when combined with resistance exercise training (38, 60).

TABLE 6 Muscle strength and physical performance before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation[1]

	Placebo ($n = 19$)			Protein ($n = 21$)			P		
	0 wk	12 wk	Difference	0 wk	12 wk	Difference	Treatment × time interaction	Treatment effect	Time effect
Leg press 1RM, kg	162 ± 6	188 ± 7	+25 ± 3	157 ± 5	180 ± 6	+24 ± 3	0.765	0.397	<0.001
Leg extension 1RM, kg	88 ± 3	105 ± 4	+16 ± 3	85 ± 3	102 ± 4	+17 ± 3	0.798	0.481	<0.001
Total 1RM, kg	250 ± 7	292 ± 9	+41 ± 5	241 ± 7	282 ± 8	+41 ± 5	0.979	0.359	<0.001
SPPB score, points	11.2 ± 0.2	11.6 ± 0.1	+0.4 ± 0.2	11.3 ± 0.1	11.5 ± 0.2	+0.2 ± 0.2	0.340	0.719	0.006
4-m Walking test, s	3.57 ± 0.12	3.60 ± 0.09	+0.03 ± 0.11	3.70 ± 0.08	3.72 ± 0.11	+0.03 ± 0.09	0.877	0.273	0.604
Sit-to-stand test time, s	12.03 ± 0.31	11.20 ± 0.32	−0.83 ± 0.37	11.57 ± 0.27	10.80 ± 0.27	−0.77 ± 0.25	0.739	0.233	<0.001

[1] Values are means ± SEMs. SPPB, short physical performance battery; 1RM, 1-repetition maximum.

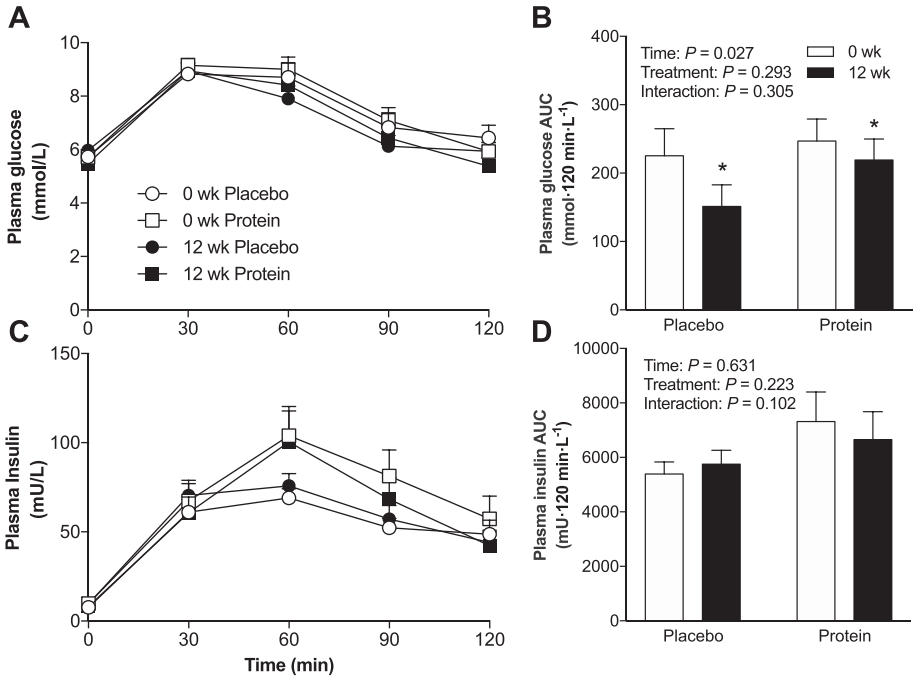

FIGURE 5 Plasma glucose (A) and insulin (C) concentrations and AUCs (B, D) in response to an OGTT before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation. Values are means ± SEMs, $n = 20$ (placebo) or 21 (protein). Data for AUCs were analyzed with the use of 2-factor ANOVA. *Different from values before exercise training, $P < 0.05$. OGTT, oral-glucose-tolerance test.

In support, we have previously shown that presleep protein supplementation (~30 g/d) augments gains in muscle mass and strength after 12 wk of resistance exercise training in healthy young men (41). In the present study, however, we failed to observe greater increases in skeletal muscle mass or strength after postexercise and presleep protein supplementation during 12 wk of resistance exercise training in active older men. The absence of any surplus benefits of protein supplementation on muscle mass and strength gains during prolonged resistance exercise training in older compared with younger adults may be explained by age-related factors, which include a lower absolute workload, lower habitual physical activity, and the prevalence of anabolic resistance. In support, exercise workload and absolute gains in muscle mass and strength tend to be greater in younger individuals than in older individuals (7, 8, 41). Consequently, protein supplementation appears to provide a greater benefit to the skeletal muscle adaptive response to resistance exercise training in younger individuals (3, 4, 6–8).

It has been suggested that the prevalence of anabolic resistance may be compensated for by ingesting even larger amounts (i.e., 40 g) of protein than what was provided in the present study (20 g whey + 1 g leucine). However, previous work has shown that the ingestion of 20 g whey should be effective at increasing postexercise muscle protein synthesis rates in the healthy older population (26). Furthermore, numerous studies have shown leucine coingestion to further augment the muscle protein synthetic response to protein ingestion (32–35). Therefore, we can only speculate on whether supplementation with a larger protein dose could have further increased muscle mass and strength gains after resistance training in these healthy, active older men.

The present findings add to the growing number of studies from our group (13, 18) as well as others (10–12, 14–17) that show that protein supplementation does not further augment skeletal muscle mass or strength gains during resistance exercise training in active older men who habitually consume ample amounts of dietary protein (1.0–1.2 g/kg body mass/d). In contrast, protein supplementation has been reported to increase muscle mass and strength gains during resistance exercise training in prefrail older men and women who consume less than optimal amounts of dietary protein (5). Therefore, protein

TABLE 7 Fasting serum hormone concentrations before and after 12 wk of resistance exercise training in active older men who did or did not receive protein supplementation[1]

	Placebo ($n = 20$)			Protein ($n = 21$)			P		
	0 wk	12 wk	Difference	0 wk	12 wk	Difference	Treatment × time interaction	Treatment effect	Time effect
Testosterone, nmol/L	17.7 ± 1.8	18.5 ± 1.8	+0.8 ± 1.0	16.0 ± 1.2	16.8 ± 1.4	+0.7 ± 0.9	0.972	0.534	0.258
Free testosterone, pmol/L	247 ± 18	251 ± 15	+5 ± 13	242 ± 15	262 ± 19	+17 ± 14	0.509	0.867	0.258
Growth hormone, μg/L	3.6 ± 1.0	4.3 ± 1.0	+0.7 ± 1.0	4.3 ± 1.3	2.6 ± 0.8	−1.6 ± 1.1	0.171	0.974	0.583
IGF-I, nmol/L	16.6 ± 1.4	17.6 ± 1.3	+0.9 ± 0.6	18.2 ± 0.8	17.9 ± 0.9	−0.1 ± 0.5	0.163	0.733	0.399
SHBG, nmol/L	50.3 ± 3.9	51.9 ± 3.9	+1.5 ± 1.2	44.3 ± 3.6	43.1 ± 3.5	−1.2 ± 1.0	0.062	0.284	0.629

[1] Values are means ± SEMs. IGF-I, insulin-like growth factor I; SHBG, sex hormone–binding globulin.

supplementation may be of greater benefit in more clinically compromised older populations when gains in muscle mass, strength, or both are restricted by habitual protein consumption (61). In conclusion, protein supplementation after exercise and before sleep does not augment skeletal muscle mass or strength gains after resistance exercise training in active older men who consume ample dietary protein.

Acknowledgments

We thank Jean Nyakayiru, Tobias Lampert, and Finn Frielink for assistance during the experimental test days. The authors' responsibilities were as follows—AMH, LBV, and LJCvL: designed the research; AMH, KJMP, MO, JSJS, and EMPB: conducted the research; AMH, JvK, APG, and JPBG: performed sample analyses; AMH: analyzed the data; AMH and LBV: performed the statistical analysis; AMH and LJCvL: wrote the manuscript and hold primary responsibility for the final content; and all authors: read and approved the final manuscript.

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