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Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy after resistance training in elderly men[1–3]

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ABSTRACT

Background: Considerable discrepancy exists in the literature on the proposed benefits of protein supplementation on the adaptive response of skeletal muscle to resistance-type exercise training in the elderly.

Objective: The objective was to assess the benefits of timed protein supplementation on the increase in muscle mass and strength during prolonged resistance-type exercise training in healthy elderly men who habitually consume adequate amounts of dietary protein.

Design: Healthy elderly men ($n = 26$) aged 72 ± 2 y were randomly assigned to a progressive, 12-wk resistance-type exercise training program with (protein group) or without (placebo group) protein provided before and immediately after each exercise session (3 sessions/wk, 20 g protein/session). One-repetition maximum (1RM) tests were performed regularly to ensure a progressive workload during the intervention. Muscle hypertrophy was assessed at the whole-body (dual-energy X-ray absorptiometry), limb (computed tomography), and muscle fiber (biopsy) level.

Results: The 1RM strength increased ≈25–35% in both groups ($P < 0.001$). Dual-energy X-ray absorptiometry and computed tomography scans showed similar increases in leg muscle mass (6 ± 1% in both groups; $P < 0.001$) and in the quadriceps (9 ± 1% in both groups), from 75.9 ± 3.7 and 73.8 ± 3.2 to 82.4 ± 3.9 and 80.0 ± 3.0 cm^2 in the placebo and protein groups, respectively ($P < 0.001$). Muscle fiber hypertrophy was greater in type II (placebo: 28 ± 6%; protein: 29 ± 4%) than in type I (placebo: 5 ± 4%; protein: 13 ± 6%) fibers, but the difference between groups was not significant.

Conclusion: Timed protein supplementation immediately before and after exercise does not further augment the increase in skeletal muscle mass and strength after prolonged resistance-type exercise training in healthy elderly men who habitually consume adequate amounts of dietary protein. This trial was registered at clinicaltrials.gov as NCT00744094. *Am J Clin Nutr* 2009;89:608–16.

INTRODUCTION

The age-related loss of skeletal muscle mass and strength, known as sarcopenia, is associated with a progressive decline in functional performance (1–4). Resistance-type exercise training has been shown to be an effective strategy to augment skeletal muscle mass and strength and improve functional capacity in the elderly (5–11). Physical activity stimulates muscle protein synthesis and accelerates protein breakdown (12–16). However, in the absence of food intake, net muscle protein balance remains negative (17). Postexercise carbohydrate ingestion attenuates the exercise-induced increase in protein breakdown (18, 19). However, amino acid and/or protein administration, with (20–22) or without carbohydrate (23, 24), is required to inhibit protein breakdown and stimulate muscle protein synthesis, resulting in a positive muscle protein balance. The timing of protein ingestion seems to represent an important factor in stimulating postexercise muscle protein accretion (25–27). Levenhagen et al (26) reported an improved postexercise net protein balance after consumption of protein and carbohydrate immediately after cessation of exercise as opposed to a more delayed supplementation regimen. Furthermore, recent studies suggest that protein co-ingestion before and/or during exercise can further augment postexercise muscle protein accretion (25, 27).

Although the results of acute studies highlight the relevance of protein ingestion before and immediately after exercise, there is considerable discussion on the proposed benefits of protein supplementation on the adaptive response to more prolonged exercise training in the elderly. From a series of well-controlled nutritional intervention studies (28–30), Campbell and Leidy (31) concluded that resistance training–induced improvements in muscle mass and strength are not enhanced when older people who consume adequate amounts of dietary protein (in excess of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$) further increase their protein intake. The latter is in line with previous studies that failed to observe benefits of nutritional co-

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intervention during long-term exercise intervention in the elderly (32, 33). The absence of any apparent benefits of protein supplementation on the adaptive response to long-term resistance exercise training might be attributed to a less than optimal timing of the applied feeding regimen. Esmarck et al (34) reported that the timing of the administration of a protein-containing supplement after resistance exercise is essential for skeletal muscle hypertrophy to occur during exercise training in the elderly. In their study, the control group, which received nutritional supplementation 2 h after cessation of exercise as opposed to immediately after, showed no improvements in muscle hypertrophy after 12 wk of training (34). However, the latter seems to be in contrast with previous studies that generally show muscle hypertrophy after resistance exercise training without dietary co-intervention (5–11). Nonetheless, recent studies in other populations (35, 36) showed that timed protein supplementation after resistance exercise might induce slight benefits over resistance training alone, although the additional effects were less marked than suggested by Esmarck et al (34).

We hypothesized that protein supplementation immediately before and after resistance exercise would augment the gain in muscle mass and strength during prolonged resistance-type exercise training in elderly people. Therefore, we assessed the impact of timed protein supplementation on the increase in muscle mass and strength during 3 mo of resistance-type exercise training in healthy elderly men who habitually consume adequate amounts of dietary protein.

SUBJECTS AND METHODS

Subjects

A total of 28 healthy elderly men aged 72 ± 2 y volunteered to participate in a 12-wk resistance-type exercise intervention program, with or without additional protein supplementation before and immediately after each exercise session (3 sessions/wk). Two subjects dropped out during the study, one because of an acute back problem that occurred during gardening and the other because of fear of re-injuring his back. The medical history of all subjects was evaluated, and an oral-glucose-tolerance test and resting echocardiograph were performed before selection. Exclusion criteria were defined that would preclude successful participation in the exercise program, which included (silent) cardiac or peripheral vascular disease and orthopedic limitations. Furthermore, because insulin resistance and/or type 2 diabetes are associated with a more progressive loss of muscle mass and strength with aging (37), type 2 diabetes patients were excluded from participation (38). All subjects were living independently and had no history of participation in any structured exercise training program in the past 5 y. All subjects were informed about the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital, Maastricht, and is part of a greater project investigating the clinical benefits of exercise and/or nutritional interventions in the elderly.

Study design

After inclusion in this study, the subjects were randomly allocated to either the protein or the placebo group. Before, during, and after exercise intervention, anthropometric measurements (height, body mass, and leg volume; 39), strength-assessment tests (one-repetition maximum), and computed tomography (CT) and dual-energy X-ray absorptiometry (DXA) scans were performed and muscle biopsy samples, blood samples, 24-h urine samples, and dietary intake records were collected.

Dietary intake and physical activity standardization

Standardized meals (≈51 kJ/kg body mass; 57% of energy as carbohydrate, 13% of energy as protein, and 30% of energy as fat) were provided to all subjects before each test day (ie, before muscle biopsy and/or blood sampling), and the subjects were instructed to refrain from strenuous physical activity for 3 d before testing. Dietary intake was recorded for 2 d before blood sample collection to standardize food intake before blood collection after cessation of the intervention program, thereby minimizing the impact of differences in food intake on blood glucose homeostasis. On all test days, the subjects arrived at the laboratory by car or public transportation after an overnight fast. Before the onset of the intervention program and in week 11 of the exercise intervention, the subjects recorded 3-d weighted dietary records (Thursday–Saturday) to assess potential changes in daily food intake that might have occurred during the intervention period. Food intake records were scrutinized by a dietitian and analyzed with Eetmeter software 2005 (version 1.4.0; Voedingscentrum, The Hague, Netherlands). Dietary intake was calculated for the entire day as well as for breakfast and lunch separately. The energy derived from the protein supplements was not included in the analysis.

Strength assessment

Maximum strength was assessed by one-repetition maximum (1RM) strength tests on leg press and leg extension machines (Technogym, Rotterdam, Netherlands). During a familiarization trial, proper lifting technique was demonstrated and practiced and maximum strength was estimated by using the multiple repetitions testing procedure (40). In an additional session, ≥1 wk before muscle biopsy collection, each subject's 1RM was determined as described previously (3). 1RM testing is preferred to evaluate changes in muscle strength during resistance-type exercise training (41). Therefore, 1RM tests were repeated after 4 and 8 wk of intervention and 2 d after the last training session of the intervention program. None of the subjects experienced any joint pain and/or muscle soreness due to the 1RM testing procedures.

Exercise intervention program

Supervised resistance-type exercise training was performed 3 times/wk for a 12-wk period. All sessions were performed in the morning, at the same time of day. Training consisted of a 5-min warm-up on a cycle ergometer, followed by 4 sets on both the leg press and leg-extension machines, followed by a 5-min cooling-down period on the cycle ergometer. During the first 4 wk of training, the workload was increased from 60% of 1RM (10–15 repetitions in each set) to 75% of 1RM (8–10 repetitions). Starting at week 5, 4 sets of 8 repetitions were performed at 75–80% of 1RM on each machine. Resting periods of 1.5 and 3 min were allowed between sets and exercises, respectively. Workload intensity was adjusted based on the 1RM tests (week 4 and 8). In addition, workload was increased when >8 repetitions could be performed

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in 3 of 4 sets. On average, the subjects attended 35 ± 1 of the 36 scheduled exercise sessions in both groups.

Protein supplementation

During the 5-min warm-up and cooling-down procedure, the subjects received 250 mL of a beverage containing either water only (placebo group) or protein (protein group). The protein beverages contained 10 g protein as casein hydrolysate (DSM, Delft, Netherlands); as such, the protein group received 20 g protein per exercise session. All beverages were flavored to mask the contents of the drinks (cream vanilla: 5 g/L; citric acid: 1.8 g/L; and sodium saccharinate: 0.28 g/L). All subjects ate breakfast ≥1.5 h before starting the exercise sessions, and lunch was eaten no less than 2 h after cessation of each session. On training days, no food or drinks were allowed other than the experimental beverages between breakfast and lunch. The subjects were allowed to drink water before, during, and after each exercise session.

CT scans

An anatomic cross-sectional area (CSA) of the quadriceps muscle was assessed by CT scanning (IDT 8000; Philips Medical Systems, Best, Netherlands) before and after cessation of the exercise intervention program (3 d after the strength assessment and before muscle biopsy collection). While the subjects were supine with their legs extended and their feet secured, a 3-mm thick axial image was taken midway between the anterior superior iliac spine and the distal end of the patella. The scanning characteristics were as follows: 120 kV, 300 mA, rotation time of 0.75 s, and a field of view of 500 mm. The exact scanning position was measured and marked for replication after cessation of the intervention program. Using the described approach, we determined the CV for repeated scans to be <0.6%. Images were loaded onto a personal computer by using IMPAX imaging software (version 5.2; AGFA Health Care, Belgium). Muscle area of the right leg was selected between −29 and 150 Hounsfield units (42), after which the quadriceps muscle was selected by manual tracing. Quadriceps area was calculated by using Lucia 4.81 software (Nikon, Badhoevedorp, Netherlands). All analyses were performed by 2 investigators blinded to subject coding; intraclass correlation coefficients for inter- and intrainvestigator reliability were 0.997 and 0.998, respectively.

DXA scans

Directly after CT scanning, body composition and bone mineral content were measured with DXA (Lunar Prodigy Advance; GE Health Care, Madison, WI). The system's software package (enCORE 2005, version 9.15.00) was used to determine whole-body and regional lean mass, fat mass, and bone mineral content. DXA scans were performed in a fasted state, after the subjects had voided. The CVs for repetitive scans ($n = 4$; 2 wk apart) were 0.4%, 1.0%, and 1.1% for whole-body lean mass, fat mass, and leg lean mass, respectively.

Blood samples

To determine glucose homeostasis and exclude insulin-resistant and/or type 2 diabetic subjects, fasting blood samples were collected before and after 4, 8, and 11 wk of intervention and 4 d after the strength assessment performed after cessation of the exercise program. In addition, a standard oral-glucose-tolerance test was performed 2 wk before and 1 wk after cessation of the intervention. Blood samples were collected in both EDTA-containing tubes and serum tubes and centrifuged at 1000 × *g* and 4°C for 10 min (plasma) or at 18°C for 15 min (serum). Aliquots of plasma and serum were frozen in liquid nitrogen and stored at −80°C. Samples were analyzed for plasma glucose and insulin to assess potential changes in whole-body insulin sensitivity using the oral glucose insulin sensitivity index (43). Plasma glucose concentrations were analyzed with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc, St Charles, MO). The blood glycated hemoglobin (Hb A_{1c}) content (3-mL blood sample, EDTA) was analyzed by HPLC (Variant II; Bio-Rad, Munich, Germany). As a measure of renal function, serum creatinine was measured by using the Jaffe rate method on a Synchron LX20 analyzer (Beckmann Coulter Inc, Fullerton, CA).

24-h Urine collection

To determine urinary nitrogen and creatinine excretion and the 3-methylhistidine concentration, 24-h urine samples were collected over the last day of the 3-d dietary intake assessment. Urine was collected, from the second voiding on day 3 until the first voiding on the day after, into containers with 10 mL of 4 mol H_2SO_4/L. After the total urine production was measured, aliquots of urine were frozen in liquid nitrogen and stored at −80°C. The nitrogen content was analyzed with an elemental analyzer (model CHN-O-RAPID; Heraeus Co, Hanau, Germany). Total nitrogen excretion was calculated from total urinary nitrogen excretion and an estimated 0.031 g/kg body mass for miscellaneous nitrogen loss (44). Nitrogen balance was calculated as the difference between nitrogen intake [protein intake (g)/6.25] and total nitrogen excretion and was used to determine nitrogen balance before and after 11 wk of intervention. Urinary creatinine excretion was measured as described above. As a measure of renal function, creatinine clearance was calculated from urinary excretion and its serum concentration and corrected for body surface area, yielding the amount of blood (in mL) that is cleared from creatinine per minute per 1.73 m^2 of total body surface area (45). As an indirect marker of myofibrillar protein degradation, 3-methylhistidine was determined by HPLC and fluorescence detection (Shimadzu Deutschland GmbH, Duisburg, Germany). The urinary 3-methylhistidine concentration was expressed relative to the creatinine concentration.

Muscle biopsy sampling

Three days before the onset of exercise training and 4 d after the postintervention strength assessment, muscle biopsy samples were taken from the right leg of each subject, in the morning after an overnight fast. After local anesthesia was induced, percutaneous needle biopsy samples (50–80 mg) were collected from the vastus lateralis muscle, ≈15 cm above the patella (46). Any visible nonmuscle tissue was removed immediately, and biopsy samples were embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, Netherlands), frozen in liquid nitrogen-cooled isopentane, and stored at −80°C until further analyses.

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Immunohistochemistry

From all biopsy samples, 5-μm thick cryosections were cut at −20°C. Pre- and postintervention samples from 2 subjects (from both the protein and placebo groups) were mounted together on uncoated glass slides. Slides were stained for muscle fiber typing as described previously (3, 47). First antibodies were directed against MHC-I (A4.951, dilution 1:20; Developmental Studies Hybridoma Bank, Iowa City, IA) and laminin (polyclonal rabbit anti-laminin, dilution 1:50; Sigma, Zwijndrecht, Netherlands). Appropriate secondary antibodies were applied: goat anti-mouse IgG1 AlexaFluor488 and goat anti-rabbit IgG AlexaFluor555 (dilutions of 1:500 and 1:200, respectively; Molecular Probes, Invitrogen, Breda, Netherlands). The staining procedures were as follows. After fixation (5 min acetone), slides were air-dried and incubated for 60 min at room temperature with primary antibodies directed against laminin and MHC-I, diluted in 0.05% Tween–phosphate-buffered saline (PBS). Slides were then washed (3 × 5 min PBS) and incubated for 30 min at room temperature with the appropriate secondary antibodies, diluted in 0.05% Tween-PBS. After a final washing step, all slides were mounted with cover glasses with the use of Mowiol (Calbiochem, Amsterdam, Netherlands).

All images were digitally captured by using fluorescence microscopy with a Nikon E800 fluorescence microscope (Nikon Instruments Europe, Badhoevedorp, Netherlands) coupled to a Basler A113 C progressive scan color CCD camera with a Bayer color filter. Epifluorescence signal was recorded by using a Texas Red excitation filter (540–580 nm) for laminin and an FITC excitation filter (465–495 nm) for MHC-I. Image processing and quantitative analyses were conducted by using the Lucia 4.81 software package (Nikon). All image recordings and analyses were performed by an investigator blinded to subject coding. Images were captured at a 120× magnification. Laminin was used to determine cell borders, and type I and type II muscle fibers were identified for all fibers within each image. Within each image, the number of fibers, the mean fiber CSA, and the percentage of area occupied per fiber type were measured for the type I and type II muscle fibers separately. As a measure of fiber circularity, form factors were calculated by using the following formula: $(4\pi \cdot \text{CSA})/(\text{perimeter})^2$. No differences in fiber circularity were observed over time or between groups. Mean numbers of 335 ± 30 and 265 ± 22 individual muscle fibers were analyzed in the pre- and postintervention biopsy samples, respectively.

Statistics

All data are expressed as means ± SEMs. Baseline characteristics between groups were compared by means of an independent *t* test. Because all data were normally distributed, training-induced changes were analyzed with mixed-model repeated-measures analysis of variance with time (before compared with after exercise training) as a within-subjects factor and group (protein compared with placebo) as a between-subjects factor. Fiber-type-specific variables were analyzed by adding a second within-subjects factor (type I or type II muscle fibers). In case of a significant interaction, paired *t* tests were performed to determine time effects within groups or within type I or II fibers and independent *t* tests for group differences in the pre- and postintervention values. Bonferroni corrections were applied when appropriate. In addition to the repeated-measures analysis, relative changes over time were calculated and analyzed by independent *t* tests to detect potential differences between groups. Because the results from both analyses were identical, we report both absolute and relative changes but only present *P* values for the repeated-measures analyses. The relation between the average habitual daily protein intake and the degree of hypertrophy was determined by correlation analyses. All analyses were performed by using SPSS version 15.0 (Chicago, IL). An α-level of 0.05 was used to determine statistical significance.

RESULTS

Subjects

The subjects' characteristics before and after the intervention are provided in **Table 1**. In total, 26 subjects completed the intervention program, 13 in each group. No differences were observed in baseline variables between groups. The mean age of the subjects was 72 ± 2 y for both groups. Total body mass, height, and BMI did not change over the intervention period in either group. Fasting blood glucose concentration and Hb A_{1c} values were within the normal range for healthy elderly individuals and did not change over time, although Hb A_{1c} tended to decline in both groups ($P = 0.057$; Table 1). Whole-body insulin sensitivity as determined by oral glucose insulin sensitivity (43) did not change over time in either group.

Skeletal muscle hypertrophy

Before the exercise intervention, no differences were observed between the placebo and protein-supplemented groups in quadriceps anatomic CSA: 75.9 ± 3.7 compared with 73.8 ± 3.2 cm^2, respectively. Over time, quadriceps CSA increased by 9 ± 1% in both groups to 82.4 ± 3.9 and 80.0 ± 3.0 cm^2 in the placebo and protein groups, respectively ($P < 0.001$), with no differences between groups (**Figure 1**).

At baseline, muscle fiber CSA was smaller in type II than in type I fibers in both groups (**Figure 2**; $P < 0.001$), with no differences between groups. For muscle fiber CSA, a significant time × fiber type interaction was observed ($P < 0.001$). After intervention, muscle fiber CSA had increased in both type I and II muscle fibers in the placebo (5 ± 4% and 28 ± 6%, respectively) and the protein (13 ± 6% and 29 ± 4%, respectively) groups. The increase in fiber CSA was greater in the type II than in type I fibers, with no differences between groups. As a consequence, differences in muscle fiber type CSA were no longer apparent after exercise intervention (Figure 2).

Muscle strength

At baseline, no differences in muscle strength (1RM) were observed between the placebo and protein groups, respectively (leg extension: 88 ± 4 and 84 ± 3 kg; leg press: 170 ± 8 and 173 ± 8 kg). After intervention, the 1RM for leg extension increased by 27 ± 3% and 38 ± 4% to 111 ± 5 and 115 ± 5 kg in the placebo and protein groups, respectively ($P < 0.001$). Likewise, the 1RM for leg press increased by 24 ± 3% and 24 ± 2% to 210 ± 10 and 215 ± 11 kg in the placebo and protein groups, respectively ($P < 0.001$). No differences were observed between groups. Repeated-measures analysis showed that the increase in 1RM strength was statistically significant for each 4-wk interval during the intervention period

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TABLE 1
Subject characteristics before and after the intervention[1]

	Placebo group ($n = 13$)		Protein group ($n = 13$)	
	Before	After	Before	After
Body mass (kg)	80.2 ± 3.4	80.1 ± 3.4	79.2 ± 2.8	78.9 ± 2.9
Height (m)	1.71 ± 0.01	1.71 ± 0.01	1.73 ± 0.02	1.73 ± 0.02
BMI (kg/m^2)	27.4 ± 1.1	27.4 ± 1.1	26.5 ± 1.0	26.4 ± 1.0
Leg volume (L)	8.2 ± 0.5	8.3 ± 0.5[2]	8.0 ± 0.3	8.2 ± 0.3[2]
Glucose (mmol/L)	5.6 ± 0.2	5.5 ± 0.1	5.9 ± 0.2	5.8 ± 0.1
Glycated hemoglobin (%)	5.8 ± 0.1	5.7 ± 0.1	5.9 ± 0.1	5.8 ± 0.1
OGIS index (mL · kg^{-1} · min^{-1})	368 ± 22	382 ± 19	365 ± 12	368 ± 16

[1] All values are means ± SEMs. OGIS, oral glucose insulin sensitivity (43). Data were analyzed by using repeated-measures ANOVA with time and group as factors. No significant differences were observed between groups before the intervention. No time × group interaction was observed for any of the variables ($P \geq 0.40$). No significant main effect of group was observed for any of the variables.
[2] Significantly different from before the intervention, $P < 0.05$.

for both exercises, with no differences between groups (data not shown).

Body composition

No significant differences were observed between groups at baseline for any of the DXA measurements. Leg lean mass increased by 6 ± 1% in both groups, from 18.3 ± 0.5 and 18.0 ± 0.6 kg to 19.3 ± 0.5 and 19.0 ± 0.6 kg in the placebo and protein groups, respectively (**Figure 3**; $P < 0.001$). Whole-body lean mass increased throughout the intervention period, from 57.4 ± 1.6 and 56.1 ± 1.4 kg to 58.0 ± 1.7 and 56.8 ± 1.4 kg in the placebo and protein groups, respectively ($P < 0.01$). Total fat mass decreased significantly ($P < 0.01$), which resulted in a significant decline in the percentage of whole-body fat (placebo group: from 23.6 ± 2.2% to 22.9 ± 2.2%; protein group: from 24.9 ± 1.4 to 23.7 ± 1.4%; $P < 0.001$). In accordance, percentage of leg fat was lower after exercise intervention ($P < 0.001$). No significant differences were observed between groups. No changes were observed in bone mineral content (data not shown).

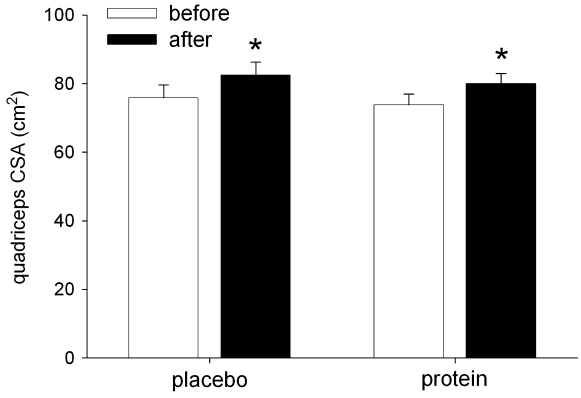

FIGURE 1. Mean (±SEM) quadriceps cross-sectional area (CSA) before and after 3 mo of resistance exercise training in elderly men with (protein group; $n = 13$) or without (placebo group; $n = 13$) protein supplementation during each exercise session. Data were analyzed by using repeated-measures ANOVA with time and group as factors. No time × group interaction ($P = 0.79$) or main group effect ($P = 0.65$) was observed. *Significantly different from before intervention, $P < 0.001$.

Muscle fiber type composition

At baseline, no group differences were observed in the percentage of type I and II muscle fibers (fiber%) and/or the percentage of muscle area occupied by type I and II fibers (area%). Type I and II muscle fiber% did not change after 3 mo of exercise intervention (**Table 2**). In contrast, type II muscle fiber area% tended to increase from 48 ± 4% and 40 ± 4% to 54 ± 3% and 47 ± 3% in the placebo and protein group, respectively ($P = 0.057$). No group differences were observed.

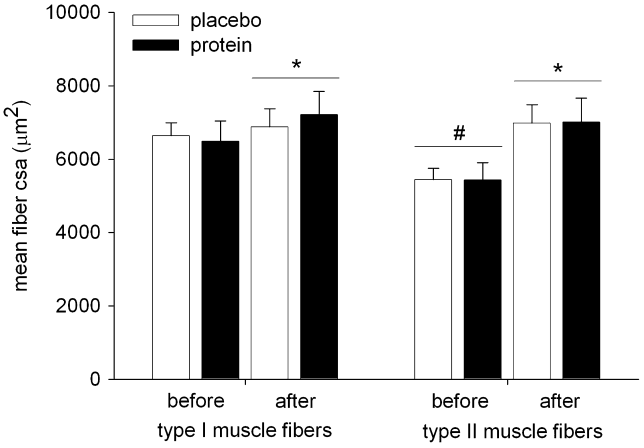

FIGURE 2. Mean (±SEM) muscle fiber cross-sectional area (CSA) for type I and II muscle fibers before and after 3 mo of resistance exercise training in elderly men with (protein group; $n = 13$) or without (placebo group; $n = 13$) protein supplementation during each exercise session. Data were analyzed by using repeated-measures ANOVA with time, group, and fiber type as factors. No time × fiber type × group ($P = 0.17$), time × group ($P = 0.54$), or fiber type × group ($P = 0.82$) interactions were observed. A significant time × fiber type interaction ($P < 0.001$) showed a difference between type I and II muscle fiber size before intervention; in addition, the increase in muscle fiber CSA over time was greater in type II than in type I muscle fibers. #Significant fiber type effect compared with type I fibers at baseline (fiber type × group interaction: $P = 0.69$; main group effect: $P = 0.90$; main fiber type effect: $P < 0.001$). *Significant time effect compared with before intervention: type I fibers (time × group interaction: $P = 0.31$; main group effect: $P = 0.90$; main time effect: $P < 0.05$) and type II fibers (time × group interaction: $P = 0.93$; main group effect: $P = 0.98$; main time effect: $P < 0.001$).

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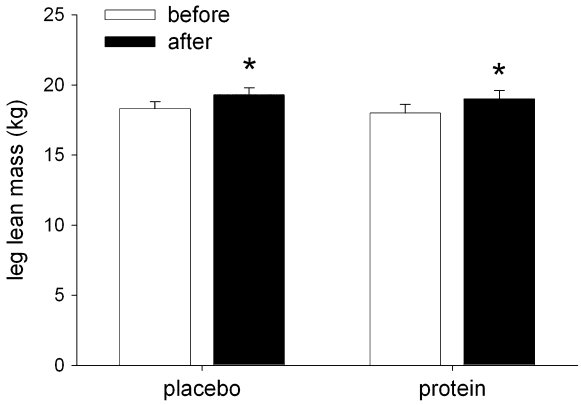

FIGURE 3. Mean (±SEM) leg lean mass before and after 3 mo of resistance exercise training in elderly men with (protein group; $n = 13$) or without (placebo group; $n = 13$) protein supplementation during each exercise session. Data were analyzed by using repeated-measures ANOVA with time and group as factors. No time × group interaction ($P = 0.79$) or main group effect ($P = 0.65$) was observed. *Significantly different from before intervention, $P < 0.001$.

Dietary intake records

Analysis of the 3-d dietary intake records collected before and after 11 wk of intervention showed no differences in total daily energy intake between groups and/or over time (9.2 ± 0.6 and 9.3 ± 0.4 MJ/d to 9.1 ± 0.4 and 9.4 ± 0.6 MJ/d in the placebo and protein groups, respectively). Macronutrient composition of the diet did not change during the intervention period and did not differ between groups (**Table 3**). Daily protein intake averaged 1.1 ± 0.1 g · kg^{-1} · d^{-1} in both groups and did not change during the intervention period.

Total energy intake and macronutrient composition of both breakfast and lunch did not differ between groups before the intervention and did not change over time in either group (data not shown). Protein intake at breakfast and lunch did not differ between groups and did not change over time (Table 3).

Correlation analyses showed that the daily dietary protein intake was positively correlated with the degree of muscle hypertrophy. Pearson correlation coefficients between total dietary protein intake (g · kg^{-1} · d^{-1}), and the increase in lean mass and leg lean mass were 0.34 and 0.33, respectively. These correlations were unchanged after adjustment for the effect of protein supplementation.

TABLE 2
Muscle fiber type composition[1]

	Placebo group ($n = 13$)		Protein group ($n = 13$)	
	Before	After	Before	After
Fiber (%)				
Type I	47 ± 4	47 ± 3	56 ± 4	52 ± 4
Type II	53 ± 4	53 ± 3	44 ± 4	48 ± 4
CSA (%)				
Type I	52 ± 4	46 ± 3	60 ± 4	53 ± 3
Type II	48 ± 4	54 ± 3	40 ± 4	47 ± 3

[1] All values are means ± SEMs. CSA, cross-sectional area. Data were analyzed by using repeated-measures ANOVA with time and group as factors. No significant differences were observed between groups before the intervention. No time × group interaction was observed ($P = 0.48$ for fiber, $P = 0.70$ for CSA). No significant main effect of group, time, or both was observed.

Blood and 24-h urine collection

Serum creatinine concentrations were within the normal range before intervention and did not change over time in either group (from 1.16 ± 0.04 and 1.10 ± 0.06 mg/dL to 1.19 ± 0.03 and 1.11 ± 0.06 mg/dL in the placebo and protein groups, respectively). No differences were observed between groups. Creatinine clearance was similar between groups before the intervention (placebo group: 59.1 ± 6.3 mL/min per 1.73 m^2; protein group: 61.0 ± 4.7 mL/min per 1.73 m^2) and did not change over time in either group. Measurement of 24-h nitrogen balance before the intervention showed that both groups were in nitrogen balance (0.25 ± 0.40 and 0.22 ± 0.92 g/d in the placebo and protein groups, respectively). No significant changes were observed over time, and the subjects were still in nitrogen balance after 11 wk of intervention (−0.03 ± 0.99 and −0.14 ± 0.87 g/d in the placebo and protein groups, respectively). No significant differences were observed in 3-methylhistidine excretion between groups before the intervention (14.3 ± 2.0 and 11.7 ± 2.1 mmol/mol creatinine in the placebo and protein groups, respectively). No significant changes were observed over time and/or between groups (mean change: 5 ± 9% and 3 ± 9% in the placebo and protein groups, respectively).

DISCUSSION

The present study showed that timed protein supplementation before and immediately after each exercise session does not further augment the increase in skeletal muscle mass and strength after 3 mo of resistance-type exercise training in healthy elderly men who habitually consumed adequate dietary protein.

Resistance-type exercise training has been shown to represent an effective interventional strategy to counteract sarcopenia (5–11). In the present study, we observed gains in whole-body lean mass of 0.6 ± 0.3 kg (placebo group) and 0.7 ± 0.2 kg (protein group) and a concomitant decrease in whole-body fat mass. The observed improvements are similar to previous findings reported after 12–16 wk of resistance exercise training in the elderly (7, 30). Improvements were predominantly located in the lower extremities, with a 6 ± 1% increase in total leg lean mass and a 9 ± 1% increase in quadriceps CSA in both groups. The increase in quadriceps CSA was very similar to the ≈9% increase in muscle area observed after exercise training in subjects aged 60–72 y (5) as well as in subjects aged >85 y (10). Skeletal muscle mass and muscle CSA are positively correlated with strength (10). In accordance, we observed substantial increases in muscle strength of 27 ± 3% and 24 ± 3% (placebo group) and of 38 ± 4% and 24 ± 2% (protein group) in 1RM leg extension and leg press. Previously, similar increases in strength (range: 25–45%) were reported (7, 8).

The loss of skeletal muscle mass with aging is associated with specific type II muscle fiber atrophy (1–3). In accordance, type II muscle fiber CSA was significantly smaller than type I fiber CSA before intervention. Consistent with previous observations (6–9), the exercise-induced increase in muscle fiber size was greater in the type II than in the type I muscle fibers in both groups (Figure 2). As a consequence, differences in muscle fiber type size before

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TABLE 3
Energy intake and macronutrient composition of the diet[1]

	Placebo group ($n = 13$)		Protein group ($n = 13$)	
	Before	After	Before	After
Total energy (MJ/d)	9.2 ± 0.6	9.1 ± 0.4	9.3 ± 0.4	9.4 ± 0.6
Carbohydrate (% of energy)	50 ± 2	52 ± 2	52 ± 2	53 ± 2
Fat (% of energy)	33 ± 2	32 ± 2	31 ± 1	31 ± 1
Protein (% of energy)	17 ± 1	16 ± 1	16 ± 1	16 ± 1
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Protein at breakfast ($g \cdot kg^{-1} \cdot d^{-1}$)	0.21 ± 0.05	0.23 ± 0.05	0.22 ± 0.02	0.23 ± 0.03
Protein at lunch ($g \cdot kg^{-1} \cdot d^{-1}$)	0.30 ± 0.02	0.32 ± 0.03	0.28 ± 0.03	0.27 ± 0.03

[1] All values are means ± SEMs. Data were analyzed by using repeated-measures ANOVA with time and group as factors. No significant differences were observed between groups before the intervention. No time × group interaction was observed for any of the variables ($P \geq 0.30$). No significant main effects of group, time, or both were observed for any of the variables.

intervention were no longer apparent after the 12-wk exercise intervention program. Taken together, these data confirm the efficacy of resistance-type exercise training to improve skeletal muscle mass and strength and reverse type II muscle fiber atrophy in the elderly.

Studies assessing the acute muscle protein synthetic response after exercise have provided ample data to suggest that muscle protein balance can be substantially increased by ingesting protein and/or amino acids before and/or immediately after exercise (18, 20–25, 27). However, long-term nutritional intervention studies have generally failed to observe additional benefits of increasing protein intake during exercise intervention in the elderly (30, 32, 33, 48). The latter observation is in line with that of Campbell and Leidy (31), who recently concluded that improvements in muscle mass and strength induced by resistance exercise training are not enhanced when older people who consume adequate dietary protein (in excess of $0.8\ g \cdot kg^{-1} \cdot d^{-1}$) further increase their protein intake. The absence of any additional benefits of nutritional cointervention during more prolonged exercise intervention programs might be attributed to an inadequate timing of the protein supplementation after each exercise bout (34). Furthermore, ingesting dietary protein before and/or during exercise has been shown to further improve the postexercise net muscle protein balance (25, 27). Therefore, we hypothesized that the intake of 10 g protein before and 10 g protein immediately after resistance exercise would increase muscle mass and strength gains during prolonged resistance-type exercise training in healthy elderly men who habitually consume adequate dietary protein. Even though we observed large increases in muscle mass and strength on a whole-body, limb, and myocellular level, no differences were observed between the groups supplemented with (protein group) or without (placebo group) additional protein (Figures 1–3). The latter occurred despite the fact that with a power of 0.80 we would have been able to detect group differences as small as 3.5%, 2.5% and 11% for the changes in quadriceps CSA, leg lean mass, and muscle fiber size, respectively. The latter would have been more than sufficient to detect even the smallest clinically relevant differences compared with previous findings (34).

The present data seem to be in contrast with the observations of Esmarck et al (34), who found that the acute postexercise ingestion of a protein-containing supplement is prerequisite for muscle hypertrophy to occur in the elderly. It might be suggested that the apparent discrepancy can be explained by differences between the supplements that were provided in these studies. In the present study we provided 20 g protein, whereas Esmarck et al (34) provided their subjects with supplements containing 10 g protein, 7 g carbohydrate, and 3 g fat. However, the lack of carbohydrate in the supplements provided in the present study would unlikely have modulated the muscle protein anabolic response, because recent work from our group (49) and from others (18) has shown that postexercise carbohydrate ingestion is not warranted when ample protein is ingested. The apparent discrepancy between studies is more likely attributed to differences in the outcome of the control groups. In the present study, we observed a substantial increase in muscle mass and strength after resistance-type exercise training without nutritional cointervention (placebo group). In contrast, Esmarck et al (34) reported no increase in leg muscle CSA and muscle fiber size when protein supplements were provided 2 h after cessation of exercise. The latter tends to disagree with the plethora of studies that report substantial increases in muscle mass and strength after 2–4 mo of resistance-type exercise training in the elderly without any dietary modulation (5–11, 32, 33). In short, timed protein supplementation before and immediately after exercise does not seem to further augment the benefits of prolonged resistance-type exercise training on muscle mass and strength in healthy elderly men.

In the present study, dietary intake remained stable throughout the intervention period (Table 3). Even without additional protein supplementation, habitual dietary protein intake averaged $1.1 \pm 0.1\ g \cdot kg^{-1} \cdot d^{-1}$ in both groups. This value is well in excess of the current Recommended Dietary Allowances values of $0.8\ g \cdot kg^{-1} \cdot d^{-1}$ (50, 51). The latter values have been suggested to be marginal or even insufficient for muscle mass maintenance (29) and/or for allowing lean mass accrual after resistance training in the elderly (28). However, when older people habitually consume adequate dietary protein (ie, $>0.9\ g \cdot kg^{-1} \cdot d^{-1}$), improvements in muscle mass and strength after long-term resistance exercise training do not seem to be further enhanced by increases in dietary protein intake (30). Yet, in line with recent observations by Campbell and Leidy (31), we also observed a positive correlation between daily dietary protein intake and the increase in lean mass with training. We can only speculate on the physiologic relevance of these correlations, which show that the regulation of the skeletal muscle adaptive response to exercise and nutritional supplementation remains far from being established.

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The additional protein ingested before and after each exercise session resulted in an average additional protein intake of 0.1 ± 0.0 $g \cdot kg^{-1} \cdot d^{-1}$. The latter induced no side effects and did not induce any changes in markers of renal function or 24 h nitrogen balance. Although we could not detect any benefits of timed protein supplementation during exercise intervention in healthy, well-nourished elderly men, it remains to be determined whether the proposed benefits of timed protein supplementation are restricted to specific elderly subpopulations, such as malnourished or frail elderly.

We conclude that prolonged resistance-type exercise training substantially improves skeletal muscle mass and strength in healthy elderly men. Timed protein supplementation immediately before and after each exercise session does not further enhance skeletal muscle mass and strength gains after prolonged resistance-type exercise training in healthy elderly men who habitually consume adequate amounts of dietary protein.

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The authors' responsibilities were as follows—LBV and LJCvL: designed the study; LBV: performed the statistical analysis, organized the data, and carried out the training and the clinical experiments; RAMJ, BGG, and WKWHW: performed the immunohistochemical and chemical analysis and quantification; LBV, LJCvL,KM, and HHCMS: wrote the manuscript; and MB and PD: provided medical assistance. None of the authors had any personal or financial conflicts of interest.

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