Protein Supplementation during Resistance-Type Exercise Training in the Elderly

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ABSTRACT

LEENDERS, M., L. B. VERDIJK, L. VAN DER HOEVEN, J. VAN KRANENBURG, R. NILWIK, W. K. W. H. WODZIG, J. M. G. SENDEN, H. A. KEIZER, and L. J. C. VAN LOON. Protien Supplementation during Resistance-Type Exercise Training in the Elderly. *Med. Sci. Sports Exerc.*, Vol. 45, No. 3, pp. 542–552, 2013. **Introduction**: Resistance training has been well established as an effective treatment strategy to increase skeletal muscle mass and strength in the elderly. We assessed whether dietary protein supplementation can further augment the adaptive response to prolonged resistance-type exercise training in healthy elderly men and women. **Methods**: Healthy elderly men (n = 31, 70 ± 1 yr) and women (n = 29, 70 ± 1 yr) were randomly assigned to a progressive, 24-wk resistance-type exercise training program with or without additional protein supplementation (15 $g \cdot d^{-1}$). Muscle hypertrophy was assessed on a whole-body Dual-energy X-ray absorptiometry (DXA), limb (computed tomography), and muscle fiber (biopsy) level. Strength was assessed regularly by 1-repetition maximum (RM) strength testing. Functional capacity was assessed with a sit-to-stand and handgrip test. **Results**: One-RM strength increased by 45% ± 6% versus 40% ± 3% (women) and 41% ± 4% versus 44% ± 3% (men) in the placebo versus protein group, respectively ($P < 0.001$), with no differences between groups. Leg muscle mass (women, 4% ± 1% vs 3% ± 1%; men, 3% ± 1% vs 3% ± 1%) and quadriceps cross-sectional area (women, 9% ± 1% vs 9% ± 1%; men, 9% ± 1% vs 10% ± 1%) increased similarly in the placebo versus protein groups ($P < 0.001$). Type II muscle fiber size increased over time in both placebo and protein groups (25% ± 13% vs 30% ± 9% and 23% ± 12% vs 22% ± 10% in the women and men, respectively). Sit-to-stand improved by 18% ± 2% and 19% ± 2% in women and men, respectively ($P < 0.001$). **Conclusion**: Prolonged resistance-type exercise training increases skeletal muscle mass and strength, augments functional capacity, improves glycemia and lipidemia, and reduces blood pressure in healthy elderly men and women. Additional protein supplementation (15 $g \cdot d^{-1}$) does not further increase muscle mass, strength, and/or functional capacity. **Key Words:** SARCOPENIA, MUSCLE MASS, STRENGTH, FUNCTIONAL CAPACITY

Aging is associated with a progressive loss of skeletal muscle mass and strength. This process is called sarcopenia and ultimately results in the loss of functional capacity and an increased risk of developing chronic metabolic diseases (12). The age-related loss of muscle mass is facilitated by a combination of factors, including a less than optimal diet and a sedentary lifestyle. Resistance-type exercise training has been well established as an effective treatment strategy to counteract the loss of skeletal muscle mass and strength in the elderly (8,14,22). Because of the efficacy of resistance-type exercise training to increase muscle mass and function up to a very old age (14,22), many attempts have been undertaken to further augment the clinical benefits of exercise training.

Dietary protein intake forms an important requirement for muscle mass maintenance. A direct relationship between dietary protein intake and the loss of muscle mass with aging has been reported previously (19). Because dietary protein intake is a prerequisite to allow net muscle protein accretion after resistance-type exercise (35), it has been suggested that dietary protein supplementation can further augment the increase in muscle mass and strength during prolonged resistance-type exercise training. In agreement, dietary protein supplementation has been shown to increase muscle mass gains during more prolonged resistance-type exercise training in healthy young adults (21,40). In contrast, studies in the elderly do not seem to confirm the proposed benefits of dietary protein supplementation (13,15,37). The apparent discrepancy between studies in the young versus elderly populations might be attributed to the reduced sensitivity of

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the muscle protein synthetic machinery to protein ingestion in the elderly when compared with the young (10,17). In this light, it has been suggested that approximately 25 g of protein should be ingested with each main meal to maximally stimulate muscle protein synthesis in the elderly (28). Interestingly, we recently reported that habitual protein intake in elderly subjects is particularly low at breakfast, providing only approximately 10 g of protein (33). Therefore, the present study was specifically designed to increase daily protein intake at breakfast with an additional 15 g, allowing ingestion of approximately 25 g of protein with each main meal. So far, most intervention studies have applied relative short intervention periods lasting between 6 and 12 wk. We speculate that a more prolonged resistance-type exercise training duration is required to allow the additional benefits of protein supplementation to become evident in an elderly population.

We hypothesize that dietary protein supplementation at breakfast during prolonged resistance-type exercise training will further augment the increase in muscle mass and strength in healthy elderly men and women. Therefore, we subjected a large group (n = 60) of healthy elderly men and women (70 ± 1 yr) to 6 months of resistance-type exercise training (three sessions per week) during which they were supplemented with dairy protein (15 $g \cdot d^{-1}$) or a placebo. Before and after 3 and 6 months, we determined muscle mass on a whole-body, limb, and muscle fiber level and assessed muscle strength and functional capacity.

METHODS

Subjects. A total of 29 healthy elderly women (70 ± 1 yr) and 31 healthy elderly men (70 ± 1 yr) volunteered to participate in a 24-wk resistance-type exercise intervention program, with or without additional protein supplementation. Seven subjects dropped out (two men and five women) during the study, one because of a heart attack that occurred at home, one because of a transient ischemic attack that occurred at home, and the other five because they underestimated the time required to participate. Medical history of all subjects was evaluated, and an oral glucose tolerance test and resting and exercise ECG were performed before inclusion. Exclusion criteria that would preclude successful participation in the exercise program were defined, and these included (silent) cardiac or peripheral vascular disease and orthopedic limitations. Furthermore, because insulin resistance and/or type 2 diabetes have been associated with a more progressive loss of skeletal muscle mass and strength with aging (30), type 2 diabetes patients were excluded from participation based on the oral glucose tolerance test data (2). All subjects were living independently and were recreationally active (i.e., walking/cycling). None of the participants had a history of participating in any structured exercise training program designed to improve performance over the past 5 yr. All subjects were informed on 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 Maastricht University Medical Centre+.

Study design. After inclusion in this study, subjects were randomly allocated to either the protein (PRO)- or the placebo (PLA)-supplemented group. Before, during, and after exercise intervention, anthropometric measurements (height, body mass, waist–hip ratio, and leg volume (20)), strength assessment (1 repetition maximum (RM)), computed tomography (CT), and Dual-energy X-ray absorptiometry (DXA) scans were performed, and muscle biopsies, blood samples, 24-h urine, and dietary intake and physical activity records were collected.

Exercise intervention program. Supervised resistance-type exercise training was performed three times a week for a 24-wk period. Training consisted of a 5-min warm-up on a cycle ergometer, followed by four sets on both the leg press and leg extension machines (Technogym, Rotterdam, the Netherlands) and three sets on the chest press and horizontal row; these four exercises were performed every training session. The vertical lat pull and abdominals were alternated with biceps curl and triceps extension between subsequent training sessions. Each session ended with a 5-min cooling down period on the cycle ergometer. During the first 4 wk of training, the workload was increased from 60% of 1 RM (10–15 repetitions in each set) to 75% of 1 RM (8–10 repetitions). Starting at week 5, four sets of eight repetitions were performed at 75%–80% of 1 RM on leg press and leg extension. For the upper body exercises, two sets were increased to three sets starting in week 5. Resting periods of 1.5 and 3 min were allowed between sets and exercises, respectively. Workload intensity was adjusted based on the 1-RM tests (performed at weeks 4, 8, 12, 16, and 20). In addition, workload was increased when more than eight repetitions could be performed in three of four sets. On average, subjects attended 90% ± 1% of the scheduled exercise sessions, with no differences between groups.

Dietary protein supplementation. Throughout the 24-wk intervention period, subjects consumed a 250-mL package containing either a placebo (placebo group, PLA) or protein drink (protein group, PRO) daily after breakfast. The protein beverages contained 15 g of protein (milk protein concentrate (MPC80); DMV International, Delhi, NY), 0.5 g of fat, 7.13 g of lactose, and 0.42 g of calcium, providing a total of 389 kJ. The milk protein consisted of 80% of casein and 20% of whey protein. The placebo beverages contained no protein or fat, only 7.13 g of lactose and 0.42 g of calcium, providing a total of 119 kJ. Placebo and protein drinks were provided in a randomized, double-blind manner.

Dietary intake and physical activity standardization. Standardized meals were provided to all subjects the evening before each test day. The subjects were instructed to refrain from strenuous physical activity for at least 3 d before testing. On all test days, subjects arrived at the laboratory by car or public transportation after an overnight fast. Subjects were encouraged to maintain their habitual dietary intake and physical activity pattern throughout the intervention program.

To assess potential changes in habitual daily food intake and physical activity during the 6-month intervention period, the subjects recorded 4-d weighted dietary intake records and 2-d physical activity records. Dietary intake was recorded before and after 4, 8, 12, 16, 20, and 24 wk of intervention. Dietary records were analyzed with Komeet (Komeet, 4.059 BaS Nutrition Software, Arnhem, the Netherlands). Supplements were not included in the dietary intake analysis. Habitual physical activity was recorded before and after 12 and 24 wk of intervention. For every type of activity, a MET score was assigned as previously defined (1). Energy expenditure is reported as mean MET hour per day (41).

Body composition. Body composition and bone mineral content were measured using DXA (Discovery A, QDR Series; Hologic, Bradford, MA). Whole-body and regional lean mass, fat mass, and bone mineral content were determined by using the system's software package Apex version 2.3 Wind River, Alameda, CA). Anthropometrics data were assessed using standardized procedures; bodyweight by digital scale to within 100 g; height by stadiometer to within 0.5 cm; and circumferences to within 1 mm using a measuring tape, with waist midway between the lowest rib and the iliac crest with the subject standing at the end of gentle expiration, and hips at the greater trochanters (16).

Anatomical cross-sectional area (CSA) of the *quadriceps* muscle was assessed by CT scanning (Philips Brilliance 64, Philips Medical Systems, Best, the Netherlands) before and after 12 and 24 wk of intervention (3 d after strength assessment and before muscle biopsy collection). The scanning characteristics were as follows: 120 kV, 300 mA, rotation time of 0.75 s, and a field of view of 500 mm. Although the subjects were lying supine, legs extended and their feet secured, a 3-mm-thick axial image was taken 15 cm proximal to the base of the patella. The exact scanning position was measured and marked for replication at subsequent visits. Muscle area of the right leg was selected between 0 and 100 Hounsfield units (16), after which, the *quadriceps* muscle was selected by manual tracing using ImageJ software (version 1.45d; National Institutes of Health, Bethesda, MD) (33). Using the described approach, we determined the coefficient of variation for repeated scans (1 wk apart) to be 0.8%. All analyses were performed by two investigators blinded to subject coding; intraclass correlation coefficients for inter- and intrainvestigator reliability were 1.000 and 0.997, respectively.

Muscle biopsy sampling. Three days before the onset of the intervention and after 12 and 24 wk of intervention (4 d after final strength testing), muscle biopsies 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, approximately 15 cm above the patella (3). Any visible nonmuscle tissue was removed immediately, and biopsy samples were embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, the Netherlands), frozen in liquid nitrogen–cooled isopentane, and stored at −80°C until further analyses.

Immunohistochemistry. From all biopsies, 5-μm-thick cryosections were cut at −20°C. Samples collected before and after 12 and 24 wk of intervention from each subject were mounted together on uncoated glass slides. Muscle biopsies were stained for muscle fiber typing as described in detail previously (37). In short, the slides were incubated with primary antibodies against MHC-I (A4.840; Developmental Studies Hybridoma Bank, Iowa City, IA) and laminin (polyclonal laminin; Sigma, Zwijndrecht, the Netherlands). After washing, appropriate secondary antibodies were applied (goat anti–mouse IgM AlexaFluor555 and goat anti–rabbit IgG AlexaFluor647, respectively; Molecular Probes, Invitrogen, Breda, the Netherlands). Images were visualized and automatically captured at 10× magnification with a fluorescent microscope equipped with an automatic stage (IX81 motorized inverted microscope; Olympus, Hamburg, Germany). Muscle fiber type (fiber%) and fiber CSA were measured for each separate muscle fiber. As such, mean muscle fiber size was calculated 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: $(4\pi \mathrm{CSA})/(\mathrm{perimeter})^2$. All image recordings and analyses were performed by an investigator blinded to subject coding. No differences in fiber circularity were observed over time or between groups. Mean numbers of 442 ± 24, 403 ± 21, and 425 ± 20 muscle fibers were analyzed in the biopsy samples collected before and after 12 and 24 wk of intervention, respectively.

Strength assessment. Maximum strength was assessed by 1-RM strength tests on leg press and leg extension machines (Technogym). During a familiarization trial, proper lifting technique was demonstrated and practiced and maximum strength was estimated using the multiple repetitions testing procedure (27). In an additional session, at least 1 week before muscle biopsy collection, each subject's 1 RM was determined as described previously (38). One-RM testing is preferred to evaluate changes in muscle strength during resistance-type exercise training (39). Therefore, 1-RM tests were repeated after 4, 8, 12, 16, and 20 wk of intervention and 2 d after the last training session of the intervention program.

Physical performance measures. To assess lower and upper extremity physical performance, a sit-to-stand test and a handgrip test were performed before and after 12 and 24 wk of intervention. For the sit-to-stand test, the participants were instructed to fold their arms across their chest and to stand up/sit down five times, as fast as possible, from a seat at 0.42 m from the floor. Time was recorded from the initial sitting to the final standing position. The fastest out of two attempts was used for analysis (18). Data on maximal grip strength were obtained using a JAMAR handheld dynamometer (model BK-7498; Fred Sammons, Inc., Burr Ridge, IL). Grip strength was measured three times with each hand. The highest value in the stronger hand was reported (7).

Blood samples. Before and after 12 and 24 wk of intervention, fasting blood samples were collected to determine basal plasma glucose and insulin concentrations, lipid

profiles, serum creatinine, and blood glycated hemoglobin (HbA1c) content. Blood (10 mL) was collected into EDTA-containing tubes and serum tubes. EDTA tubes were immediately centrifuged at 1000*g* for 10 min at 4°C, and the serum tubes were centrifuged at 1000*g* for 15 min at 21°C after allowing the blood to clot for 90 min at 21°C. Aliquots of plasma and serum were immediately frozen in liquid nitrogen and stored at −80°C until further analysis. Plasma insulin concentrations were determined by using an Insulin RIA Kit (LINCO Research Inc., St Charles, MO). Plasma glucose, triglycerides, total cholesterol, and HDL cholesterol were analyzed with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland) with test kits from ABX Diagnostics (Montpellier, France). As plasma triacylglycerol concentrations were below 4.5 $mmol \cdot L^{-1}$, plasma LDL cholesterol could be calculated by LDL cholesterol = total cholesterol − HDL cholesterol − triacylglycerol/2.2 (in $mmol \cdot L^{-1}$). Serum creatinine concentrations were determined using the Jaffe rate method on a Synchron LX Systems analyzer (Beckmann Coulter Inc., Fullerton, CA). To determine blood HbA1c content, 3 mL of blood was collected in EDTA containing tubes and analyzed by high-performance liquid chromatography (Bio-Rad Variant II 4, Munich, Germany).

Twenty-four–hour urine collection. To determine urinary nitrogen and creatinine excretion, 24-h urine was collected over the last day of the 4-d dietary intake assessment. Urine was collected from the second voiding on day 4 until the first voiding on the day after. Nitrogen content was analyzed with an elemental analyzer (model CHN-ORAPID; Heraeus Co., Hanau, Germany). Total nitrogen excretion was calculated from total urinary nitrogen excretion and an estimated 0.031 $g \cdot kg^{-1}$ body mass for miscellaneous nitrogen loss (24). Nitrogen balance was calculated as the difference between nitrogen intake (protein intake (g)/6.25) and total nitrogen excretion before and after 4, 12, and 24 wk of intervention. Urinary creatinine excretion was measured as described previously. 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 was cleared from creatinine per minute per 1.73 m^2 of total body surface area (23).

Statistics. Data are expressed as means ± SEM. Based on a Type I error probability of 0.05, a power of 80%, and a drop-out rate of 20%, a total of 60 subjects was included in the present study to detect relevant differences in the primary outcome parameters: lean mass determined by DXA and quadriceps CSA determined by CT scan. For various reasons (not related to the study), seven subjects (12%) dropped out during the first month of the study. Because no follow-up measurements could be performed for these subjects, they were excluded from the analyses. Baseline characteristics between groups were compared using an independent samples *t*-test. Pre- versus 12-wk versus postintervention data were analyzed using repeated-measures ANOVA with time as within-subjects factor and gender and treatment as between-subjects factor. In case of significant main effects or interactions, *post hoc* testing with Bonferroni correction and/or separate analyses within groups were performed where appropriate. Significance was set at $P < 0.05$. All calculations were performed using SPSS version 17.0 (Chicago, IL).

RESULTS

Subjects. Subjects' characteristics are provided in Table 1. In total, 53 subjects completed the intervention program: 27 subjects in the protein group (12 women and 15 men) and 26 subjects in the placebo group (12 women and 14 men). Within gender subgroups, no differences were observed in baseline variables between PLA and PRO. No significant changes over time were observed for weight, height, waist–hip ratio, and body mass index. Systolic blood pressure significantly decreased between 12 and 24 wk of intervention in both the PLA and PRO group (women: from 133 ± 3 to 131 ± 5 and 143 ± 5 to 136 ± 7 mm Hg, respectively; men: from 142 ± 3 to 133 ± 3 and 139 ± 4 to 134 ± 3 mm Hg, respectively; $P < 0.001$), with no differences between groups. Diastolic blood pressure decreased between 12 and 24 wk of intervention in both groups ($P < 0.001$).

Body composition. At baseline, no significant differences were observed between the PLA and PRO group for any of the DXA measurements. Whole-body lean mass increased throughout the intervention period in the women, from 43.3 ± 1.2 to 44.4 ± 1.3 and 41.7 ± 1.5 to 43.0 ± 1.5 kg

TABLE 1. Subjects' characteristics.

	Women		Men	
	Placebo ($n = 12$)	Protein ($n = 12$)	Placebo ($n = 14$)	Protein ($n = 15$)
Age (yr)	69 ± 1	72 ± 2	70 ± 1	70 ± 1
Body weight (kg)	67.9 ± 1.8	63.3 ± 2.5	84.3 ± 2.4	84.0 ± 2.3
Height (m)	1.65 ± 0.02	1.62 ± 0.02	1.78 ± 0.02	1.76 ± 0.01
Waist–hip ratio	0.88 ± 0.01	0.88 ± 0.02	0.98 ± 0.01	0.98 ± 0.01
BMI ($kg \cdot m^{-2}$)	25.0 ± 0.4	24.2 ± 0.7	26.7 ± 0.6	27.2 ± 0.7
Basal plasma glucose ($mmol \cdot L^{-1}$)	5.4 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
HbA1c (%)	5.8 ± 0.1	5.8 ± 0.1	5.5 ± 0.1	5.5 ± 0.1
Lean mass (kg)	43.3 ± 1.2	41.7 ± 1.5	62.4 ± 1.5	61.5 ± 1.3
Fat (%)	33 ± 1	31 ± 1	22 ± 1	23 ± 1
Systolic blood pressure (mm Hg)	133 ± 4	141 ± 7	140 ± 4	146 ± 4
Diastolic blood pressure (mm Hg)	73 ± 3	76 ± 3	74 ± 3	77 ± 3

No differences between the protein and placebo group. All values represent means ± SEM.
BMI, body mass index; HbA1c: blood glycosylated hemoglobin.

in the PLA and the PRO group, respectively ($P < 0.001$). A similar increase was observed for the men, from 62.4 ± 1.5 to 63.4 ± 1.6 and 61.5 ± 1.3 to 62.9 ± 1.5 kg, respectively ($P < 0.001$). Leg lean mass had increased by 3% ± 1% in both the women and men ($P < 0.001$, Fig. 1A, B). Total fat mass decreased significantly in all groups ($P < 0.001$), resulting in a significant decline in body fat percentage (women: from 33.3% ± 1.1% to 31.9% ± 1.1% and 30.9% ± 1.2% to 29.2% ± 1.2%; men: from 22.3% ± 1.2% to 21.1% ± 1.0% and 23.1% ± 1.2% to 21.6% ± 1.1% in the PLA and PRO group, respectively; ($P < 0.001$)). In accordance, leg fat mass decreased during the intervention ($P < 0.01$). No significant differences were observed for the intervention effects between treatments and/or genders for any of the DXA variables. No changes were observed in bone mineral content (*data not shown*).

Skeletal muscle hypertrophy. At baseline, no significant differences in *quadriceps* CSA were observed between the PLA and PRO group. CSA had increased by 8% ± 1% and 7% ± 1% after 12 wk of intervention in the women and men, respectively ($P < 0.001$, Fig. 1 C, D), with no differences between groups. In the subsequent 12 wk of intervention, no significant further increase was observed in the women (Fig. 1C). In the men, a significant 2% ± 1% increase in CSA was observed between 12 and 24 wk of intervention, with no differences between groups ($P < 0.001$).

Muscle fiber–type composition in the PRO (women and men 42% ± 5% and 46% ± 5% Type II fibers, respectively) and PLA group (women and men 42% ± 4% and 45% ± 4% Type II fibers, respectively) did not differ between groups. Before intervention, Type II muscle fiber size was significantly smaller than Type I muscle fibers size (Fig. 2A, B). For muscle fiber CSA, a significant "time × fiber type" interaction ($P < 0.05$) and a significant "gender × fiber type" interaction ($P < 0.001$) were observed. Separate analyses showed that Type I muscle fiber CSA did not significantly change in any of the groups. In contrast, Type II muscle fiber size significantly increased in all groups ($P < 0.001$, Fig. 2A, B), with no differences between the PRO- and PLA-supplemented groups. *Post hoc* analyses showed the majority of the increase to occur between week 0 and 12. As a consequence of the Type II muscle fiber hypertrophy, the total area percentage occupied by Type II muscle fibers had increased from 38% ± 2% at baseline to 43% ± 2% after 24 wk of training, with no differences between groups.

Muscle strength. At baseline, no significant differences in muscle strength (1 RM) were observed between the PLA and PRO group (Fig. 3A). After 12 wk of intervention, leg extension strength had increased by 22% ± 2% in the women

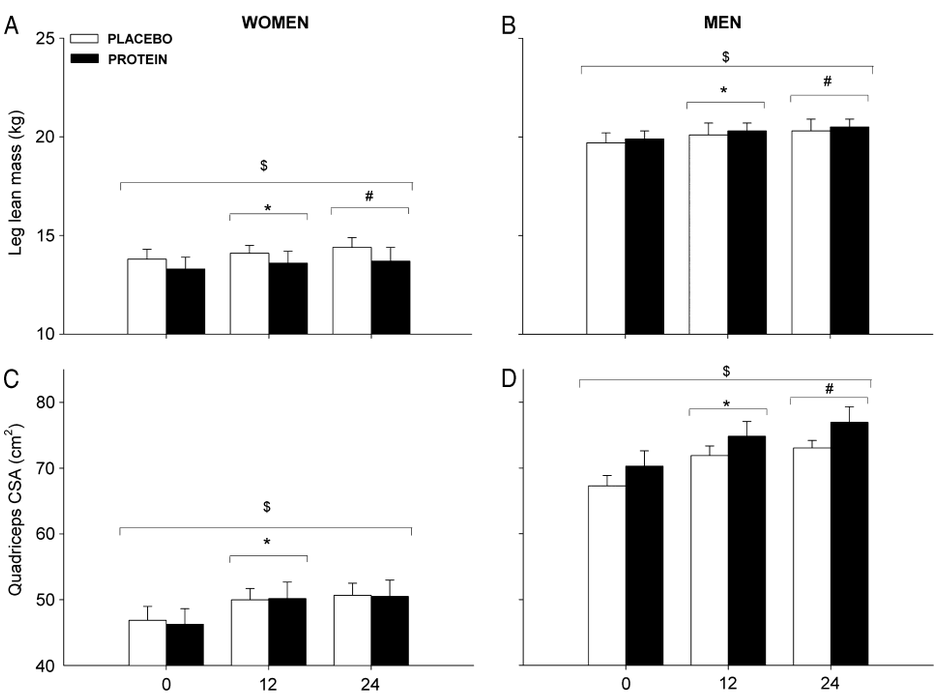

FIGURE 1—Mean ± SEM leg lean mass before and after 12 and 24 wk of resistance-type exercise training in elderly women (A) and men (B) with or without protein supplementation. Data were analyzed using repeated-measures ANOVA with time as within-subjects factor and gender and group as between-subjects factor. No time × gender × group ($P = 0.69$), time × gender ($P = 0.50$), and time × group ($P = 0.61$) interactions were observed. *Significantly different from baseline, $P < 0.001$. #Significantly different from baseline and status after 12 wk of intervention, $P < 0.005$. $Significant increase over time $P < 0.001$. Mean ± SEM quadriceps CSA before and after 12 and after 24 wk of resistance-type exercise training in elderly women (C) and men (D) with or without protein supplementation. Data were analyzed using repeated-measures ANOVA with time as within-subjects factor and gender and group as between-subjects factor. No time × gender × group ($P = 0.69$), and time × group ($P = 0.60$) interactions were observed. A time × gender interaction was observed ($P < 0.05$).

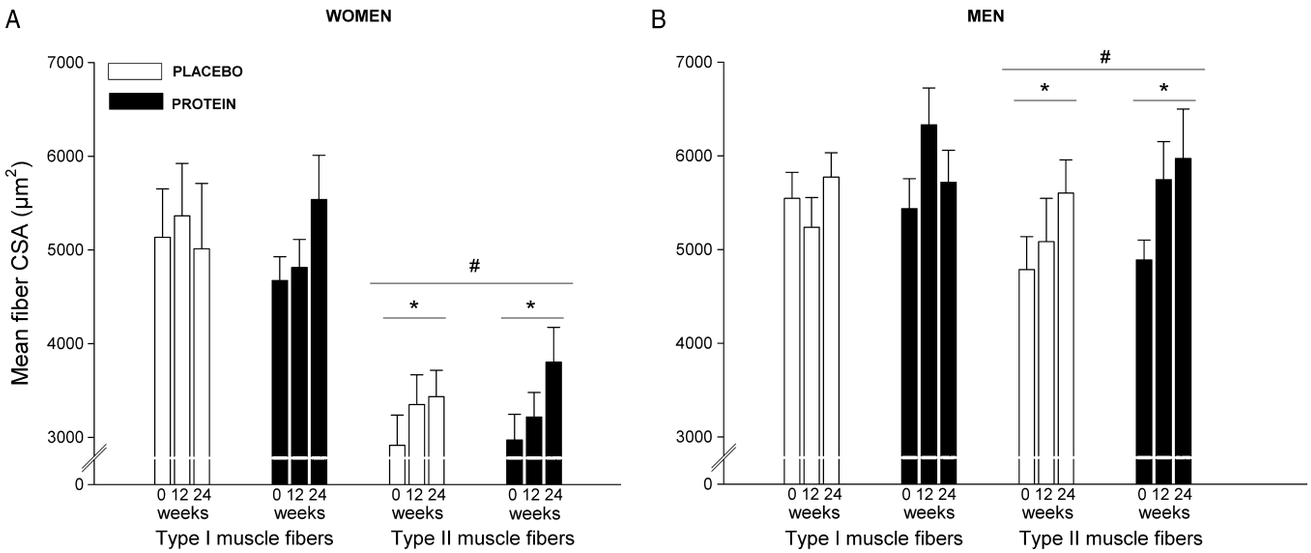

FIGURE 2—Mean ± SEM muscle fiber CSA for Type II muscle fibers before and after 12 and 24 wk of resistance-type exercise training in elderly women (A) and men (B) with or without protein supplementation. Data were analyzed using repeated-measures ANOVA with time as within-subjects factor and gender and group as between-subjects factor. No time × gender × group ($P = 0.57$), time × group ($P = 0.25$), and time × gender ($P = 0.26$) interactions were observed. *Significant increase over time, $P < 0.01$. #Significantly different from Type I fibers before intervention, $P < 0.001$.

and 23% ± 2% in the men ($P < 0.001$), with no differences between groups. Between 12 and 24 wk of intervention, leg extension strength increased by another 17% ± 1% in the women and 16% ± 1% in the men. No significant differences were observed between groups. For the leg press, similar gains in strength were observed; 1 RM leg press strength had increased by 31% ± 3% and 26% ± 2% after 24 wk of intervention in the women and men, respectively ($P < 0.001$), with no differences between groups.

Physical performance. At baseline, no significant differences were observed in sit-to-stand time between the PLA and PRO groups (Fig. 3B). After 12 wk of intervention, sit-to-stand time had decreased by 8% ± 2% in the women and 9% ± 2% in the men ($P < 0.001$). Between 12 and 24 wk of intervention, sit-to-stand time decreased with another 10% ± 2% and 11% ± 2%, respectively ($P < 0.001$). No significant differences were observed between the PLA and PRO group. Before the exercise intervention, no differences were observed in handgrip strength between the PLA and PRO group (27 ± 1 and 25 ± 1 kg in the women and 41 ± 2 and 45 ± 2 kg in the men, respectively). No significant changes were observed in handgrip strength over time in either the PLA or PRO group.

Dietary intake and physical activity. Analysis of the 3-d dietary intake records collected before the exercise training regimen showed no significant differences between the PLA and the PRO group at baseline (Table 2). No changes in total energy intake or macronutrient composition of the diet were observed over time. Daily protein intake averaged 1.2 ± 0.1 $g \cdot kg^{-1} \cdot d^{-1}$ in the women and 1.1 ± 0.0 $g \cdot kg^{-1} \cdot d^{-1}$ in the men. Daily protein intake increased significantly by 0.24 $g \cdot kg^{-1} \cdot d^{-1}$ in the women and by 0.18 $g \cdot kg^{-1} \cdot d^{-1}$ in the men after daily protein supplementation in the PRO group. Habitual physical activity levels and mean energy expenditure at baseline did not differ between groups (Table 2). Habitual physical activity levels did not change over time.

Glycemia and lipidemia. Measures of glycemic control did not differ between the PLA and PRO group before intervention (Table 3). After 24 wk of intervention, HbA1c had decreased in both the PLA and PRO group. In accordance, insulin sensitivity parameters (oral glucose insulin sensitivity (OGIS) and insulin sensitivity index (ISI)) increased over time (Table 3). Fasting plasma glucose and insulin concentrations and homeostatic model assessment (HOMA) index remained stable throughout the intervention period. No significant differences were observed between groups. At baseline, no group differences were observed in plasma lipid concentrations. Triglycerides and HDL were within the normal range (1.2 ± 0.1 and 1.7 ± 0.1 $mmol \cdot L^{-1}$ (normal value: triglycerides <1.7 and HDL >1.5 $mmol \cdot L^{-1}$)) before intervention and did not change over time. No differences were observed between groups. Total cholesterol and LDL were above the normal range (6.3 ± 0.2 and 4.1 ± 0.2 $mmol \cdot L^{-1}$ (normal value: total cholesterol <5.2 and LDL <2.6 $mmol \cdot L^{-1}$)) before intervention. In both the PLA and the PRO group, total cholesterol had decreased significantly after 12 wk of intervention (from 6.84 ± 0.19 to 6.58 ± 0.20 $mmol \cdot L^{-1}$ in women and from 5.83 ± 0.21 to 5.70 ± 0.20 $mmol \cdot L^{-1}$ in men; $P < 0.001$). No further decrease was observed between 12 and 24 wk. In both the PLA and the PRO group, LDL had decreased from 4.46 ± 0.18 to 4.14 ± 0.18 $mmol \cdot L^{-1}$ in the women and from 3.76 ± 0.18 to 3.60 ± 0.17 $mmol \cdot L^{-1}$ in the men after 12 wk of intervention ($P < 0.001$). Between 12 and 24 wk, LDL further decreased

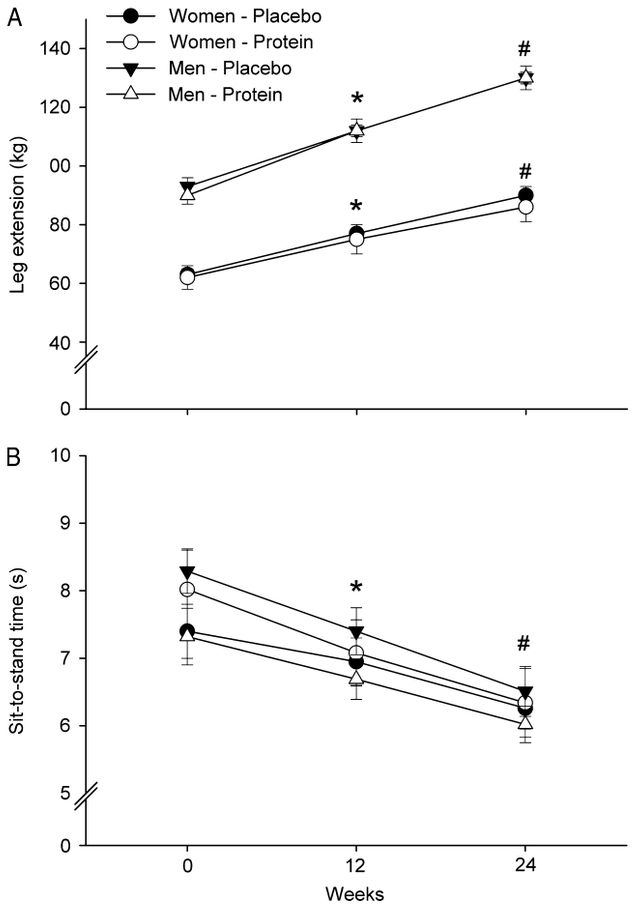

FIGURE 3—A, Mean ± SEM leg extension 1 RM after 12 and 24 wk of resistance-type exercise training in elderly women and men with or without protein supplementation. Data were analyzed by using repeated-measures ANOVA with time, as within-subjects factor and gender and group as between-subjects factor. No time × gender × group ($P = 0.37$) and time × group ($P = 0.86$) interactions were observed. A time × gender interaction was observed ($P < 0.001$). *Significantly different from baseline, $P < 0.001$. #Significantly different from baseline and status after 12 wk of intervention, $P < 0.001$. B, Mean ± SEM sit-to-stand time after 12 and 24 wk of resistance-type exercise training in elderly women and men with or without protein supplementation. Data were analyzed by using repeated-measures ANOVA with time, as within-subjects factor and gender and group as between-subjects factor. No time × gender × group ($P = 0.14$), time × gender ($P = 0.71$), and time × group ($P = 0.73$) interactions were observed. *Significantly different from baseline, $P < 0.001$. #Significant improvement different from baseline and status after 12 wk of intervention, $P < 0.001$.

from 4.14 ± 0.18 to 3.98 ± 0.18 mmol·L^{-1} in the women and from 3.60 ± 0.17 to 3.53 ± 0.17 mmol·L^{-1} in the men ($P < 0.05$).

Creatinine and urinary nitrogen. Serum creatinine concentrations were within the normal range (>60 μmol·L^{-1}) before the intervention. At baseline, no significant differences were observed between the PLA and PRO groups (women: 76.0 ± 4.2 and 77.9 ± 3.3 μmol·L^{-1}; men: 88.9 ± 4.5 and 93.9 ± 3.7 μmol·L^{-1}, respectively). No changes were observed over time in either group. Creatinine clearance was within the normal range (>60 mL·min^{-1} per 1.73 m^2) before intervention. At baseline, no significant differences were observed between the PLA and PRO group (women: 83.2 ± 3.7 and 81.3 ± 4.9 mL·min^{-1} per 1.73 m^2; men: 85.5 ± 6.8 and 89.4 ± 6.8 mL·min^{-1} per 1.73 m^2, respectively). No changes were observed over time in either group. Before the intervention, 24-h nitrogen balance was 1.30 ± 0.44 g·d^{-1} in the women and −0.14 ± 0.53 g·d^{-1} in the men. Nitrogen balance did not change over time in either group.

DISCUSSION

The present study shows that prolonged resistance-type exercise training increases muscle mass and strength, augments functional capacity, improves glycemia and lipidemia, and reduces blood pressure in healthy elderly men and women. Additional protein supplementation at breakfast (15 g·d^{-1}) did not further augment the increase in muscle mass, strength, and/or functional capacity in these healthy elderly subjects.

Resistance-type exercise training has been well established as an effective treatment strategy to counteract the loss of muscle mass and strength in the elderly (8,14,22). In the present study, we observed substantial gains in whole-body lean mass of 1.2 ± 0.2 and 1.3 ± 0.4 kg in the women and 1.0 ± 0.3 and 1.4 ± 0.4 kg in the men in the PLA- and PRO-supplemented groups, respectively. These findings tend to be in line with previous observations in shorter intervention studies (22,37). The increase in whole-body lean mass was largely attributed to an increase in leg lean mass. The latter was also evident from the quadriceps CSA that had increased by approximately 10% after 6 months of training in both the women and men. The greater part of the increase in muscle mass occurred during the first 12 wk of the intervention. The latter is in line with findings by Frontera et al. (14), who observed a 9% increase in quadriceps CSA after 12 wk of resistance-type exercise training in elderly men.

Apart from the changes in whole-body and leg lean mass and quadriceps CSA with aging and exercise, specific alterations likely also occur at the myocellular level. The age-related loss of muscle mass is largely attributed to the specific Type II muscle fiber atrophy (38). In accordance, we observed a significantly smaller Type II muscle fiber size when compared with Type I muscle fibers in these older women and men at baseline (Fig. 2A, B). Resistance-type exercise training strongly increased Type II muscle fiber size with 28% ± 7% in the women and 23% ± 7% in the men. These findings are in line with earlier reports from Kosek et al. (22), who observed an approximately 23% increase in Type II muscle fiber size after 16 wk of resistance-type exercise training (three times a week). Also in accordance with the observations of Kosek et al. (22), Type I muscle fiber size did not change in response to training. In agreement with muscle mass data, Type II muscle fiber size had increased most during the first 12 wk of intervention.

The increase in leg lean mass, quadriceps CSA, and muscle fiber size was accompanied by a substantial increase in leg muscle strength. Interestingly, both the increase in leg lean mass ($r = 0.307$, $P < 0.05$) and quadriceps CSA ($r = 0.44$,

TABLE 2. Food intake and habitual physical activity levels.

	Women (n = 24)		Men (n = 29)	
Week	**0**	**24**	**0**	**24**
Energy intake ($MJ \cdot d^{-1}$)	8.2 ± 0.2	8.7 ± 0.4	10.0 ± 0.4	10.1 ± 0.4
Carbohydrate (%)	44 ± 1	44 ± 1	45 ± 1	45 ± 1
Fat (%)	36 ± 1	36 ± 1	34 ± 1	35 ± 1
Protein (%)	16 ± 1	15 ± 1	15 ± 1	14 ± 1
Alcohol (%)	4 ± 1	5 ± 1	6 ± 1	6 ± 1
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Mean energy expenditure ($MET \cdot h \cdot d^{-1}$)	1.44 ± 0.15	1.49 ± 0.11	1.48 ± 0.19	1.50 ± 0.18

All values represent means ± SEM. No significant differences were observed between the protein and the placebo group before intervention. No time × group interaction or main group effects were observed.

$P < 0.001$) correlated significantly with the concomitant increase in 1 RM strength. Furthermore, Type II muscle fiber size was strongly correlated with leg lean mass ($r = 0.70$, $P < 0.001$) and quadriceps CSA ($r = 0.79$, $P < 0.001$) and also with leg press and leg extension 1 RM ($r = 0.79$ and $r = 0.74$, respectively; $P < 0.001$). Whereas leg press strength had increased by 25%–30% in both the men and the women, leg extension strength had increased by as much as 40% after 6 months of training. Although similar improvements in muscle strength have been reported previously after 8–16 wk of resistance-type knee extension exercise training (22,25), this is the first study to assess the effect of 6 months of whole-body resistance-type exercise training in elderly men and women. The observed increase in leg muscle strength was shown to be of important clinical relevance as it translated to substantial improvements in functional capacity (Fig. 3). The sit-to-stand test showed an 18% ± 1% improvement in both the women and the men ($P < 0.001$). This is in accordance with Capodaglio et al. (6) who observed an approximately 23% improvement in chair rise time after 1 yr of strength training in the elderly. The latter clearly shows the clinical relevance of increasing leg muscle strength in the elderly, which generally translates to improvements in functional capacity. Despite the whole-body resistance-type exercise training, we failed to detect any changes in handgrip strength over time. In fact, handgrip strength was shown to be highly reproducible (with a coefficient of variation of 0.06%) between measurements taken at 0, 3, and 6 months and had clearly not been affected by the resistance-type exercise training. Although handgrip strength has been shown to represent a useful tool to assess physical performance capacity in both healthy and compromised populations in cross-sectional studies (9,36), handgrip strength should not be used as a parameter to assess changes in muscle strength and/or function over time in the individual patient when the training program does not include a specific handgrip exercise.

Our data show that prolonged resistance-type exercise training represents an effective strategy to augment muscle mass and strength and improve functional performance in the elderly. It has been suggested that dietary protein supplementation can further augment the skeletal muscle adaptive response to resistance-type exercise training. However, long-term nutritional intervention studies have generally failed to observe additional benefits of increasing protein intake during exercise intervention in elderly populations (13,15,37). It should be noted, however, that these combined exercise and nutritional interventions in the elderly generally lasted between 6 and 12 wk (13,15,37). Considering the blunted muscle protein synthetic response to protein intake in the elderly, it could be speculated that more prolonged exercise intervention periods are required for any benefits of dietary protein supplementation to become apparent. Another aspect that may well influence the benefits of additional protein intake is the timing of the supplementation (11). We have previously shown that the ingestion of a protein supplement immediately before and after each exercise session (i.e., three times per week for 12 wk) does not further augment muscle hypertrophy after resistance-type exercise training in elderly men (37). Therefore, in the present study, we applied a different dietary protein supplementation strategy. Because it has been proposed that a sufficient amount of protein (i.e., 25–30 g) should be ingested with each main meal to allow proper muscle maintenance, we assessed normal dietary habits in various elderly subpopulations in the Netherlands (34). These data show that dietary protein intake in community-dwelling elderly is particularly low at breakfast (approximately 10 g) and, as such, insufficient to allow normal postprandial stimulation of myofibrillar muscle protein

TABLE 3. Glycemic control.

	Women (n = 24)		Men (n = 29)	
Week	**0**	**24**	**0**	**24**
Plasma glucose ($mmol \cdot L^{-1}$)	5.4 ± 0.1	5.2 ± 0.1	5.6 ± 0.1	5.7 ± 0.1
Plasma insulin ($mU \cdot L^{-1}$)	13.7 ± 1.2	13.2 ± 1.3	14.5 ± 0.9	14.5 ± 0.9
HbA1c (%)	5.8 ± 0.0	5.7 ± 0.0[a]	5.5 ± 0.1	5.4 ± 0.1[a]
ISI	3.8 ± 0.4	4.2 ± 0.3[a]	3.1 ± 0.3	3.4 ± 0.4[a]
OGIS	385 ± 15	418 ± 14[a]	345 ± 15	361 ± 15[a]

All values represent means ± SEM. No significant differences were observed between the protein and the placebo group before the intervention. No time × group interaction or main group effects were observed.
[a] Significantly different from wk 0.
HbA1c, blood glycosylated hemoglobin; ISI, insulin sensitivity index; OGIS, oral glucose insulin sensitivity.

synthesis (29). We speculated that provision of additional protein with breakfast during a prolonged period of resistance-type exercise training would represent a more effective strategy to support net muscle protein accretion. Therefore, a drink containing 15 g of milk protein was supplemented at breakfast to ensure the ingestion of an optimal amount of protein (i.e., approximately 25 g) with all main meals.

Despite substantial increases in skeletal muscle mass in both women and men, we observed no differences between the PRO- and PLA-supplemented groups. Importantly, muscle mass, muscle strength, and muscle function continued to increase between 12 and 24 wk of training. As such, prolonging exercise training in the elderly clearly has the capacity to induce further clinical benefits. However, daily protein supplementation with breakfast did not further augment the increase in muscle mass and strength during prolonged resistance-type exercise training in this large group of elderly men and women. To provide an in-depth insight in the hypertrophic response to training, muscle mass was measured on a whole-body (DXA), limb (CT), and myocellular level (biopsy). Furthermore, muscle strength and function were extensively assessed with both leg extension and leg press 1-RM testing and with handgrip strength and sit-to-stand time. On each of these outcome parameters, we did not observe any additional benefits of protein supplementation during 6 months of resistance-type exercise training in elderly women and men. These results extend on several relative short-term intervention studies that failed to observe additional benefits of protein supplementation during resistance-type exercise training in the elderly (13,15,37). Taken together, these data clearly indicate that there is no rationale for protein supplementation even during prolonged resistance-type exercise training in healthy elderly men and women.

The apparent absence of any additional benefits of protein supplementation implies that optimal dietary protein requirements to allow skeletal muscle hypertrophy to occur were already met within the habitual diet of the participants. In the present study, dietary intake remained stable throughout the intervention period. Even without additional protein supplementation, habitual protein intake averaged 1.2 ± 0.1 $g \cdot kg^{-1} \cdot d^{-1}$ in the women and 1.1 ± 0.0 $g \cdot kg^{-1} \cdot d^{-1}$ in the men in both groups. These values are well above the current Recommended Daily Allowance of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ (4). Hence, we conclude that protein supplementation does not modify improvements in muscle mass, strength, and function during prolonged resistance-type exercise training when ample protein is already ingested in the normal diet (>1.0 $g \cdot kg^{-1} \cdot d^{-1}$). These findings are in line with observations from Campbell and Leidy (5), who concluded that improvements in muscle mass and strength induced by resistance-type 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. Apparently, a low-protein intake at breakfast does not preclude muscle hypertrophy to occur in healthy elderly men and women. However, it remains to be addressed whether dietary protein supplementation is of greater clinical relevance in more compromised (frail) elderly populations in which daily protein intake is likely insufficient (34).

Besides muscle mass, strength, and functional capacity, we observed many other benefits of prolonged resistance-type exercise training that are normally attributed to the effect of prolonged endurance-type exercise. In the present study, we observed substantial improvements in glycemic control in our healthy elderly subjects. The latter was evident by both a decrease in HbA1c (0.1%) and an increase in insulin sensitivity, i.e., a 7% ± 2% increase in OGIS and a 6% ± 4% increase in ISI, with no differences between the placebo- and protein-supplemented groups. These results extend on previous findings from several studies in middle-age subjects (28,31,32), showing that resistance-type exercise training improves whole-body insulin sensitivity and improves glycemic control in elderly men and women. In addition to the improvement in glucose tolerance, prolonged resistance-type exercise also improves blood lipid profile. We observed a decrease in total cholesterol and LDL in the men and even to a larger extent in the women. Furthermore, systolic and diastolic blood pressure was significantly reduced over the course of the intervention. These findings are in line with previous observations (26) and underline the clinical relevance of prolonged resistance-type exercise training to improve metabolic health in the elderly. Because the resistance-type exercise program was highly appreciated by the subjects and accompanied by excellent adherence and compliance, we suggest that exercise intervention programs for the elderly should focus on the implementation of resistance-type exercise.

One of the clear advantages of the present study is the prolonged timeline and the ability to compare the adaptive response during the first 3 months with the subsequent 3 months of intervention in both older men and women. The greater increase in muscle mass and muscle strength was observed during the initial 12 wk of training, representing 85% and 53% of the change in muscle mass and strength over the entire 6-month period. The latter clearly shows that the first few months of resistance-type exercise training should be highly supervised to allow proper and safe training responses, with concomitant increases in muscle mass and strength. The subsequent period should focus on muscle mass maintenance and likely requires less effort than implemented in the present study. Clearly, future studies should establish how resistance-type exercise training can be prescribed to elderly people and how we can set up an exercise regimen that will allow maintenance of the initial increase in muscle mass and strength that is obtained during the initial approximately 3 months of supervised resistance-type exercise training.

We conclude that prolonged resistance-type exercise training increases skeletal muscle mass and strength, augments functional capacity, improves glycemia and lipidemia, and reduces blood pressure in healthy elderly men and women. Additional

protein supplementation (15 $g \cdot d^{-1}$) does not further augment the gains in skeletal muscle mass, strength, and functional capacity in healthy elderly men and women.

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