

# Protein Ingestion before Sleep Increases Muscle Mass and Strength Gains during Prolonged Resistance-Type Exercise Training in Healthy Young Men<sup>1–3</sup>

Tim Snijders,<sup>4,7</sup> Peter T Res,<sup>4</sup> Joey SJ Smeets,<sup>4</sup> Stephan van Vliet,<sup>4,8</sup> Janneau van Kranenburg,<sup>4</sup> Kamiel Maase,<sup>5</sup> Arie K Kies,<sup>6</sup> Lex B Verdijk,<sup>4</sup> and Luc JC van Loon<sup>4\*</sup>

<sup>4</sup>Department of Human Movement Sciences, Faculty of Health, Medicine, and Life Sciences, School for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, The Netherlands; <sup>5</sup>Unit Elite Sports, Dutch Olympic Committee\* Dutch Sports Federation, Arnhem, The Netherlands; and <sup>6</sup>DSM Biotechnology Center, Department of Applied Biochemistry, Delft, The Netherlands

## Abstract

**Background:** It has been demonstrated that protein ingestion before sleep increases muscle protein synthesis rates during overnight recovery from an exercise bout. However, it remains to be established whether dietary protein ingestion before sleep can effectively augment the muscle adaptive response to resistance-type exercise training.

**Objective:** Here we assessed the impact of dietary protein supplementation before sleep on muscle mass and strength gains during resistance-type exercise training.

**Methods:** Forty-four young men ( $22 \pm 1$  y) were randomly assigned to a progressive, 12-wk resistance exercise training program. One group consumed a protein supplement containing 27.5 g of protein, 15 g of carbohydrate, and 0.1 g of fat every night before sleep. The other group received a noncaloric placebo. Muscle hypertrophy was assessed on a whole-body (dual-energy X-ray absorptiometry), limb (computed tomography scan), and muscle fiber (muscle biopsy specimen) level before and after exercise training. Strength was assessed regularly by 1-repetition maximum strength testing.

**Results:** Muscle strength increased after resistance exercise training to a significantly greater extent in the protein-supplemented (PRO) group than in the placebo-supplemented (PLA) group ( $+164 \pm 11$  kg and  $+130 \pm 9$  kg, respectively;  $P < 0.001$ ). In addition, quadriceps muscle cross-sectional area increased in both groups over time ( $P < 0.001$ ), with a greater increase in the PRO group than in the PLA group ( $+8.4 \pm 1.1$  cm<sup>2</sup> vs.  $+4.8 \pm 0.8$  cm<sup>2</sup>, respectively;  $P < 0.05$ ). Both type I and type II muscle fiber size increased after exercise training ( $P < 0.001$ ), with a greater increase in type II muscle fiber size in the PRO group ( $+2319 \pm 368$  μm<sup>2</sup>) than in the PLA group ( $+1017 \pm 353$  μm<sup>2</sup>;  $P < 0.05$ ).

**Conclusion:** Protein ingestion before sleep represents an effective dietary strategy to augment muscle mass and strength gains during resistance exercise training in young men. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT02222415. *J Nutr* 2015;145:1178–84.

**Keywords:** exercise training, protein, muscle mass, strength, fiber size

## Introduction

Resistance-type exercise training represents an effective interventional strategy to augment skeletal muscle protein accretion

(1, 2). A single bout of resistance-type exercise stimulates both muscle protein synthesis and breakdown rates, albeit the latter to a lesser extent (2–4). Although exercise improves net muscle protein balance, net muscle balance remains negative in the

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\* To whom correspondence should be addressed. E-mail: [L.vanLoon@maastrichtuniversity.nl](mailto:L.vanLoon@maastrichtuniversity.nl).

<sup>3</sup> Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

<sup>7</sup> Present address: Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada.

<sup>8</sup> Present address: Department of Kinesiology and Community Health, Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, 906 S. Goodwin Avenue, Champaign, IL 61801.

absence of nutrient intake (2, 3). Protein ingestion after exercise stimulates muscle protein synthesis and inhibits muscle protein breakdown, resulting in net muscle protein accretion during the acute stages of postexercise recovery (5–7). Therefore, it has been suggested that protein supplementation can further augment the muscle adaptive response to prolonged resistance-type exercise training. However, studies investigating the impact of protein supplementation on muscle mass and strength gains during more prolonged resistance-type exercise training tend to report discrepant findings. Although some studies report greater gains in muscle mass, muscle fiber size, and/or muscle strength after dietary protein supplementation during prolonged resistance-type exercise training (8–16), others have failed to confirm such findings (17–24). In a recent meta-analysis, Cermak et al. (25) showed that these discrepant findings may be largely explained by differences in study design and/or the number of participants included in the study. Other important factors that may contribute to the observed discrepancy on the proposed benefits of protein supplementation are the source and quantity of protein that is provided as well as the timing of protein supplementation (25).

In recent studies, we have demonstrated that protein provided before sleep is properly digested and absorbed resulting in muscle protein accretion throughout overnight sleep (26, 27). When athletes were provided with a bolus of dietary protein immediately before sleep, muscle protein synthesis rates were ~22% higher during postexercise overnight sleep when compared to the ingestion of a placebo (27). Consequently, we concluded that protein feeding before sleep may represent an effective interventional strategy to further augment the skeletal muscle adaptive response to exercise training and, as such, to improve exercise training efficiency.

We hypothesized that dietary protein supplementation provided before sleep will further augment the gains in muscle mass, strength, and muscle fiber size during more prolonged resistance-type exercise training in healthy young men. Therefore, we subjected 44 healthy young men to a 12-wk resistance-type exercise training program (3 exercise sessions per week) during which they were provided with a protein supplement (27.5 g/d of protein) or a noncaloric placebo. Before and after the intervention period, we determined muscle mass on a whole-body, limb, and muscle fiber level and assessed muscle strength.

## Methods

**Subjects.** A total of 44 healthy young men ( $22 \pm 1$  y) volunteered to participate in a 12-wk resistance-type exercise training intervention program, with or without additional protein supplementation. Three subjects dropped out during the study, 1 because of a road accident, 1 because of pneumonia, and 1 because of time availability. Medical history was evaluated and a blood sample was taken to assess blood glycated hemoglobin content and fasting plasma glucose concentrations. Participants were excluded when glycated hemoglobin content exceeded 6.5% or fasting plasma glucose was  $>7$  mmol/L. Additional exclusion criteria that would preclude successful participation in the intervention program included (diagnosed) lactose intolerance and/or dairy protein allergy, chronic obstructive pulmonary disease, and/or orthopedic limitations. All subjects were recreationally active, performing sports on a noncompetitive basis between 2 and 5 h/wk. None of the participants had a history of participating in a structured resistance-type exercise training program to improve performance over the past 2 y. All subjects were informed of the nature and possible risks of the experimental procedures before their written informed consents were obtained. This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center and complied

with the guidelines set by the Declaration of Helsinki of 1975 as revised in 1983. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT02222415.

**Study design.** After inclusion, participants were randomly allocated to either a protein-supplemented (PRO)<sup>9</sup> or placebo-supplemented (PLA) group. Before, during, and after the exercise training program, anthropometric measurements (height, body mass, and leg volume), strength assessment [1-repetition maximum (1RM)], and computed tomography and DXA scans were performed and muscle biopsy specimens and dietary intake records were collected.

**Exercise intervention program.** Supervised resistance-type exercise training was performed 3 times/wk for a 12-wk period. After a 5-min warm-up on a cycle ergometer, the training session consisted of 4 sets on both the leg press and the leg extension machines (Technogym); these 2 exercises were performed every training session. In addition, 2 sets on the chest press and horizontal row were alternated with vertical pull-down and shoulder press between every training session. Each exercise session ended with a 5-min cooling down period on the cycle ergometer. During the first week of the training period, the workload was gradually increased from 70% (10–15 repetitions) of 1RM to 80% of 1RM (8–10 repetitions). Thereafter, training was always performed at 80% 1RM. Resting periods were allowed between sets and different exercises for 1.5 and 3 min, respectively. Workload intensity was adjusted based on the outcome of the successive 1RM tests (performed at weeks 4 and 8). In addition, workload was increased when  $>8$  repetitions could be performed in 3 of 4 sets. All training sessions were performed in the evening between 2000 and 2100 or 2100 and 2200. On average, subjects attended  $91\% \pm 1\%$  and  $90\% \pm 1\%$  of the scheduled exercise sessions in the PLA and PRO groups, respectively, with no differences between groups.

**Dietary protein supplementation.** Throughout the 12-wk intervention period, subjects consumed a 300-mL bottle containing either a placebo drink (PLA group) or protein drink (PRO group) daily immediately before sleep. The protein beverage contained 13.75 g of casein hydrolysate (Peptopro), 13.75 g of casein, 15 g of carbohydrate, and 0.1 g of fat (DSM), providing 746 kJ of energy. The control drink was a noncaloric placebo beverage. Beverages were masked for taste and smell by adding citric and vanilla additives. In addition, beverages were masked for color by adding titanium dioxide (food-grade E171) to the placebo drink. Placebo and protein drinks were provided in a randomized, double-blind manner. On average, subjects consumed  $98\% \pm 1\%$  of the beverages, with no differences between groups.

**Dietary intake, physical activity standardization, and sleep records.** All participants received a snack immediately after every training session, including a cheese sandwich, an apple, and a noncaloric beverage (total energy intake, 1151 kJ; 37 g of carbohydrates, 10 g of protein, and 9 g of fat). Furthermore, all participants consumed a standardized meal the evening before each test day. Participants were instructed to refrain from vigorous physical activity for at least 5 d before testing. At the different test days, participants arrived at the laboratory by car or public transportation after an overnight fast. Throughout the intervention program, participants were encouraged to maintain their habitual dietary intake and physical activity pattern. Participants recorded 3-d (Thursday–Saturday) weighted dietary intake records to assess potential changes in daily food intake that might have occurred during the intervention period before the onset of the intervention program and in week 11 of the exercise intervention. Dietary intake records were analyzed with Etmeter Software 2005 (version 1.4.0; Voedingscentrum). All participants recorded the time they went to sleep at night and woke up in the morning, on both training and nontraining days, throughout the entire intervention period.

<sup>9</sup> Abbreviations used: CSA, cross-sectional area; PLA, placebo supplemented; PRO, protein supplemented; 1RM, 1-repetition maximum.

**TABLE 1** Participant characteristics of healthy young men who performed 12 wk of resistance-type exercise training who did or did not receive protein supplementation<sup>1</sup>

	PLA group (n = 19)	PRO group (n = 20)
Age, y	21 ± 1	23 ± 1
Height, m	1.85 ± 0.02	1.82 ± 0.02
Body mass, kg	80.0 ± 2.5	76.9 ± 2.1
BMI, kg · m <sup>-2</sup>	23.4 ± 0.8	23.2 ± 0.6
Leg volume, L	9.3 ± 0.3	9.0 ± 0.3

<sup>1</sup> Values are means ± SEMs. No significant differences were observed between groups. PLA, placebo supplemented; PRO, protein supplemented.

**Body composition.** Body composition was measured using DXA (Discovery A, QDR Series; Hologic). Whole-body and regional lean mass and fat mass were determined by using the system's software package Apex version 2.3 (Wind River). Anthropometrics were assessed using standardized procedures, body weight by digital scale to within 100 g, and height by stadiometer to within 0.5 cm. Anatomic cross-sectional area (CSA) of the quadriceps muscle was assessed by computed tomography scanning (Philips Brilliance 64; Philips Medical Systems) before and after 12 wk of intervention, as described previously (28).

**Muscle biopsy sampling.** Seven days before the onset of the intervention and after 12 wk of intervention (5 d after final strength testing), percutaneous needle muscle biopsy specimens (29) were taken from the right leg of each participant in the morning after an overnight fast, as described previously (30, 31).

**Strength assessment.** Maximum strength was assessed by 1RM strength tests on leg press, leg extension, chest press, shoulder press, vertical pull-down, and horizontal row machines (Technogym). During a familiarization trial, proper lifting technique was demonstrated and practiced and maximum strength was estimated using the multiple-repetitions testing procedure. In an additional session, at least 1 wk before muscle biopsy specimen collection, each subject's 1RM was determined as described previously (32). 1RM tests for leg press and leg extension machines were repeated after 4 and 8 wk of intervention to adjust training weights. In addition, all 1RM tests were repeated 4 d after the last training session of the intervention program.

**Immunohistochemistry.** From all biopsy specimens 5- $\mu$ m-thick cryosections were cut at -20°C. Samples collected before and after 12 wk of intervention from each subject were mounted together on uncoated glass slides. Muscle biopsy specimens were stained for muscle fiber typing as described in detail previously (30, 31). No differences in fiber circularity were observed in response to training or between groups. Mean numbers of 149 ± 11 and 182 ± 14 muscle fibers were analyzed in the biopsy samples collected before and after 12 wk of intervention, respectively.

**Statistics.** All data are expressed as means ± SEMs. Baseline characteristics between groups were compared by means of an independent-samples *t* test. Because all data were normally distributed, training-induced changes

were analyzed with a split-plot model with treatment (PRO group vs. PLA group) and training (before training vs. after training) as fixed factors and subject as a within-treatment random factor. Muscle fiber type-specific variables were analyzed by adding another fixed factor (type I fibers vs. type II fibers). In case of a significant interaction, paired-samples *t* tests were performed to determine training effects within treatment groups or within types I or II fibers. Independent-samples *t* tests were used to determine group differences in either the pre- or the postintervention values. Bonferroni corrections were applied where appropriate. All analyses were performed by using SAS 9.3 (SAS Institute, Inc.). An  $\alpha$ -level of 0.05 was used to determine statistical significance.

## Results

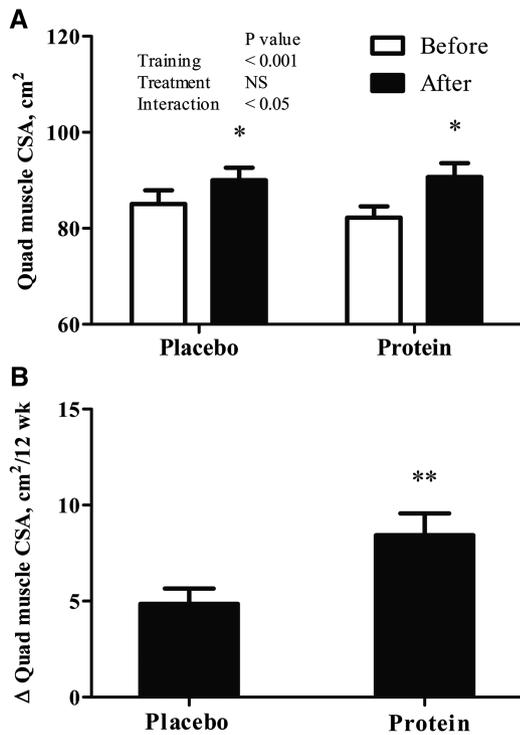
**Participants.** Participant characteristics are provided in Table 1. In total, 41 participants completed the intervention program. Two participants were excluded from the analysis; 1 participant missed too many training sessions (>10% was the predefined exclusion criteria) and 1 participant missed too many test beverages (>20% was the predefined exclusion criteria). Subsequently, analysis was performed on 39 participants, 20 in the PLA group and 19 in the PRO group. At baseline no differences in age, body mass, height, BMI, and leg volume were observed between the PLA and PRO groups (Table 1). We observed a significant increase in body mass from 80.0 ± 2.5 kg to 81.0 ± 2.7 kg and from 76.9 ± 2.1 kg to 78.9 ± 2.3 kg in response to 12 wk of resistance-type exercise training in both the PLA and the PRO groups, respectively ( $P < 0.05$ ). BMI increased significantly over time (from 23.4 ± 0.8 kg · m<sup>-2</sup> to 23.7 ± 0.8 kg · m<sup>-2</sup> and from 23.2 ± 0.6 kg · m<sup>-2</sup> to 23.8 ± 0.7 kg · m<sup>-2</sup> in the PLA and PRO groups, respectively;  $P < 0.05$ ). Furthermore, leg volume increased significantly in both groups in response to resistance-type exercise training (from 9.3 ± 0.3 L to 9.7 ± 0.3 L and from 9.0 ± 0.3 L to 9.1 ± 0.3 L in the PLA and PRO groups, respectively;  $P < 0.05$ ). No significant differences were observed between treatments.

**Body composition.** At baseline, no significant differences were observed between the PLA and PRO groups for any of the DXA scan measurements. Whole-body lean mass increased throughout the intervention period in both the PLA and the PRO groups ( $P < 0.001$ ), with no differences between groups (Table 2). Leg lean mass (left + right) increased significantly by 607 ± 121 g and 842 ± 129 g after 12 wk of resistance-type exercise training in the PLA and PRO groups, respectively, with no difference between the PLA and PRO groups (training × treatment interaction,  $P = 0.19$ ; Table 2). Although no significant changes were observed in total fat mass in the PLA and PRO groups, we did show a significant decline in percentage of whole-body fat and leg fat mass in response to 12 wk of resistance-type exercise training ( $P < 0.05$ ); no significant differences were observed between groups (data not shown).

**TABLE 2** Body composition before and after 12 wk of resistance-type exercise training in healthy young men who did or did not receive protein supplementation<sup>1</sup>

	PLA group (n = 19)		PRO group (n = 20)		<i>P</i>		
	Before	After	Before	After	Training	Treatment	Interaction
Whole-body lean mass, kg	63.6 ± 1.6	65.3 ± 1.7	62.9 ± 1.3	64.8 ± 1.4	<0.001	NS	NS
Trunk lean mass, kg	30.7 ± 0.8	31.4 ± 0.8	30.6 ± 0.7	31.2 ± 0.7	<0.001	NS	NS
Leg lean mass, kg	22.3 ± 0.6	23.0 ± 0.7	21.6 ± 0.5	22.5 ± 0.5	<0.001	NS	NS

<sup>1</sup> Values are means ± SEMs. NS,  $P \geq 0.05$ . PLA, placebo supplemented; PRO, protein supplemented.



**FIGURE 1** Quadriceps muscle CSA before and after 12 wk of resistance-type exercise training (A) and changes during the 12 wk (B) in healthy young men who did or did not receive protein supplementation. Values are means  $\pm$  SEMs,  $n = 19$  (placebo) or 20 (protein). NS,  $P \geq 0.05$ ; \*Different from before the intervention,  $P < 0.001$ ; \*\*Different from PLA group,  $P < 0.05$ . CSA, cross-sectional area; PLA, placebo supplemented.

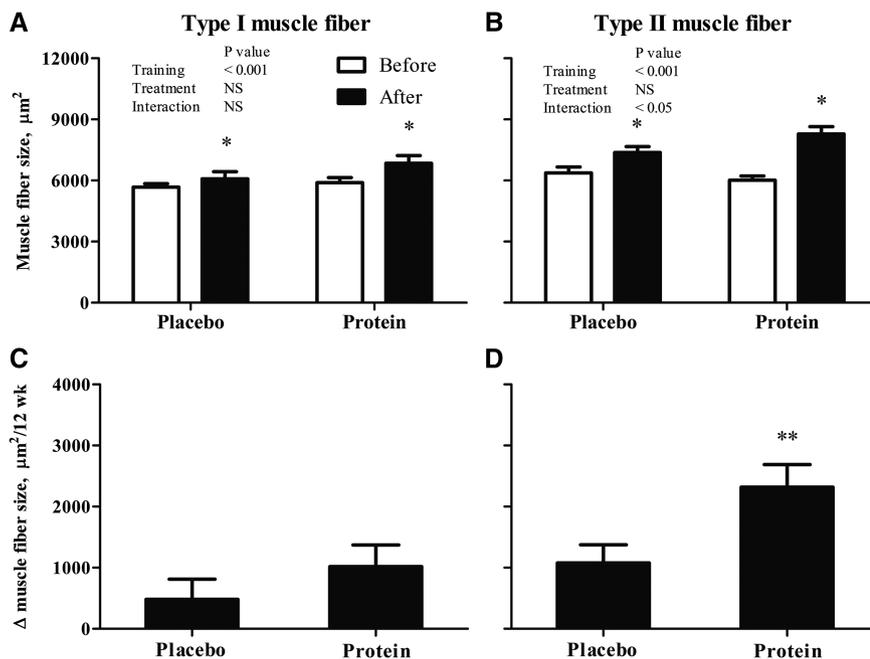
**Skeletal muscle hypertrophy.** At baseline, no significant difference in quadriceps muscle CSA was observed between the PLA and PRO groups (Figure 1). Quadriceps muscle CSA increased in both groups ( $P < 0.001$ ), with a greater increase in the PRO group than in the PLA group ( $P < 0.05$ ; Figure 1). Before intervention, no significant differences were observed in type I and type II muscle fiber size between PLA and PRO

groups. We observed a significant fiber type  $\times$  training  $\times$  treatment interaction ( $P < 0.05$ ). Separate analyses showed that type I muscle fiber size had increased in both the PLA and the PRO groups in response to 12 wk of resistance-type exercise training ( $P < 0.05$ ), with no differences between groups (training  $\times$  treatment interaction,  $P = 0.23$ ; Figure 2). Type II muscle fiber size increased in both groups ( $P < 0.001$ ), with a greater increase in the PRO group than in the PLA group (training  $\times$  treatment interaction,  $P < 0.05$ ; Figure 2).

**Muscle fiber type composition.** At baseline, no group differences were observed in the percentages of type I and type II muscle fibers and/or the percentage of muscle area occupied by types I and II fibers. Percentage of types I and II muscle fiber did not change in either group after 12 wk of exercise intervention (Supplemental Table 1). In contrast, percentage of type II muscle fiber area increased significantly from  $54\% \pm 3\%$  to  $65\% \pm 3\%$  in the PRO group after resistance-type exercise training ( $P < 0.05$ ), whereas no changes were observed in the PLA group.

**Muscle strength.** At baseline no significant differences in 1RM muscle strength were observed between the PLA and PRO groups (Table 3). After 12 wk of resistance-type exercise training leg press and leg extension muscle strength had increased significantly in both groups ( $P < 0.001$ ), with no differences between groups (Table 3). Similarly, for the upper body exercises (chest press, shoulder press, and horizontal row) we observed a significant increase in 1RM muscle strength over time ( $P < 0.001$ ), with no differences between the PLA and PRO groups (Table 3). On the lateral pull-down machine we found significantly greater muscle strength gains in the PRO group than in the PLA group (main effect of training,  $P < 0.001$ ; training  $\times$  treatment interaction,  $P < 0.05$ ; Table 3). Furthermore, we observed that the sum of all 1RM measurements increased to a larger extent in the PRO than in the PLA group (main effect of training,  $P < 0.001$ ; training  $\times$  treatment interaction,  $P < 0.05$ ; Figure 3).

**Dietary intake records.** Analysis of the 3-d dietary intake records collected before and after 11 wk of resistance-type exercise



**FIGURE 2** Muscle fiber size before and after 12 wk of resistance-type exercise training (A, B) and changes during 12 wk (C, D) in healthy young men who did or did not receive protein supplementation. Values are means  $\pm$  SEMs,  $n = 19$  (placebo) or 20 (protein). NS,  $P \geq 0.05$ ; \*Different from before the intervention,  $P < 0.001$ ; \*\*Different from PLA group,  $P < 0.05$ . PLA, placebo supplemented.

**TABLE 3** Muscle strength before and after 4, 8, and 12 wk of resistance-type exercise training in healthy young men who did or did not receive protein supplementation<sup>1</sup>

	PLA group (n = 19)				PRO group (n = 20)				P		
	0 wk	4 wk	8 wk	12 wk	0 wk	4 wk	8 wk	12 wk	Training	Treatment	Interaction
Leg press, kg	197 ± 9 <sup>a</sup>	220 ± 10 <sup>b</sup>	226 ± 9 <sup>c</sup>	239 ± 10 <sup>c</sup>	195 ± 6 <sup>a</sup>	228 ± 10 <sup>b</sup>	243 ± 10 <sup>c</sup>	244 ± 9 <sup>c</sup>	<0.001	NS	NS
Leg extension, kg	123 ± 5 <sup>a</sup>	134 ± 5 <sup>b</sup>	144 ± 6 <sup>c</sup>	151 ± 6 <sup>d</sup>	115 ± 5 <sup>a</sup>	133 ± 4 <sup>b</sup>	143 ± 6 <sup>c</sup>	146 ± 5 <sup>d</sup>	<0.001	NS	NS
Chest press, kg	86 ± 5 <sup>a</sup>	—	—	109 ± 5 <sup>b</sup>	88 ± 5 <sup>a</sup>	—	—	116 ± 7 <sup>b</sup>	<0.001	NS	NS
Shoulder press, kg	80 ± 5 <sup>a</sup>	—	—	93 ± 4 <sup>b</sup>	85 ± 5 <sup>a</sup>	—	—	104 ± 6 <sup>b</sup>	<0.001	NS	NS
Horizontal row, kg	62 ± 2 <sup>a</sup>	—	—	72 ± 3 <sup>b</sup>	62 ± 2 <sup>a</sup>	—	—	75 ± 2 <sup>b</sup>	<0.001	NS	NS
Vertical pull-down, kg	67 ± 3 <sup>a</sup>	—	—	76 ± 3 <sup>b</sup>	66 ± 2 <sup>a</sup>	—	—	80 ± 3 <sup>b</sup>	<0.001	NS	<0.05

<sup>1</sup> Values are means ± SEMs. Means within a group without a common letter are significantly different (a < b < c < d),  $P < 0.05$ . NS,  $P \geq 0.05$ . PLA, placebo supplemented; PRO, protein supplemented.

training showed no differences in total daily energy intake (excluding supplement intake) between groups and/or over time (Table 4). We observed a significant increase in the percentage of energy of protein intake during the 12-wk intervention period ( $P < 0.05$ ), with no differences between groups (Table 4). Daily protein intake averaged  $1.3 \pm 0.1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  in both groups and did not change significantly during the intervention period. As a result of the supplementation regime, protein intake increased to  $1.9 \pm 0.1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  in the PRO group (Table 4).

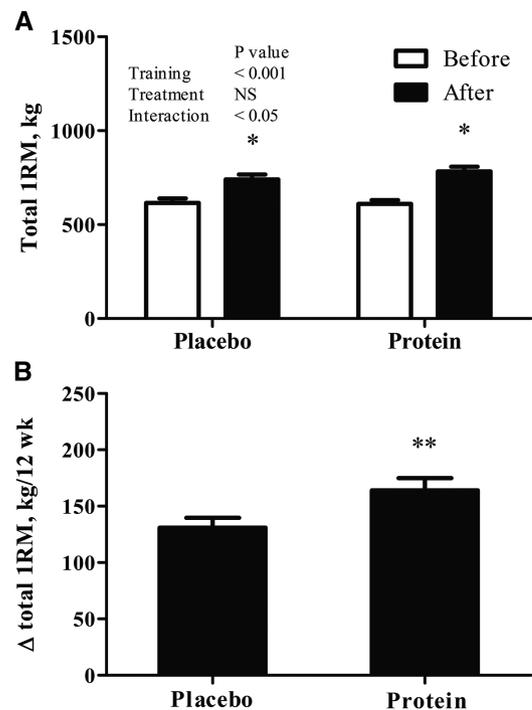
## Discussion

The present study shows that 3 mo of resistance-type exercise training increases skeletal muscle mass, strength, and muscle fiber size in young men. The exercise training-induced gains in muscle mass and strength are shown to be further increased after daily supplementation with 27.5 g of dietary protein consumed before sleep.

It has been well established that long-term resistance-type exercise training is an effective intervention strategy to increase skeletal muscle mass and strength (8–24). In the present study, we show substantial increases in whole-body lean mass after 12 wk of resistance-type exercise training (Table 2). The observed gains in muscle mass are in line with previous findings reported after 8–16 wk of resistance-type exercise training in healthy young men (25). The increase in whole-body lean mass was mainly attributed to an increase in leg and trunk lean mass (Table 2). These gains in muscle mass were accompanied by a  $6\% \pm 1\%$  increase in quadriceps muscle CSA after 12 wk of resistance-type exercise training (Figure 1). In agreement, on a myocellular level, we also observed a significant increase in muscle fiber size in response to exercise training. Previous studies have reported mixed results on either type I or type II muscle fiber hypertrophy after prolonged resistance-type exercise training. Although some demonstrate an increase in type I and/or type II muscle fiber size (8–10, 33), others have been unable to demonstrate muscle fiber hypertrophy in response to prolonged resistance-type exercise training (15, 34). In the present study, we report a substantial increase in both type I and type II muscle fiber size in response to resistance-type exercise training (Figure 2). Skeletal muscle mass and/or CSA are known to be positively correlated with skeletal muscle strength (35, 36). In accordance, we observed a substantial  $23\% \pm 2\%$  and  $25\% \pm 2\%$  increase in leg extension and leg press strength, respectively.

It has been suggested that protein supplementation can further augment the skeletal muscle adaptive response to prolonged resistance-type exercise training (25). However, there is much discrepancy in the literature on the proposed benefits of

protein supplementation to increase muscle mass and strength, with most studies unable to report significantly greater increases in muscle mass or strength after prolonged resistance-type exercise training (17–24). In the present study, we investigated the impact of daily protein supplementation, provided before sleep, on muscle mass and strength gains during 12 wk of resistance-type exercise training. Participants in the PRO group showed greater gains in muscle quadriceps CSA ( $+10\% \pm 1\%$ ) when compared with the PLA group ( $+6\% \pm 1\%$ ) after the exercise intervention (Figure 1). In agreement, increase in leg lean mass tended to be greater in the PRO group vs. the PLA group, but this difference did not reach statistical significance ( $P = 0.19$ ; Table 2). The latter is not unexpected because of the greater variation in the estimation of muscle mass by DXA. On the muscle fiber level, we observed a significantly greater increase in type II muscle fiber size in the PRO group than in



**FIGURE 3** Total 1RM (sum of all 1RM tests) strength before and after 12 wk of resistance-type exercise training (A) and changes during 12 wk (B) in healthy young men who did or did not receive protein supplementation. Values are means ± SEMs,  $n = 19$  (placebo) or 20 (protein). NS,  $P \geq 0.05$ ; \*Different from before the intervention,  $P < 0.001$ ; \*\*Different from PLA group,  $P < 0.05$ . PLA, placebo supplemented; 1RM, 1-repetition maximum.

**TABLE 4** Energy intake and macronutrient composition of the diet before and after 12 wk of resistance-type exercise training in healthy young men who did or did not receive protein supplementation<sup>1</sup>

	PLA group (n = 19)		PRO group (n = 20)		P		
	Before	After	Before	After	Training	Treatment	Interaction
Total energy, MJ/d	12.0 ± 0.7	10.8 ± 0.6	11.5 ± 0.7	11.4 ± 0.7	NS	NS	NS
Carbohydrate, % of energy	47 ± 1	48 ± 1	45 ± 2	43 ± 3	NS	NS	NS
Fat, % of energy	35 ± 1	34 ± 1	36 ± 2	35 ± 2	NS	NS	NS
Alcohol, % of energy	3 ± 1	1 ± 1	5 ± 1	5 ± 2	NS	NS	NS
Protein, % of energy	15 ± 1	17 ± 1	15 ± 1	17 ± 1	<0.05	NS	NS
Protein intake, g/d	101 ± 6	103 ± 7	99 ± 6	106 ± 8	NS	NS	NS
Protein intake, g · kg <sup>-1</sup> · d <sup>-1</sup>	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	NS	NS	NS
Protein intake including supplement, g · kg <sup>-1</sup> · d <sup>-1</sup>	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.9 ± 0.1*	<0.05	<0.001	<0.05

<sup>1</sup> Values are means ± SEMs. NS,  $P \geq 0.05$ . \*Significantly different compared with before,  $P < 0.05$ . PLA placebo supplemented; PRO, protein supplemented.

the PLA group after resistance-type exercise training (Figure 2). The greater gains in muscle mass also translated into increased functional capacity, with greater gains in 1RM muscle strength after protein supplementation (Figure 3). These results clearly show that protein supplementation, as performed in the present study, augments the skeletal muscle adaptive response to prolonged resistance-type exercise training.

These findings seem to be in line with some (8–16) but certainly not all studies (17–24) that investigated the impact of protein supplementation on muscle mass and strength gains during prolonged resistance-type exercise training. The obvious discrepancy in the literature is likely attributed to differences in the applied research design. Factors such as subject cohort size and applied training regimen, as well as the supplemented protein may strongly modulate the outcome of such studies. We speculate that the applied type, dose, and timing of the protein supplement chosen may have contributed largely to the surplus benefits of protein supplementation observed in the present study. Previous work has shown that ingestion of milk protein, or its main constituents whey and casein, give rise to a greater postexercise muscle protein synthetic response (37, 38) and greater gains in muscle mass and strength during prolonged resistance-type exercise training when compared with the ingestion of soy protein (10). Furthermore, it has been demonstrated that postexercise muscle protein synthesis rates increase more with the ingestion of greater amounts of protein, reaching maximal stimulation after ingestion of ~20 g of protein (39, 40). In addition, recent work from our group has shown that dietary protein provided before sleep results in proper overnight dietary protein digestion and absorption, allowing net muscle protein accretion during overnight sleep (26, 27, 41).

In the present study, all volunteers were subjected to resistance-type exercise training 3 times/wk, performed in the evening. All participants consumed a snack containing ~10 g of protein immediately after every exercise session to support postexercise recovery and to standardize postexercise dietary intake. In the PRO group we provided additional protein before sleep on all days of the week. The supplement was on average taken  $3.9 \pm 0.2$  h after cessation of exercise, allowing net muscle protein accretion during overnight sleep. This nutritional strategy was feasible and well tolerated by the participants. The protein supplementation effectively increased gains in muscle mass and strength despite the fact that participants in both the PRO and the PLA groups already received a protein snack after each training session and consumed a relative high-protein diet ( $1.3 \pm 0.1$  g protein/kg body weight; Table 4).

Consequently, it is quite remarkable that dietary protein supplementation before sleep further increased the gains in muscle mass and strength during prolonged resistance-type exercise training in these young, healthy adults. In the present study, ingestion of the protein supplement before sleep was not compared with other time points of ingestion. As such, we can only speculate on the surplus benefit(s) of the protein supplement being provided before sleep as opposed to other time points throughout the day.

In the present study, we provided participants in the PRO group with a beverage containing both carbohydrates (15 g) and protein (27.5 g), providing ~746 kJ. In the control group, participants received a noncaloric placebo. We chose to provide a noncaloric placebo as opposed to an isocaloric supplement providing carbohydrate and fat only. Providing such an isocaloric supplement could lower dietary protein intake in the control group, making a possible surplus benefit of protein supplementation less convincing. We observed no changes in total energy intake over time in either group, but a significant increase in the amount of protein consumed expressed as a percentage of daily energy intake was observed in both groups (Table 4).

The present study confirms our observations that provision of dietary protein before sleep is well received and well tolerated by athletes. We conclude that protein ingestion before sleep represents an effective dietary strategy to augment skeletal muscle mass and strength gains during prolonged resistance-type exercise training in healthy, young men.

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TS, KM, AKK, LBV, and LJCvL designed the research; TS, PTR, JSJS, and SvV conducted the research; TS, JSJS, SvV, and JvK performed immunohistochemical analyses; TS, AKK, and LBV analyzed the data; TS, LBV, and LJCvL wrote the paper. TS had primary responsibility for final content. All authors read and approved the final manuscript.

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