Physical Activity Performed in the Evening Increases the Overnight Muscle Protein Synthetic Response to Presleep Protein Ingestion in Older Men1–3

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Abstract

Background: The age-related decline in skeletal muscle mass is partly attributed to anabolic resistance to food intake. Dietary protein ingestion before sleep could be used as a nutritional strategy to compensate for anabolic resistance.

Objective: The present study assessed whether physical activity performed in the evening can augment the overnight muscle protein synthetic response to presleep protein ingestion in older men.

Methods: In a parallel group design, 23 healthy older men (mean ± SEM age: 71 ± 1 y) were randomly assigned to ingest 40 g protein intrinsically labeled with L-[1-13C]-phenylalanine and L-[1-13C]-leucine before going to sleep with (PRO+EX) or without (PRO) performing physical activity earlier in the evening. Overnight protein digestion and absorption kinetics and myofibrillar protein synthesis rates were assessed by combining primed, continuous infusions of L-[ring-2H5]-phenylalanine, L-[1-13C]-leucine, and L-[ring-2H2]-tyrosine with the ingestion of intrinsically labeled casein protein. Muscle and blood samples were collected throughout overnight sleep.

Results: Protein ingested before sleep was normally digested and absorbed, with 54% ± 2% of the protein-derived amino acids appearing in the circulation throughout overnight sleep. Overnight myofibrillar protein synthesis rates were 31% (0.058% ± 0.002%/h compared with 0.044% ± 0.003%/h; P < 0.01; based on L-[ring-2H5]-phenylalanine) and 27% (0.074% ± 0.004%/h compared with 0.058% ± 0.003%/h; P < 0.01; based on L-[1-13C]-leucine) higher in the PRO+EX than in the PRO treatment. More dietary protein-derived amino acids were incorporated into de novo myofibrillar protein during overnight sleep in PRO+EX than in PRO treatment (0.042 ± 0.002 compared with 0.033 ± 0.002 mole percent excess; P < 0.05).

Conclusions: Physical activity performed in the evening augments the overnight muscle protein synthetic response to presleep protein ingestion and allows more of the ingested protein-derived amino acids to be used for de novo muscle protein synthesis during overnight sleep in older men. This trial was registered at Nederlands Trial Register as NTR3885. J Nutr 2016;146:1307–14.

Keywords: muscle protein synthesis, sarcopenia, dietary protein, exercise, overnight

Introduction

The age-related decline in muscle mass and strength, termed sarcopenia, is accompanied by impairments in functional capacity and an increased risk of developing chronic metabolic diseases (1, 2). With no apparent differences in basal, postabsorptive muscle protein synthesis rates between young and older individuals (3, 4), many research groups have started to investigate the muscle protein synthetic response to the main anabolic stimuli, such as food intake and physical activity. This has led to the observation of an attenuated muscle protein synthetic response to food intake in

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3 Supplemental Figures 1 and 2, Supplemental Table 1, and Supplemental Methods are 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.
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older individuals (5, 6), a condition that has been coined anabolic resistance (7).

Effective strategies are needed to augment the muscle protein synthetic response to feeding as a means to compensate for the presence of anabolic resistance. Therefore, our laboratory and many others are investigating nutritional strategies to increase the muscle protein synthetic response to meal ingestion. Such interventions may include modulating the amount (8–10), type (11–13), and timing (14, 15) of protein ingestion, as well as combining protein ingestion with various food compounds (16, 17). Besides enhancing the muscle protein synthetic response to the main meals (i.e., breakfast, lunch, and dinner), we have proposed the ingestion of dietary protein before sleep with the aim of providing dietary-derived amino acids to support an increase in overnight muscle protein synthesis (18, 19). In a proof-of-principle study, Groen et al. (19) found that enteral administration of 40 g protein during sleep via a nasogastric tube was followed by proper protein digestion and absorption, thereby increasing overnight amino acid availability and stimulating overnight muscle protein synthesis rates in older individuals. Therefore, we proposed dietary protein ingestion before sleep as a practical and effective strategy to improve overnight protein balance and to stimulate overnight muscle protein synthesis.

Physical activity is an important factor responsible for the degree of anabolic resistance (7, 20). Previous work has established that physical activity or exercise can robustly increase muscle protein synthesis rates (21–24). Moreover, physical activity augments the postprandial muscle protein synthetic response to protein feeding, thereby compensating for anabolic resistance (9, 10, 22). Here, we hypothesized that physical activity performed during the evening would augment the overnight muscle protein synthetic response to presleep protein ingestion, allowing more of the ingested protein to be used for de novo myofibrillar protein accretion during sleep.

To test our hypothesis that physical activity can augment the impact of presleep protein ingestion on overnight muscle protein synthesis, we selected 23 older men (71 ± 6 y) who ingested 40 g casein protein intrinsically labeled with L-[1-13C]-phenylalanine and L-[1-13C]-leucine before going to sleep with (PRO+EX; n = 11) or without (PRO; n = 12) a bout of physical activity being performed earlier in the evening. By combining the ingestion of specifically produced casein intrinsically labeled with L-[1-13C]-phenylalanine and L-[1-13C]-leucine with the administration of primed continuous infusions of L-[ring-2H5]-phenylalanine, L-[ring-2H5]-leucine, and L-[ring-2H5]-tyrosine we were able to assess overnight protein digestion and amino acid absorption kinetics, whole-body protein metabolism, muscle protein synthesis rates, and the metabolic fate of the dietary protein-derived amino acids during overnight sleep.

Methods

Subjects. A total of 24 healthy, normoglycemic, older men (71 ± 1 y) were selected to participate in the present study. Subject characteristics of the study participants are presented in Table 1. Subjects randomly assigned to the PRO+EX treatment were cleared to perform physical activity by a cardiologist who examined electrocardiograms measured at rest and during submaximal cycling (performed at 70% of age-predicted heart rate maximum). The subjects were then familiarized with the exercise equipment and physical activity protocol. Subjects first performed a 10-min cycling warm-up at 70% of their age-predicted heart rate maximum before completing an estimation of their 1 repetition maximum (1RM) on the leg press and leg extension exercises with the use of the multiple repetitions testing procedure (26). For each exercise, subjects performed 10 submaximal, or warm-up, repetitions to become familiarized with the equipment and to have lifting technique critiqued and corrected. Subjects then performed sets at progressively increasing loads until failing to complete a valid repetition, judged by their inability to complete the full range of motion for an exercise. Ideally, subjects failed within 3–6 repetitions during the last and heaviest set. A 2-min resting period between subsequent attempts was allowed. The pretesting and experimental trials were separated by a period of ≥7 d.

Table 1: Subject characteristics of healthy older men who ingested 40 g protein before sleep after either completing a session of resistance exercise or remaining at rest

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Body weight, kg</th>
<th>BMI, kg/m²</th>
<th>Body fat, %</th>
<th>Lean body mass, kg</th>
<th>Appendicular lean mass, kg</th>
<th>Leg volume, L</th>
<th>HbA1c, %</th>
<th>Basal plasma glucose, mmol/L</th>
<th>Basal plasma insulin, mU/L</th>
<th>HOMA-IR</th>
<th>1RM leg press, kg</th>
<th>1RM leg extension, kg</th>
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<td>PRO</td>
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<td>70 ± 1</td>
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<td>25.6 ± 0.7</td>
<td>20.8 ± 0.9</td>
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<td>26.2 ± 0.7</td>
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<td>6.4 ± 0.3</td>
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<td>77 ± 5</td>
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<td>PRO+EX</td>
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<tr>
<td>71 ± 1</td>
<td>79.0 ± 2.3</td>
<td>26.0 ± 0.7</td>
<td>20.3 ± 1.3</td>
<td>66.1 ± 1.8</td>
<td>25.8 ± 0.9</td>
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<td>5.5 ± 0.1</td>
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<td>8.5 ± 1.1</td>
<td>3.1 ± 0.4</td>
<td>170 ± 8</td>
<td>77 ± 5</td>
<td>2.8 ± 0.5</td>
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Table 1 includes subject characteristics of older men who ingested 40 g protein before sleep after either completing a session of resistance exercise or remaining at rest. Values are expressed as means ± SEMs. No differences were observed between treatment groups. HbA1c, glycated hemoglobin; PRO, 40 g protein in rested state; PRO+EX, 40 g protein after resistance-type exercise; PSQI, Pittsburgh Sleep Quality Index; 1RM, 1 repetition maximum.
calculated with the Harris-Benedict equation and adjusted with a physical activity factor of 1.4 to ensure ample energy intake. The mean ± SEM dietary protein intake was 1.1 ± 0.01 g/kg body weight, with 35% ± 1% of the protein consumed at dinner.

**Experimental protocol.** At 1730, participants reported to the laboratory and had Teflon catheters inserted into the antecubital veins of each arm. At 1830 (t = −300 min), all subjects consumed the same standardized dinner meal under supervision (2.5 ± 0.1 MJ, providing 62% ± 0.2% of energy as carbohydrate, 19% ± 0.1% of energy as fat, and 19% ± 0.1% of energy as protein). Subjects in the PRO+EX group performed a single physical activity session between 1945 and 2045. After the physical activity session, and at 2100 (t = −150 min), a background blood sample was taken before the initiation of the tracer infusion protocol. The plasma and intracellular phenylalanine and leucine pools were primed with a single intravenous dose (priming dose) of i-[ring-2H3]-phenylalanine (2.0 μmol/kg), i-[ring-2H3]-tyrosine (0.615 μmol/kg), and i-[1-13C]-leucine (4.0 μmol/kg). Once primed, the continuous stable isotope infusion was initiated (infusion rate: 0.05 μmol · kg⁻¹ · min⁻¹ i-[ring-2H3]-phenylalanine, 0.015 μmol · kg⁻¹ · min⁻¹ i-[ring-2H3]-tyrosine, and 0.1 μmol · kg⁻¹ · min⁻¹ i-[1-13C]-leucine; Cambridge Isotopes Laboratories). Participants rested for 2.5 h until 2330 (t = 0 min), when the first muscle biopsy sample was taken. Subsequently, subjects ingested within 5 min a 450-mL beverage that contained 40 g casein intrinsically labeled with L-[1-13C]-phenylalanine and L-[1-13C]-leucine and 1.5 mL vanilla extract, added to improve palatability (Dr. Oetker, Amersfoort, Netherlands). Subjects went to sleep at 0000. During the night, blood samples (10 mL) were taken without waking the subjects at t = 30, 60, 90, 150, 210, 270, 330, 390, 450 min relative to the intake of the protein drink. A second muscle biopsy was obtained from the contralateral leg 7.5 h later at 0700 (t = 450 min).

Blood samples were collected in EDTA-containing tubes and were centrifuged at 1000 g for 10 min at 4°C. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C. Muscle biopsies were obtained from the middle region of the M. vastus lateralis, 15 cm above the patella and ~4 cm below entry through the fascia, using the percutaneous needle biopsy technique (27). Muscle samples were dissected carefully and freed from any visible nonmuscle material. The muscle samples were immediately frozen in liquid nitrogen and stored at −80°C until further analysis. A graphical representation of the experimental protocol is shown in **Supplemental Figure 1**.

**Physical activity protocol.** The physical activity protocol consisted of 60 min of moderate-intensity, lower-body, resistance-type exercise. After 15 min of self-paced cycling at 100 W with a cadence of 60–80 rpm, subjects performed 6 sets of 10 repetitions on the horizontal leg press machine (Technogym BV) and 6 sets of 10 repetitions on the leg extension machine (Technogym BV). The first 2 sets of both exercises were performed at 55% and 65% 1RM, respectively, and sets 3–6 were performed at 75% 1RM. Subjects were allowed to rest for 2 min between all sets.

**Sleep quality assessment.** The Pittsburgh Sleep Quality Index (Sleep Medicine Institute, University of Pittsburgh) was used to assess habitual sleep quality during pretesting (28). Pittsburgh Sleep Quality Index scoring (global scores 0–21 points) was used to classify all subjects to very good, good, poor, or very poor sleepers. Subjects that scored >5 (poor sleepers) were not included in the trial. Sleep behavior during the test night was monitored with wrist activity monitors and analyzed with Actiware software (Philips Respironics). Time was scored as awake unless the following 2 conditions were met simultaneously: participant was lying down attempting to sleep and the activity counts from the monitor were sufficiently low to indicate that the participant was immobile. In addition, the start and end times of sleep were recorded throughout the trial. The following variables were derived from sleep records and activity monitors: bed time (clock time), get up time (clock time), sleep onset latency (the period of time between bedtime and sleep start), sleep duration (h), time awake/flight sleep (h), sleep efficiency (sleep duration expressed as a percentage of time in bed), and wake bouts.

**Preparation of tracer and production of intrinsically labeled protein.** The stable isotope tracers i-[ring-2H3]-phenylalanine, i-[1-13C]-leucine, and i-[ring-2H3]-tyrosine were purchased from Cambridge Isotopes and were dissolved in 0.9% saline before infusion (Basic Pharma). Continuous intravenous infusions were performed with a calibrated IVAC 598 pump. Casein protein intrinsically labeled with i-[1-13C]-phenylalanine and i-[1-13C]-leucine was extracted from whole milk obtained during the constant infusion of i-[1-13C]-phenylalanine (455 μmol/min) and i-[1-13C]-leucine (200 μmol/min) for 96 h in a lactating dairy cow. The milk was collected, processed, and fractionated into the casein protein concentrate as previously described (16, 18, 29). The i-[1-13C]-phenylalanine and i-[1-13C]-leucine enrichments in casein protein were measured by gas chromatography–mass spectrometry isotope ratio mass spectrometry (MAT 253, Finnigan) and averaged 38.7 mol percent excess (MPE) and 9.3 MPE, respectively. The proteins met all chemical and bacteriologic specifications for human consumption.

**Plasma and muscle analysis.** Plasma glucose and insulin concentrations were analyzed with commercially available kits (GLUC, Roche, Ref: 05168791 190, and Immunologic, Roche, Ref: 12017547 122, respectively). Plasma amino acid concentrations and enrichments were determined by gas chromatography–mass spectrometry analysis (Agilent 7890A GC/5975C, MSD). Myofibrillar protein-bound i-[ring-2H3]-phenylalanine enrichments were determined by gas chromatography–mass spectrometry analysis, whereas the i-[1-13C]-phenylalanine and i-[1-13C]-leucine enrichments were determined by gas chromatography–combustion isotope ratio mass spectrometry analysis (Trace GC Ultra, IRMS model MAT 253; Thermo Scientific). For complete details, see the **Supplemental Methods.**

**Calculations.** Ingestion of i-[1-13C]-phenylalanine–labeled protein, intravenous infusion of i-[ring-2H3]-phenylalanine, and blood sample enrichment values were used to assess whole-body amino acid kinetics in non–steady state conditions. Total, exogenous, and endogenous phenylalanine rate of appearance (Ra) and rate of plasma availability of dietary protein-derived phenylalanine that appeared in the systemic circulation as a fraction of total amount of phenylalanine that was ingested was calculated with modified Steele’s equations (30–32). Total rate of disappearance of phenylalanine equals the rate of phenylalanine hydroxylation (first step in phenylalanine oxidation) and utilization for protein synthesis. Myofibrillar protein fractional synthetic rate (FSR) was calculated with the standard precursor-product method. For complete details, see the **Supplemental Methods.**

**Statistical analysis.** All data are expressed as means ± SEMs. Baseline characteristics between groups were compared with a Student’s unpaired t test. A 2-factor repeated-measures ANOVA (time × treatment) with time as within-subjects factor and treatment group as between-subjects factor was performed for the analysis of plasma amino acid concentrations, plasma tracer enrichments, whole-body kinetics, and glucose and insulin concentrations. The analysis was performed for the period that started at the time of protein administration, between t = 0 and 450 min. On identification of a statistically significant time × treatment interaction, Bonferroni post hoc testing was used to identify time points in which the treatments differed. Non–time-dependent variables (i.e., whole-body metabolism, FSR values, i-[1-13C]-phenylalanine myofibrillar enrichments) were compared between treatment groups with the use of Student’s unpaired t test. Statistical significance was set at P < 0.05. All calculations were performed with SPSS 21.0 (SPSS Inc.).

**Results**

**Plasma analysis.** Overnight plasma glucose (**Supplemental Figure 2A**) and insulin (**Supplemental Figure 2B**) concentrations after presleep protein ingestion did not differ between the PRO and PRO+EX groups (P > 0.05). Plasma insulin concentrations increased after protein ingestion in both treatments, reaching peak concentrations after 30 min.
Plasma concentrations of phenylalanine (Figure 1A), leucine (Figure 1B), and tyrosine (Figure 1C) increased over time, with peak concentrations being reached 210 min after protein ingestion, which did not differ between treatments ($P > 0.05$).

Plasma enrichments from infused L-[ring-$^2$H$_5$]-phenylalanine (Figure 2A), infused and ingested L-[1-$^{13}$C]-leucine (Figure 2B), and ingested L-[1-$^{13}$C]-phenylalanine (Figure 2C) did not differ between treatments before ingesting the protein ($t = 0$ min; $P > 0.05$). Plasma L-[ring-$^2$H$_5$]-phenylalanine enrichments declined after protein ingestion; plasma L-[1-$^{13}$C]-leucine enrichment increased before reaching a steady state for the duration of the trial. After protein ingestion, plasma L-[1-$^{13}$C]-phenylalanine enrichments, originating from the ingested protein, increased in both groups, reaching maximal values at $t = 210$ min in the PRO group and $t = 150$ min in the PRO+EX group and remained elevated for the duration of the night. A main effect for time was detected across groups ($P < 0.05$), but no significant interaction effects were found for treatment $\times$ time.

**Whole-body amino acid kinetics.** Exogenous phenylalanine $R_a$ (Figure 3B) increased after protein ingestion with peak rates being reached at $t = 150$ min in the PRO and PRO+EX treatment groups ($P > 0.05$). As a result of increased exogenous $R_a$, endogenous phenylalanine $R_a$ (Figure 3C) declined after protein ingestion in both groups, with no differences detected ($P > 0.05$). The ingested dietary protein-bound phenylalanine that appeared in the circulation over the entire 7.5-h postprandial period did not differ between groups ($P > 0.05$) and was $54 \pm 2\%$ overall.

Protein ingestion before sleep resulted in positive overnight whole-body protein net balance (Figure 4), with no differences observed between treatment groups ($P > 0.05$). Furthermore, physical activity did not appear to further influence any other variables of whole-body protein metabolism: synthesis rates ($P > 0.05$), breakdown rates ($P > 0.05$), oxidation rates ($P > 0.05$).
Myofibrillar FSRs and protein-bound enrichments. Myofibrillar L-\([\text{ring}^{-2}\text{H}_5]\)-phenylalanine and L-\([1-^{13}\text{C}]\)-leucine enrichments were measured in muscle samples collected immediately before protein ingestion and immediately after waking. The mean postprandial increase in myofibrillar protein-bound L-\([\text{ring}^{-2}\text{H}_5]\)-phenylalanine and L-\([1-^{13}\text{C}]\)-leucine enrichments was 0.0230±0.0015 compared with 0.0294±0.0010 MPE and 0.0334±0.0020 compared with 0.0417±0.0018 MPE in PRO compared with PRO+EX, respectively (P < 0.05). Myofibrillar L-\([1-^{13}\text{C}]\)-phenylalanine (Figure 6) protein-bound enrichment (MPE) was 28% higher after the ingestion of protein intrinsically labeled with L-\([1-^{13}\text{C}]\)-phenylalanine in the PRO+EX treatment group than in the PRO treatment group (P < 0.05).

Sleep data. Sleep onset latency and sleep efficiency data collected during the overnight sleep test are displayed in Supplemental Table 1. Sleep onset latency, a measure of the time taken to fall asleep, was 6 min 12 s ± 2 min 4 s compared with 7 min ± 2 min 9 s in the PRO compared with the PRO+EX treatment groups, respectively (P ≥ 0.05). The amount of time that subjects spent awake or in light sleep throughout the sleeping period was 1 h 12 s ± 11 min 28 s compared with 38 min 15 s ± 6 min 45 s in the PRO and PRO+EX treatment groups, respectively (P ≥ 0.05). Sleep efficiency, a measure of sleep quality throughout the night, also did not differ between treatment groups (81.4%±3.1% compared with 82.3%±8.7% in the PRO and PRO+EX groups, respectively; P ≥ 0.05).

Discussion

In the present study, we assessed whether physical activity performed in the evening after a full day of standardized dietary intake and physical activity could augment the overnight muscle protein synthetic response to presleep protein ingestion in older men. We observed that protein ingested before sleep was normally digested and absorbed, with 54%±2% of the ingested protein-derived amino acids appearing in the circulation throughout overnight sleep. Myofibrillar protein synthesis rates during overnight sleep were substantially higher when physical activity was performed earlier that evening, with 28% more of the presleep dietary protein-derived amino acids being directed toward de novo overnight muscle protein synthesis.

We administered a primed, continuous intravenous infusion of L-\([\text{ring}^{-2}\text{H}_5]\)-phenylalanine and L-\([1-^{13}\text{C}]\)-leucine for 2 h and measured the rates of appearance (Ra) and disappearance (Rd) of phenylalanine and leucine throughout the overnight sleep period. The calculated rates of whole-body protein synthesis, breakdown, oxidation, and net protein balance after PRO+EX (n = 11) or PRO (n = 12) presleep treatment in older men are displayed in Figure 4. No significant differences were detected. PRO, 40 g protein in rested state; PRO+EX, 40 g protein after resistance-type exercise.
the night and provided all participants with 40 g casein protein intrinsically labeled with L-[1-13C]-phenylalanine before sleep. With this experimental protocol, we were able to assess overnight protein digestion and amino acid absorption kinetics, whole-body protein metabolism, myofibrillar protein synthesis, and the specific utilization of dietary protein-derived amino acids for de novo muscle protein synthesis (16, 33). On protein ingestion, we observed a rapid rise in circulating plasma amino acids (Figure 1) and L-[1-13C]-phenylalanine enrichments (Figure 2C), indicating proper protein digestion and subsequent amino acid absorption during sleep. Exogenous dietary protein-derived phenylalanine \( R_0 \) remained elevated throughout overnight sleep (Figure 3B). Over the entire 7.5-h overnight period, 53% ± 2% of the ingested protein-derived amino acids appeared in the circulation, with the other 47% ± 2% of the protein-derived amino acids being retained in the gut to support turnover of splanchnic tissues. These data are in line with previous work that quantified first-pass amino acid extraction conducted during the day time (8, 22, 34) and extend on these findings with the observation that physical activity performed earlier during the evening does not modulate overnight protein digestion and amino acid absorption after presleep protein ingestion.

Protein ingestion before sleep has previously been shown to improve whole-body protein synthesis and to reduce whole-body protein breakdown, allowing for a positive net protein balance during overnight sleep (18, 19). In the present study, we confirmed our previous findings by showing that presleep protein ingestion resulted in a (more) positive overnight whole-body protein balance (Figure 4). Physical activity performed earlier that day did not seem to have a substantial impact on overnight whole-body protein synthesis, breakdown, amino acid oxidation, or net balance (Figure 4). However, note that whole-body protein metabolism does not necessarily reflect skeletal muscle protein turnover. Because we aimed to assess the impact of physical activity on the overnight muscle protein synthetic response, we collected muscle biopsies immediately before and after overnight sleep.

With the present amino acid tracer method, we were able to assess rates of muscle protein synthesis under steady state (L-[1-13C]-leucine) and non–steady state (L-[ring-2H5]-phenylalanine) precursor conditions (33). As hypothesized, myofibrillar protein synthesis rates were 31% (L-[ring-2H5]-phenylalanine; Figure 5A) and 27% (L-[1-13C]-leucine; Figure 5B) higher with the PRO+EX treatment than with the PRO treatment. These findings extend on our previous work showing that administration of 40 g protein before sleep increases overnight muscle protein synthesis rates compared with a placebo (19). Furthermore, these findings are in line with previous work that investigated the impact of physical activity or exercise on the postprandial muscle protein synthetic response to protein or meal feeding after an overnight fast, showing that physical activity further augments the postprandial rise in muscle protein synthesis rate by ~25% (22, 35). This supports the concept that physical activity increases the sensitivity of skeletal muscle tissue to the anabolic properties of protein ingestion and that this response may extend into overnight sleep. In the present study, we combined continuous infusions of L-[ring-2H5]-phenylalanine and L-[1-13C]-leucine with the ingestion of protein intrinsically labeled with L-[1-13C]-phenylalanine (29, 33). Because this specifically produced protein is highly enriched (>35 MPE), we are able to directly assess the metabolic fate of the dietary protein-derived amino acids. Here, we demonstrate that the ingested protein was used for de novo skeletal muscle protein synthesis throughout overnight sleep (Figure 6). Substantially more (28%) of the dietary protein-derived phenylalanine was incorporated into skeletal muscle protein when physical activity was performed earlier in the day. Therefore, physical activity throughout the day increases the efficiency by which protein-derived amino acids provided before sleep are directed toward overnight de novo muscle protein synthesis. Consequently, the combination of physical activity or exercise with presleep protein ingestion can augment the overnight muscle protein synthetic response and increase the efficacy by which presleep protein supplementation may help to preserve muscle mass and strength in the older population. In support, we recently reported greater gains in skeletal muscle mass and strength after 3 mo of evening resistance-type exercise training in young men when subjects were provided with additional protein before sleep (36).

It is well established that the anabolic response to protein ingestion is impaired in older (5, 7, 37) and/or more clinically compromised (20, 37–39) populations. Previous work from our group (8) and from others (9, 10) has shown that increasing protein intake can compensate for anabolic resistance. However, ingesting larger protein doses may not be feasible or practical in all older and/or clinically compromised populations.
ingestion before sleep may represent an effective nutritional strategy to preserve muscle mass by stimulating and supporting muscle protein accretion during overnight sleep. The current data extend on previous observations and are the first, to our knowledge, to show that physical activity performed throughout the day increases the efficiency by which dietary protein ingested before sleep is directed toward de novo muscle protein synthesis in older individuals. Therefore, older individuals who are unable to ingest large amounts of protein can still benefit from ingesting smaller amounts of protein (<40 g) before sleep by performing physical activity beforehand. As such, a physical activity program should be implemented in combination with presleep protein ingestion to benefit from the synergy between physical activity and protein to increase overnight muscle protein accretion to support healthy aging.

In conclusion, physical activity performed in the evening augments the overnight muscle protein synthetic response to presleep protein ingestion and allows more of the ingested protein-derived amino acids to be used for de novo muscle protein synthesis during overnight sleep in older men. Combining presleep protein ingestion with physical activity may aid in preserving skeletal muscle mass and, as such, support healthy aging.

Acknowledgments

AMH, IWKK, and LJCvL designed the research; AMH conducted the research with the assistance of IWKK and JT, and performed the statistical analysis; LJCvL provided essential reagents and materials; AMH, IWKK, JT, SLH, WKWHW, and LBV analyzed the data; and AMH and LJCvL wrote the paper and held primary responsibility for the final content. All authors read and approved the final manuscript.

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