Resistance Exercise Augments Postprandial Overnight Muscle Protein Synthesis Rates

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ABSTRACT
TROMMELEN, J., A. M. HOLWERDA, I. W. K. KOUW, H. LANGER, S. L. HALSON, I. ROLLO, L. B. VERDIJK, and L. J. C. VAN LOON. Resistance Exercise Augments Postprandial Overnight Muscle Protein Synthesis Rates. Med. Sci. Sports Exerc., Vol. 48, No. 12, pp. 2517–2525, 2016. Introduction: We have previously shown that protein ingestion before sleep increases overnight muscle protein synthesis rates. Whether prior exercise further augments the muscle protein synthetic response to presleep protein ingestion remains to be established. Objective: This study aimed to assess whether resistance-type exercise performed in the evening increases the overnight muscle protein synthetic response to presleep protein ingestion. Methods: Twenty-four healthy young men were randomly assigned to ingest 30 g intrinsically L-[1-13C]-phenylalanine and L-[1-13C]-leucine-labeled casein protein before going to sleep with (PRO + EX, n = 12) or without (PRO, n = 12) prior resistance-type exercise performed in the evening. Continuous intravenous L-[ring-2H5]-phenylalanine, L-[1-13C]-leucine, and L-[ring-2H5]-tyrosine infusions were applied. Blood and muscle tissue samples were collected to assess whole-body protein balance, myofibrillar protein synthesis rates, and overnight incorporation of dietary protein-derived amino acids into de novo myofibrillar protein. Results: A total of 57% ± 1% of the ingested protein-derived phenylalanine appeared in the circulation during overnight sleep. Overnight myofibrillar protein synthesis rates were 37% (0.055%/h-1 ± 0.002%/h-1 vs. 0.040%/h-1 ± 0.003%/h-1, P < 0.001, based on L-[ring-2H5]-phenylalanine) and 31% (0.073%/h-1 ± 0.004%/h-1 vs. 0.055%/h-1 ± 0.006%/h-1, P = 0.024, based on L-[1-13C]-leucine) higher in PRO + EX compared with PRO. Substantially more of the dietary protein-derived amino acids were incorporated into de novo myofibrillar protein during overnight sleep in PRO + EX compared with PRO (0.026 ± 0.003% vs. 0.015 ± 0.003 molar percent excess, P = 0.012). Conclusions: Resistance-type exercise 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 myofibrillar protein synthesis during overnight sleep. Key Words: SLEEP, RECOVERY, EXERCISE, MUSCLE, STABLE ISOTOPES, CASEIN

A single session of resistance-type exercise stimulates both muscle protein synthesis and breakdown rates (4,23,28). 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 (5,7,15). Therefore, postexercise protein ingestion is widely applied as a strategy to increase postexercise muscle protein synthesis rates and, as such, to stimulate postexercise recovery and facilitate skeletal muscle reconditioning. Various factors have been identified that can modulate the postexercise muscle protein synthetic response including the amount (20,31), type (27,30), and timing (1,18) of protein ingestion.

Previously, we have demonstrated that protein ingested before sleep is properly digested and absorbed, thereby increasing overnight amino acid availability and stimulating muscle protein synthesis during overnight sleep (13,24). On the basis of these findings, we suggested that protein ingestion before sleep may represent a practical and effective interventional strategy to support muscle mass maintenance and/or stimulate muscle hypertrophy. In this regard, presleep protein supplementation has recently been applied in a prolonged resistance-type exercise training regimen, allowing greater gains in muscle mass and strength (26). In this study, subjects ingested either ~30 g of additional protein or a noncaloric placebo before sleep during a 3-month resistance-type exercise training program, with supplements ingested on both training days (three times per week) and nontraining days. Although presleep protein supplementation was effective in further increasing muscle mass and strength.
gains when compared with a placebo, the acute effects of resistance-type exercise on the muscle protein synthetic response to protein ingestion before sleep remain to be determined.

The aim of the present study was to assess whether resistance-type exercise performed in the evening increases the overnight muscle protein synthetic response to presleep protein ingestion. We hypothesized that resistance-type exercise performed in the evening augments the muscle protein synthetic response to presleep protein ingestion, allowing more of the ingested protein to be used for de novo myofibrillar protein accretion during overnight sleep. Therefore, we studied 24 recreationally active young males who ingested 30 g intrinsically l-\([1-^{13}C]\)-phenylalanine and l-\([1-^{13}C]\)-leucine-labeled casein protein before going to sleep with (PRO + EX) or without prior exercise (PRO) being performed in the evening. By combining the use of specifically produced intrinsically l-\([1-^{13}C]\)-phenylalanine and l-\([1-^{13}C]\)-leucine-labeled casein with primed continuous infusion of l-\([\text{ring-}^{2}{^2}H_{2}]\)-phenylalanine, l-\([1-^{13}C]\)-leucine, and l-\([\text{ring-}^{2}{^2}H_{2}]\)-tyrosine, we were able to assess 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 toward de novo myofibrillar protein accretion during overnight sleep.

**METHODS**

**Subjects.** A total of 24 healthy, recreationally active (participating in exercise other than structured resistance-type exercise, for 1–3 d·wk\(^{-1}\) for ≥12 months) young men were selected to participate in this study. Subjects’ characteristics are presented in Table 1. Subjects were randomly assigned to ingest 30 g intrinsically l-\([1-^{13}C]\)-phenylalanine and l-\([1-^{13}C]\)-leucine-labeled casein protein before going to sleep with (PRO + EX, \(n = 12\)) or without a bout of resistance-type exercise (PRO, \(n = 12\)) being performed in the evening (1945–2045 h). All subjects were fully informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study is part of a greater project investigating the effect of presleep protein feeding on overnight muscle protein synthesis. The project was registered at Netherlands Trial Registry as NTR3885, was approved by the Medical Ethical Committee of the Maastricht University Medical Centre, The Netherlands, and conformed to standards for the use of human subjects in research as outlined in the most recent version of the Helsinki Declaration.

**Pretesting.** Bodyweight and body composition (fat and fat-free mass) were determined by dual-energy x-ray absorptiometry; Discovery A; Hologic, Bedford). Leg volume was determined by anthropometry measurements as described by Jones and Pearson (14). The subjects were then familiarized with the resistance-type exercise protocol and the exercise equipment. All exercises during pretesting and experimental trials were supervised by trained personnel. Subjects started by performing a 10-min cycling warm-up at 150 W before completing an estimation of their 1-repetition maximum (1-RM) on the leg press and leg extension exercises using the multiple repetitions testing procedure (19). For each exercise, subjects performed 10 submaximal repetitions to become familiarized with the equipment and to have lifting technique critiqued and properly adjusted. Sets were then performed at progressively increasing loads until failure to perform a valid estimation within 3–6 repetitions of the set. A repetition was valid if the subject was able to complete the entire lift in a controlled manner without assistance. A 2-min resting period between subsequent attempts was allowed. The pretesting and experimental trials were separated by at least 7 d.

**Diet and physical activity.** All subjects were instructed to refrain from exhaustive physical labor and exercise and to keep their diet as constant as possible for 2 d before the experimental day. Food intake and physical activity questionnaires were collected for 2 d before the experiment. All subjects received a standardized diet throughout the experimental day (0.16 MJ·kg\(^{-1}\)·day\(^{-1}\), providing 62 energy percentage (En%) carbohydrate, 13 En% protein, and 22 En% fat). The energy content of the standardized diet was based on individual energy requirements based on the Harris–Benedict equation and adjusted using a physical activity factor of 1.6 to ensure ample energy intake. During the experimental day, participants ingested 1.2 ± 0.01 g protein per kilogram of bodyweight via the standardized diet with an additional 20 g (0.27 ± 0.01 g·kg\(^{-1}\)) of protein provided at 2045 h and an additional 30 g of protein (0.40 ± 0.01 g·kg\(^{-1}\)) provided before sleep (2330 h).

**Experimental protocol.** An outline of the study protocol is provided in Figure 1. At 1730 h, participants reported to the laboratory and had Teflon catheters inserted into the antecubital veins of each arm. At 1830 h (\(t = –300\) min), all the subjects consumed a standardized dinner (Sligro, Maastricht, the Netherlands) under supervision (0.04 MJ·kg\(^{-1}\)), providing 55 En% carbohydrate, 21 En% protein, and 20 En% fat), after which no more solid food was consumed. Subjects in the PRO + EX group subsequently performed a resistance-type exercise bout between 1945 and 2045 h. Subjects in the PRO group rested in a sitting position during this period. Immediately after the exercise or rest session, both the PRO and the PRO + EX groups received drinks providing 20 g protein and 45 g carbohydrate (Gatorade G-series 03 Recover protein recovery shake; the Gatorade Company, Chicago, IL), which were ingested within 2 min. The purpose of this recovery drink was to optimize muscle protein synthesis rates.

<table>
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<th>Table 1. Subjects’ characteristics.</th>
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<tr>
<td><strong>PRO ((n = 12))</strong></td>
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<td>Age (yr)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg·m(^{-2}))</td>
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<td>LBM (kg)</td>
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Values represent mean ± SEM. Data were analyzed using Student’s t-test.

*Significantly different from PRO (\(P = 0.036\)).

PRO, presleep protein ingestion without prior exercise; PRO + EX, presleep protein ingestion with prior exercise; BMI, body mass index.
in the hours before sleep (2). After protein ingestion, a background blood sample was taken before the initiation of the tracer infusion protocol, which was started at 2100 h ($t = -150$ min). The plasma and intracellular phenylalanine and leucine pools were primed with a single intravenous dose (priming dose) of $\text{L-}[\text{ring}^2\text{H}_2]$-phenylalanine (2.0 $\mu$mol kg$^{-1}$), $\text{L-}[\text{ring}^2\text{H}_2]$-tyrosine (0.615 $\mu$mol kg$^{-1}$), and $\text{L-}[^{15}\text{C}]$-leucine (4.0 $\mu$mol kg$^{-1}$). Once primed, the continuous stable isotope infusion was initiated (infusion rate: 0.05 $\mu$mol kg$^{-1}$ min$^{-1}$ $\text{L-}[\text{ring}^2\text{H}_2]$-phenylalanine, 0.015 $\mu$mol kg$^{-1}$ min$^{-1}$ $\text{L-}[\text{ring}^2\text{H}_2]$-tyrosine, 0.1 $\mu$mol kg$^{-1}$ min$^{-1}$ $\text{L-}[^{15}\text{C}]$-leucine; Cambridge Isotopes Laboratories, Andover, MA). Participants rested in a supine position for 2.5 h until 2330 h ($t = 0$ min), after which the first muscle biopsy was taken. Subsequently, subjects ingested a 450-mL beverage containing 30 g intrinsically labeled casein protein and 45 g of carbohydrate (Gatorade G-series 03 Recover protein recovery shake, Gatorade Company).

### Production of intrinsically labeled protein

Intrinsically, $\text{L-}[^{15}\text{C}]$-phenylalanine and $\text{L-}[^{15}\text{C}]$-leucine-labeled casein protein were obtained during the constant infusion of $\text{L-}[^{15}\text{C}]$-phenylalanine (455 $\mu$mol min$^{-1}$) and $\text{L-}[^{15}\text{C}]$-leucine (200 $\mu$mol min$^{-1}$) maintained for 96 h in a lactating dairy cow. The milk was collected, processed, and fractionated into the casein protein concentrate as previously described (8,22,29). The $\text{L-}[^{15}\text{C}]$-phenylalanine and $\text{L-}[^{15}\text{C}]$-leucine enrichments in casein protein were measured by gas chromatography–combustion–isotope ratio mass spectrometry (GC-IRMS; MAT 252, Finnigan, Breman, Germany) and averaged 38.7 molar percent excess (MPE) and 9.3 MPE, respectively. The proteins met all chemical and bacteriological specifications for human consumption.

#### Tracer preparation

The stable isotope tracers $\text{L-}[\text{ring}^2\text{H}_2]$-phenylalanine, $\text{L-}[^{15}\text{C}]$-leucine, and $\text{L-}[\text{ring}^2\text{H}_2]$-tyrosine were purchased from Cambridge Isotopes (Andover, MA) and dissolved in 0.9% saline before infusion (Basic Pharma, Geleen, The Netherlands). Continuous intravenous infusions were performed using a calibrated IVAC 598 pump (San Diego, CA).

#### Plasma analysis

Plasma glucose and insulin concentrations were analyzed using commercially available kits (GLUC3, Roche, ref. no. 05168791 190, and Immunologic, Roche, ref. no. 12017547 122, respectively). Plasma amino acid concentrations and enrichments were determined by gas chromatography–mass spectrometry analysis (GC-MS; Agilent 7890A GC/5975C; MSD, Wilmington, Delaware). Specifically, internal standards of $[\text{U-}^{15}\text{C}_9]$-leucine, $[\text{U-}^{15}\text{C}_9\text{N}]$-phenylalanine, and $[\text{U-}^{15}\text{C}_9\text{N}]$-tyrosine were added to the samples. The plasma was deproteinized on ice with dry 5-sulfosalicylic acid. Free amino acids were purified using cation exchange chromatography (AG 50 W-X8 resin; mesh size, 100–200 $\mu$m; ionic form, hydrogen; Bio-Rad Laboratories,
Hercules, CA). The purified amino acids were converted into tert-butyl dimethylsilyl (tert-BDMS) derivatives with MTBSTFA before analysis by GC-MS. The amino acid concentrations were determined using electron impact ionization by monitoring ions at mass/charge (m/z) 302 and 308 for unlabeled and [U-13C6]-labeled-leucine, 336 and 346 for unlabeled and [U-13C15N]-labeled-phenylalanine respectively, and 466 and 476 for unlabeled and [U-13C15N]-tyrosine. The plasma leucine, phenylalanine, and tyrosine 13C and 2H enrichments were determined using selective ion monitoring at m/z 302 and 303 for unlabeled and labeled (1-13C) leucine, respectively; m/z 336, 337, and 341 for unlabeled and labeled (1-13C and ring-2H3) phenylalanine, respectively; m/z 466, 467, 468, and 470 for unlabeled and labeled (1-13C, ring-3,5-2H2, and ring-2H4) tyrosine, respectively. Standard regression curves were applied from a series of known standard enrichment values against the measured values to assess the linearity of the mass spectrometry and to account for any isotopic fractionation.

Muscle analysis. Myofibrillar protein enriched fractions were extracted from ~60 mg of wet muscle tissue by hand-homogenizing on ice using a pestle in a standard extraction buffer (7 μL·mg⁻¹) (16). The samples were spun at 2500 g and 4°C for 5 min. The pellet was washed with 500 μL ddH2O and centrifuged at 250 g and 4°C for 10 min. The myofibrillar protein was solubilized by adding 1 mL of 0.3 M NaOH and heating at 50°C for 30 min with vortex mixing every 10 min. Samples were centrifuged at 9500g and 4°C for 5 min, the supernatant containing the myofibrillar proteins was collected, and the collagen pellet was discarded. Myofibrillar proteins were precipitated by the addition of 1 M of 1 M PCA and spinning at 700g and 4°C for 10 min. The myofibrillar protein was washed twice with 70% ethanol and hydrolyzed overnight in 2 mL of 6 M HCL for 10 min. The free amino acids from the hydrolyzed myofibrillar protein pellet were dried under nitrogen stream while being heated to 120°C. The free amino acids were then dissolved in 25% acetic acid solution, passed over cation exchange AG 50 W-X8 resin columns (mesh size, 100–200; ion form, hydrogen; Bio-Rad Laboratories), and eluted with 2 M NH4OH. The purified amino acids were divided into two aliquots to determine the 1-[ring-2H5]-phenylalanine enrichments by GC-MS analysis and the 1-[1-13C]-phenylalanine and 1-[1-13C]-leucine enrichments by GC-IRMS analysis. To reduce the signal-to-noise ratio during GC-MS analysis at low tracer enrichments, the phenylalanine from the myofibrillar protein hydrolysates was enzymatically decarboxylated to β-phenethylamine before derivatization with MTBSTFA. To determine myofibrillar protein 1-[1-13C]-phenylalanine and 1-[1-13C]-leucine enrichments by GC-IRMS analysis, the purified amino acids were converted into N-ethoxy carbonyl ethyl ester derivatives with ethyl chloroformate. The derivatives were then measured by GC-IRMS (MAT 253, Finnigan) using a DB5-MS-column (no. 122–5532; Agilent J+W scientific GC Column), GC Isolink, and monitoring of ion masses 44, 45 and 46. Standard regression curves were applied to assess the linearity of the mass spectrometer and to account for isotopic fractionation.

Calculations. Ingestion of 1-[1-13C]-phenylalanine-labeled protein, intravenous infusion of 1-[ring-2H5]-phenylalanine and 1-[ring-3,5-2H2]-tyrosine, 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 (R0) and plasma availability of dietary protein-derived phenylalanine that appeared in the systemic circulation as a fraction of total amount of phenylalanine that was ingested (Pheplasma) were calculated using modified Steele's equations (6,10).

These parameters were calculated as follows:

\[ \text{Total } R_a = \frac{F - \int pV C(t) \, dt}{E_{iv}(t)} \]  
\[ \text{Exo } R_a = \text{Total } R_a - \text{Endo } R_a - F \]  
\[ \text{Pheplasma} = \left( \frac{\text{AUC}_{\text{Exo}R_a}}{\text{Pheprot}} \right) \times 100 \]

where \( F \) is the intravenous tracer infusion rate (μmol·kg⁻¹·min⁻¹) and \( pV \) (0.125 L·kg⁻¹) is the distribution volume for phenylalanine (6). \( C(t) \) is the mean plasma phenylalanine concentration between two consecutive time points. \( dE_{iv}/dt \) represents the time-dependent variations of plasma phenylalanine enrichment derived from the intravenous tracer, and \( E_{iv}(t) \) is the mean plasma phenylalanine enrichment from the intravenous tracer between two consecutive time points. \( \text{Exo } R_a \) represents the plasma entry rate of dietary phenylalanine, \( E_{po}(t) \) is the mean plasma phenylalanine enrichment for the ingested tracer, \( dE_{po}/dt \) represents the time-dependent variations of plasma phenylalanine enrichment derived from the oral tracer, and \( E_{prot} \) is the 1-[1-13C]-phenylalanine enrichment in the dietary protein. \( \text{Pheplasma} \) is the percentage of ingested dietary phenylalanine that becomes available in the plasma and is calculated using Pheprot and AUC_{ExoR_a}. Pheprot is the amount of dietary phenylalanine ingested, and AUC_{ExoR_a} represents the area under the curve (AUC) of ExoR_a, which corresponds to the amount of dietary phenylalanine that appeared in the blood during a 7.5-h period after ingestion.

Total rate of disappearance of phenylalanine equals the rate of phenylalanine hydroxylation (first step in phenylalanine oxidation) and use for protein synthesis. This parameter is calculated as follows:

\[ \text{Total } R_d = \text{Total } R_a \times pV \frac{dC}{dt}. \]

Because whole-body \( R_d \) comprises the rate of phenylalanine disappearance from the free amino acid pool in the blood due to protein synthesis and oxidation, whole-body protein synthesis can be calculated as \( R_d \) minus oxidation.
Whole-body phenylalanine oxidation can be determined from the conversion (hydroxylation) of L-[ring-2H5]-phenylalanine to L-[ring-2H4]-tyrosine. The rate of hydroxylation was calculated by using the following formula:

$$\text{Phenylalanine hydroxylation} = \text{Tyrr}_E(t) \frac{E(t) - E(0)}{E(t)} \frac{\text{Phe}R_d}{(F + \text{Phe}R_d)}.$$  \[6\]

Tyrr$_E$ represents the rate of appearance of L-[ring-2H4]-tyrosine; $E(t)$ and $E(0)$ are the L-[ring-2H4]-tyrosine and the L-[ring-2H4]-phenylalanine enrichments in plasma between two consecutive time points, respectively; and $F$ is the infusion rate of phenylalanine. Whole-body protein synthesis was calculated using the following:

$$\text{protein synthesis} = R_d - \text{Phenylalanine hydroxylation}.$$  \[7\]

Whole-body protein net balance was calculated as AUC during the 7.5-h postprandial phase using whole-body protein synthesis minus endogenous $R_d$:

$$\text{Phe net balance} = \text{Protein synthesis} - \text{Endo}R_d.$$  \[8\]

The fractional synthetic rate (FSR) of myofibrillar protein was calculated by dividing the increment in enrichment in the product, i.e., protein-bound L-[1-13C]-leucine or L-[1-13C]-phenylalanine, by the enrichment of the respective precursor amino acid enrichments (i.e., plasma free amino acids). Weighted mean plasma L-[ring-2H4]-phenylalanine and L-[1-13C]-leucine enrichments were used as the preferred precursor pools to estimate myofibrillar protein FSR from the continuously infused L-[ring-2H3]-phenylalanine and L-[1-13C]-leucine tracers. Consequently, myofibrillar FSR was calculated as follows (17):

$$\text{FSR} = \frac{E_t(t) - E_t(0)}{\int_{t_0}^{t} E_t(t) \, dt} \times 100$$  \[9\]

where $E_B$ represents muscle protein-bound L-[ring-2H5]-phenylalanine or L-[1-13C]-leucine, $E_t$ represents the average plasma L-[ring-2H3]-phenylalanine or L-[1-13C]-leucine enrichment during the tracer incorporation period, and $t$ indicates the time interval (h) between biopsies.

**Sleep quality assessment.** The Pittsburg Sleep Quality Index (Sleep Medicine Institute, University of Pittsburgh) was used to assess habitual sleep quality during pretesting (9). The Pittsburg Sleep Quality Index scoring (global scores 0–21 points; higher scores indicate worse sleep quality) was used to assess habitual sleep quality during pretesting. Baseline characteristics between groups were compared using Student’s $t$-test. Time-dependent variables (i.e., plasma metabolite concentrations, plasma enrichments, and whole-body protein kinetics) were analyzed by a two-factor repeated-measures ANOVA with time as a within-subjects factor and treatment group as a between-subjects factor. The analysis was carried out for the period starting at the time of protein administration, between $t = 0$ and 450 min. Non-time-dependent variables (i.e., FSR values) were compared between treatment groups using Student’s $t$-tests. Statistical significance was set at $P < 0.05$. All calculations were performed using SPSS 21.0 (SPSS Inc., Chicago, IL).

**RESULTS**

**Plasma analysis.** Plasma glucose and insulin concentrations are shown in (see Figure, Supplemental Digital Content 1, plasma glucose and insulin concentrations, http://links.lww.com/MSS/A759). Both plasma glucose and plasma insulin concentrations were slightly lower in the PRO-EX versus PRO group throughout the overnight period (treatment effect: $P < 0.05$). However, changes in plasma glucose and plasma insulin over time were not different between groups (time–treatment interaction: $P = 0.476$). Plasma insulin concentrations peaked at 30 min after protein ingestion at 9.1 ± 2.2 and 14.3 ± 1.2 mU·L$^{-1}$ in the PRO and PRO + EX treatment, respectively.

Plasma phenylalanine (A), leucine, and tyrosine concentrations are displayed in Figure 2A–C. Plasma amino acid concentrations increased over time, with no significant differences in plasma phenylalanine and leucine concentrations between treatments (time–treatment interaction: $P > 0.05$). By contrast, a significant time–treatment interaction was found for plasma tyrosine ($P = 0.005$).

Figure 3 shows the plasma L-[ring-2H5]-phenylalanine, L-[1-13C]-leucine, and L-[1-13C]-phenylalanine enrichments (MPE). Plasma tracer enrichments did not differ between treatments before ingesting the protein ($t = 0$ min, $P > 0.05$). After protein ingestion, plasma L-[ring-2H5]-phenylalanine and L-[1-13C]-leucine enrichments increased slightly over time, but this increase over time did not differ between treatments (time–treatment interaction, $P > 0.05$). Plasma L-[1-13C]-phenylalanine enrichments, originating from the ingested protein, increased in both treatments, reaching maximal values of 9.39 ± 0.42 MPE at $t = 90$ min in PRO and 9.89 ± 0.44 MPE at $t = 150$ min in the PRO + EX treatment, and remained elevated above basal levels for the duration of the night with no differences over time between treatments (time–treatment interaction: $P = 0.365$).

**Whole-body amino acid kinetics.** Plasma amino acid kinetics data are presented in (see Figure, Supplemental Digital Content 2, plasma amino acid kinetics, http://links.lww.com/ MSS/A760). Exogenous phenylalanine appearance rates increased with time in bed, and wake bouts.

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after protein ingestion with no differences between treatments (time–treatment interaction: $P = 0.334$). Endogenous phenylalanine appearance rates declined after protein ingestion, with no differences between treatments (time–treatment interaction: $P = 0.179$). During the 7.5-h postprandial period, 59.0% ± 1.8% and 55.8% ± 1.7% (PRO and PRO + EX, respectively) of the ingested dietary protein-bound phenylalanine appeared in the plasma circulation, with no differences between treatments ($P = 0.205$).

Whole-body protein metabolism data are expressed in Figure 4. Protein ingestion before sleep resulted in positive overnight whole-body protein net balance with no differences observed between treatments (PRO: 51.1 ± 1.5 vs PRO + EX: 53.0 ± 1.9 μmol phenylalanine·kg$^{-1}$·per 7.5 h, $P = 0.436$). Furthermore, resistance-type exercise did not appear
to further influence any other parameters of whole-body protein metabolism.

**Myofibrillar protein FSR and protein-bound enrichments.** Myofibrillar protein FSR based on L-[ring-2H₅]-phenylalanine enrichment and plasma L-[ring-2H₅]-phenylalanine infusion with plasma L-[ring-2H₅]-phenylalanine as precursor (A, FSR in % h⁻¹) or using L-[1-¹³C]-leucine ingestion and infusion with plasma L-[1-¹³C]-leucine enrichments as precursor (B, FSR in % h⁻¹) are displayed in Figure 5. Exercise before protein ingestion before sleep resulted in greater stimulation of overnight myofibrillar FSR calculated based on L-[ring-2H₅]-phenylalanine (0.055% h⁻¹ ± 0.002% h⁻¹ vs 0.040% h⁻¹ ± 0.003% h⁻¹, P < 0.001) as well as L-[1-¹³C]-leucine (0.073% h⁻¹ ± 0.004% h⁻¹ vs 0.055% h⁻¹ ± 0.006% h⁻¹, P = 0.024). Overnight FSR were 37% or 31% higher in the PRO + EX compared with the PRO treatment, based on L-[ring-2H₅]-phenylalanine and L-[1-¹³C]-leucine infusion, respectively.

Myofibrillar L-[1-¹³C]-phenylalanine enrichments (MPE) increased after the ingestion of intrinsically L-[1-¹³C]-phenylalanine-labeled protein, reaching values of 0.026 ± 0.003 versus 0.015 ± 0.003 MPE in the PRO + EX and PRO treatment, respectively (P = 0.012; Fig. 5C).

**Sleep data.** Sleep analysis data are presented in (see Table, Supplemental Digital Content 3, sleep analysis data http://links.lww.com/MSS/A792). Total sleep duration did not differ between the treatments (PRO: 6:05 ± 0:10 vs. PRO + EX: 6:00 ± 0:11 [h:mm], P = 0.794). In addition, there were no significant differences between the treatments in sleep time, wake time, sleep onset latency (the period between bedtime and sleep start), or sleep efficiency (% sleep duration expressed as a percentage of time in bed).

**DISCUSSION**

The present study demonstrates that protein ingested before sleep is properly digested and absorbed, with ~55% of the ingested protein-derived amino acids appearing in the systemic circulation throughout overnight sleep. Resistance-type exercise performed earlier in the evening increases myofibrillar protein synthesis rates during overnight sleep and improves the efficiency by which the presleep dietary protein-derived amino acids are directed toward overnight de novo myofibrillar protein synthesis.

In the present study, we combined the ingestion of specifically produced intrinsically L-[1-¹³C]-phenylalanine-labeled...
protein with continuous intravenous infusion of L-\([\text{ring-}^{2}\text{H}_3]\)-phenylalanine to allow us to assess dietary protein digestion and amino acid absorption kinetics during overnight sleep. Ingestion of 30 g casein protein was followed by proper protein digestion and subsequent amino acid absorption, as indicated by the postprandial increase in plasma phenylalanine concentrations (Fig. 2A) and L-\([^{13}\text{C}]\)-phenylalanine enrichments (Fig. 3C). Exogenous dietary protein-derived phenylalanine appearance rates remained elevated throughout overnight sleep (see Figure, Supplemental Digital Content 2, plasma amino acid kinetics, http://links.lww.com/MSS/A760), with 57\% ± 1\% of the ingested protein-derived amino acids being released in the circulation during the 7.5-h overnight period. These data are in line with previous observations showing ~50\% of ingested protein becoming available in the systemic circulation during a 5- to 7-h postprandial period (11,12,24). We extend on our previous work, with the observation that resistance-type exercise performed earlier in the evening does not impair the digestion and absorption of protein ingested before sleep.

We have previously shown that ingestion of 40 g protein before sleep increases whole-body protein synthesis, resulting in a positive protein net balance during overnight sleep (24). In the current study, we observed that a more moderate 30 g protein dose likewise improves whole-body protein synthesis rates and allows for a positive whole-body protein net balance (Fig. 4). Prior resistance-type exercise did not further modulate overnight whole-body protein synthesis, breakdown, amino acid oxidation, or net balance after presleep protein ingestion (Fig. 4). As whole-body protein kinetics do not necessarily reflect skeletal muscle metabolism, we also collected muscle biopsies before and after overnight sleep to assess the effect of resistance-type exercise on the overnight muscle protein synthetic response to presleep protein feeding.

As hypothesized, resistance-type exercise augmented overnight skeletal muscle reconditioning, with muscle protein synthesis rates being 37\% (L-\([\text{ring-}^{2}\text{H}_3]\]-phenylalanine; Fig. 5A) and 31\% (L-\([^{13}\text{C}]\)-leucine; Fig. 5B) higher in the exercise compared with the nonexercise control group. These data are in line with the greater anabolic response to protein ingestion after exercise observed in the morning after an overnight fast (21,25,31). The present study extends on these findings with the observation that resistance-type exercise increases the overnight muscle protein synthetic response to presleep protein ingestion. In addition to the applied continuous infusions of L-\([\text{ring-}^{2}\text{H}_3]\)-phenylalanine and L-\([^{13}\text{C}]\)-leucine to measure myofibrillar protein synthesis rates, the ingestion of highly enriched (>35\%) intrinsically L-\([^{13}\text{C}]\)-phenylalanine-labeled protein allowed us to also directly assess the metabolic fate of the presleep dietary protein-derived amino acids. We were able to measure L-\([^{13}\text{C}]\)-phenylalanine enrichments in myofibrillar protein after presleep protein ingestion (Fig. 5C), demonstrating that the presleep protein-provided amino acids as precursors for de novo myofibrillar protein synthesis during overnight sleep. Substantially more (76\%) more of the dietary protein-derived phenylalanine was incorporated in myofibrillar protein when resistance-type exercise was performed earlier in the evening. These data indicate that prior resistance-type exercise increases the efficiency by which presleep protein-derived amino acids are directed toward de novo muscle protein synthesis during overnight sleep.

We have previously shown that protein ingestion before sleep increases muscle mass and strength gains during a 12-wk resistance-type exercise training program (26). Our current data show that resistance-type exercise performed earlier in the evening enhances the muscle protein synthetic response to presleep protein ingestion. Therefore, protein ingestion before sleep represents an effective strategy to augment overnight skeletal muscle reconditioning and is even more relevant on exercise training days.

In conclusion, resistance-type exercise 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 myofibrillar protein synthesis during overnight sleep. Combining presleep protein ingestion with resistance-type exercise represents an effective strategy to maximize overnight skeletal muscle reconditioning.

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