

Protein Ingestion before Sleep Improves Postexercise Overnight Recovery

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ABSTRACT

RES, P. T., B. GROEN, B. PENNING, M. BEELEN, G. A. WALLIS, A. P. GIJSEN, J. M. G. SENDEN, and L. J. C. VAN LOON. Protein Ingestion before Sleep Improves Postexercise Overnight Recovery. *Med. Sci. Sports Exerc.*, Vol. 44, No. 8, pp. 1560–1569, 2012. **Introduction:** The role of nutrition in modulating postexercise overnight recovery remains to be elucidated. We assessed the effect of protein ingestion immediately before sleep on digestion and absorption kinetics and protein metabolism during overnight recovery from a single bout of resistance-type exercise. **Methods:** Sixteen healthy young males performed a single bout of resistance-type exercise in the evening (2000 h) after a full day of dietary standardization. All subjects were provided with appropriate recovery nutrition (20 g of protein, 60 g of CHO) immediately after exercise (2100 h). Thereafter, 30 min before sleep (2330 h), subjects ingested a beverage with (PRO) or without (PLA) 40 g of specifically produced intrinsically [^{1-¹³C}]phenylalanine-labeled casein protein. Continuous intravenous infusions with [ring-²H₅]phenylalanine and [ring-²H₂]tyrosine were applied with blood and muscle samples collected to assess protein digestion and absorption kinetics, whole-body protein balance and mixed muscle protein synthesis rates throughout the night (7.5 h). **Results:** During sleep, casein protein was effectively digested and absorbed resulting in a rapid rise in circulating amino acid levels, which were sustained throughout the remainder of the night. Protein ingestion before sleep increased whole-body protein synthesis rates (311 ± 8 vs 246 ± 9 $\mu\text{mol}\cdot\text{kg}^{-1}$ per 7.5 h) and improved net protein balance (61 ± 5 vs -11 ± 6 $\mu\text{mol}\cdot\text{kg}^{-1}$ per 7.5 h) in the PRO vs the PLA experiment ($P < 0.01$). Mixed muscle protein synthesis rates were $\sim 22\%$ higher in the PRO vs the PLA experiment, which reached borderline significance ($0.059\% \cdot \text{h}^{-1} \pm 0.005\% \cdot \text{h}^{-1}$ vs $0.048\% \cdot \text{h}^{-1} \pm 0.004\% \cdot \text{h}^{-1}$, $P = 0.05$). **Conclusions:** This is the first study to show that protein ingested immediately before sleep is effectively digested and absorbed, thereby stimulating muscle protein synthesis and improving whole-body protein balance during postexercise overnight recovery. **Key Words:** SLEEP, RECOVERY, EXERCISE, MUSCLE, STABLE ISOTOPES, CASEIN

Resistance-type exercise training represents an effective strategy to augment muscle protein accretion (13,31). A single session of resistance-type exercise stimulates both protein synthesis and breakdown rates (6,31,37). Although resistance-type exercise stimulates protein synthesis to a greater extent than protein degradation, protein balance will remain negative in the absence of nutrient intake (6,8,31). Consequently, both exercise and nutrition are required to obtain a positive protein balance, allowing muscle hypertrophy to occur. CHO ingestion during postexercise recovery attenuates the exercise-induced

increase in protein breakdown (11,28) but does not affect protein synthesis rates (10,21,28). Intake of protein and/or amino acids is required to stimulate postexercise protein synthesis thereby allowing protein balance to become positive (8,12,23,28,32,36). In accordance, dietary protein administration with or without CHO before (35,38), during (3), and/or immediately after exercise (23,28,32,35,38) has been shown to stimulate net muscle protein accretion during postexercise recovery.

In general, most studies assess the effect of food intake on the muscle protein synthetic response to exercise in an overnight fasted state (10,12,21,23,26,28,32,35,36). Under these conditions, it seems reasonable to assume that the limited endogenous availability of amino acids from the gut and/or the intramuscular free amino acid pool prevents a substantial rise in postexercise muscle protein synthesis rate. Such postabsorptive conditions differ from normal everyday practice in which recreational sports activities are often performed in postprandial conditions, such as in the evening. Recently, we evaluated the effect of exercise in the evening and the efficacy of protein ingestion immediately after exercise on protein synthesis during subsequent overnight

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recovery (4). We observed an increase in protein synthesis during the first few hours of postexercise recovery when protein was ingested. However, muscle protein synthesis rates during subsequent overnight sleep were found to be unexpectedly low with values being even lower than those observed in the normal basal postabsorptive state (4). It was speculated that the lack of an increase in plasma amino acid and/or insulin concentration prevents muscle protein synthesis rates to be elevated throughout the night.

The present study was undertaken to assess whether provision of dietary protein before sleep leads to adequate dietary protein digestion and absorption thereby increasing plasma amino acid availability. In addition, we assessed whether such a proposed increase in plasma amino acid availability throughout the night would stimulate protein synthesis and/or attenuate protein breakdown, thereby improving protein balance during overnight recovery from exercise. It was hypothesized that the provision of dietary protein before sleep could be an effective nutritional intervention to increase plasma amino acid availability, stimulate skeletal muscle protein synthesis, and, as such, improve whole-body protein balance during overnight sleep.

Therefore, 16 recreationally active young males were studied during overnight recovery from a single bout of resistance-type exercise performed in the evening after a full day of dietary standardization. Subjects were provided with a bolus of protein or placebo (PLA) immediately before sleep after which protein digestion and absorption kinetics and subsequent overnight muscle protein synthesis rates were assessed during sleep. For this purpose, a specifically produced intrinsically [$1-^{13}\text{C}$]phenylalanine-labeled protein was provided. Combined with continuous intravenous infusion of [ring- $^2\text{H}_5$]phenylalanine and [ring- $^2\text{H}_2$]tyrosine, this allows direct assessment of *in vivo* dietary protein digestion and absorption kinetics, whole-body protein balance, and muscle protein synthesis rates during overnight sleep. This is the first study to assess the effect of protein ingestion immediately before sleep as a dietary strategy to stimulate muscle protein synthesis and, as such, to improve protein balance during postexercise overnight recovery.

METHODS

Subjects. A total of 16 recreationally active men were selected to participate in this study. One trial in the PLA experiment had to be excluded because of a problem with a catheter that obstructed isotope infusion. Subjects were randomly assigned to either the protein (PRO: $n = 8$) or the placebo experiment (PLA: $n = 7$) experiment. Subjects' characteristics are presented in Table 1. All subjects were fully informed on the nature and possible risks of the experimental procedures before their written informed consent was obtained. The study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre, The Netherlands.

Pretesting. All subjects participated in two screening sessions, separated by at least 7 d. Body weight was mea-

TABLE 1. Subjects' characteristics.

	PRO ($n = 8$)	PLA ($n = 7$)
Age (yr)	22.9 \pm 0.7	22.4 \pm 0.8
Height (m)	1.81 \pm 0.02	1.84 \pm 0.02
Weight (kg)	74.2 \pm 2.3	76.3 \pm 2.6
BMI ($\text{kg}\cdot\text{m}^{-2}$)	22.7 \pm 0.6	22.5 \pm 0.8
Body fat (%)	13.7 \pm 0.9	13.0 \pm 0.9
Trunk fat (%)	11.8 \pm 0.8	11.5 \pm 0.9
Leg fat (%)	15.2 \pm 0.9	13.8 \pm 0.9
Lean mass (kg)	63.9 \pm 1.6	66.4 \pm 2.3
Leg volume (L)	9.0 \pm 0.4	9.5 \pm 0.3
1RM leg press (kg)	201 \pm 9	220 \pm 15
1RM leg extension (kg)	133 \pm 6	132 \pm 9
Physical activity ($\text{h}\cdot\text{wk}^{-1}$)	6.3 \pm 1.1	5.2 \pm 1.0

Values are expressed as means \pm SEM.

PRO, protein experiment; PLA, placebo experiment; BMI, body mass index.

sured with a digital balance with an accuracy of 0.1 kg (Seca GmbH, Hamburg, Germany). Body composition (fat and fat-free mass) was determined by a dual-energy x-ray absorptiometry scan (Hologic, Inc., Bedford, MA). With the dual-energy x-ray absorptiometry scan, the lean mass and percent body fat were determined on a whole-body level and for specific regions (trunk and legs). Leg volume was determined by anthropometry as described by Jones and Pearson (20). All subjects participated in a familiarization trial where a safe lifting technique was demonstrated and practiced for the two exercises (leg press and leg extension). Thereafter, maximum strength was estimated using the multiple-repetition testing procedure (27). In another session, at least 1 wk before the first experimental trial, subjects' one repetition maximum (1RM) was determined (25). After warming up, the load was set at 97.5% of the estimated 1RM and increased after each successful lift until failure. Five-minute rest periods were allowed between attempts. A repetition was regarded valid when the subject used proper form and was able to complete the entire lift in a controlled manner without assistance.

Diet and physical activity. All subjects received the same standardized meal the evening before the test and a standardized diet throughout the subsequent experimental day ($0.16 \text{ MJ}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, consisting of 57 Energy percentage (En%) CHO, 13 En% protein, and 30 En% fat). The energy content of the standardized diet was based upon energy requirements estimated using the Harris-Benedict equation (18) and adjusted using a physical activity factor of 1.6 to ensure ample energy intake (29). Breakfast, lunch, and dinner were provided at the laboratory and were eaten under supervision. During the experimental day, participants ingested $1.2 \text{ g}\cdot\text{kg}^{-1}$ protein via the standardized diet with an additional 40 g ($0.54 \pm 0.01 \text{ g}\cdot\text{kg}^{-1}$) of protein provided in the PRO experiment. All subjects were instructed to refrain from exhaustive physical labor and exercise and to keep their diet as constant as possible 2 d before the experimental day. Food intake and physical activity questionnaires were collected for 2 d before the experiment.

Experimental protocol. An overview of the experimental protocol is given in Figure 1. At 1630 h, subjects reported to the laboratory where, at 1645 h, a standardized

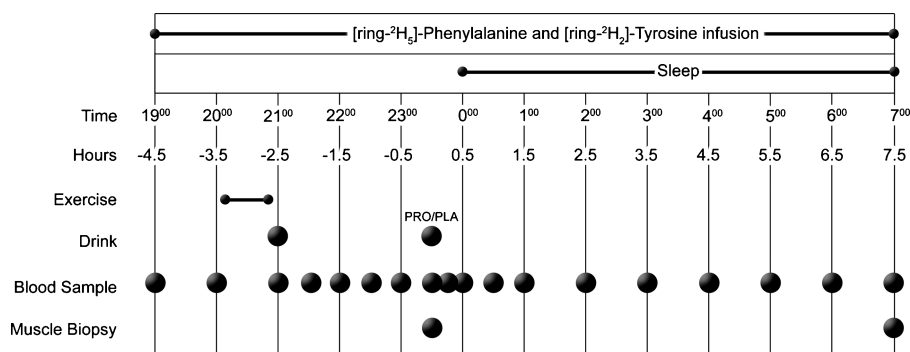


FIGURE 1—Protocol for the measurement of the effect of protein ingestion before sleep on subsequent overnight recovery. A drink with 60 g of CHO and 20 g of whey protein was provided immediately after exercise. Subjects were randomly assigned to receive either 40 g of casein protein or placebo (water only) at 2330 h.

meal was provided. At 1830 h, a Teflon catheter was inserted into an antecubital vein for the primed continuous infusion of [ring-²H₅]phenylalanine and [ring-²H₂]tyrosine. A second Teflon catheter was inserted in the contralateral hand vein for arterialized blood sampling. Blood was arterialized by placing the hand in a hotbox (1), and a resting/background blood sample was drawn. After a background blood sample was collected ($t = -4.5$ h), the primed continuous tracer infusion was started (1900 h), and subjects rested in a supine position for 1 h. HR was monitored using a portable HR monitor (Polar Electro Oy). The exercise protocol was started at 2000 h and consisted of a bilateral lower limb resistance-type exercise workout with eight sets of eight repetitions on the horizontal leg press machine (Technogym BV, Rotterdam, The Netherlands) and eight sets of eight repetitions on the leg extension machine (Technogym BV). On both machines, one set was performed at 55% of the individual 1RM, one was performed at 65%, and six were performed at 75%. There was a resting period of 2 min between sets and a 5-min rest between exercises. The entire protocol required ~45 min to complete. All subjects were verbally encouraged during the test to complete the whole protocol. Water was provided *ad libitum* during exercise. After the exercise protocol (2100 h, $t = -2.5$ h), subjects remained in a supine position, and a blood sample from the arterialized hand vein was obtained. Drinks providing 60 g of CHO and 20 g of whey protein (Lucozade Sport Body Fuel and Lucozade Sport Recovery; GlaxoSmithKline Nutrition, Brentford, United Kingdom) were ingested immediately after exercise. At 2330 h, a muscle biopsy from the vastus lateralis muscle was taken, after which subjects received the PRO or the PLA drink. Subsequently, subjects remained in a supine position until 2400 h. Thereafter, subjects slept for 7 h, after which participants were awoken the next morning at 0700 h for a second muscle biopsy. Blood samples (8 mL) were taken from the arterialized hand vein at $t = -4.5, -3.5, -2.5, -2, -1.5, -1, -0.5,$ and 0 h; 15, 30, and 60 min; and 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 h after test drink ingestion. Muscle biopsies were taken at $t = 0$ and 7.5 h after PRO or PLA ingestion.

PRO administration. Subjects ingested 450 mL water with (PRO) or without (PLA) 40 g of intact intrinsically [1-¹³C]phenylalanine-labeled casein protein. Intrinsically [1-¹³C]phenylalanine-labeled casein protein was obtained by infusing a Holstein cow with large quantities of [1-¹³C]phenylalanine, collecting milk, and purifying the casein fraction as described previously (39). The [1-¹³C]phenylalanine enrichment in the casein fraction averaged 6.1 mole percent excess (MPE). The casein protein met all chemical and bacteriological specifications for human consumption. All solutions were flavored by adding strawberry flavor and artificial sweetener (GlaxoSmithKline).

Stable isotope tracers. The stable isotope tracers [ring-²H₅]phenylalanine and [ring-²H₂]tyrosine were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA), and dissolved in 0.9% saline before infusion. Continuous intravenous infusion (during a period of 12 h, $0.06 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of [ring-²H₅]phenylalanine, $0.023 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of [ring-²H₂]tyrosine) of the labeled isotopes was performed using a calibrated IVAC 598 pump (San Diego, CA). Both the phenylalanine and tyrosine pools were primed ($2.4 \mu\text{mol}\cdot\text{kg}^{-1}$ of [ring-²H₅]phenylalanine, $0.925 \mu\text{mol}\cdot\text{kg}^{-1}$ of [ring-²H₂]tyrosine) such that whole-body phenylalanine kinetics could be calculated using established tracer models (17,33,34). Infusates were prepared by the pharmacy of the Maastricht University Medical Centre.

Muscle biopsies. Muscle biopsies were obtained from the middle region of the vastus lateralis muscle (15 cm above the patella) and approximately 3 cm below the entry through the fascia with the percutaneous needle biopsy technique described by Bergstrom (5). The first muscle biopsy was taken from the right leg. The biopsy taken 7.5 h after test drink ingestion was collected from the left leg. All muscle samples were carefully freed from any visible fat and blood, rapidly frozen in liquid nitrogen, and stored at -80°C until subsequent analysis.

Plasma analysis. Blood samples (8 mL) were collected in EDTA-containing tubes and centrifuged at 1000g and 4°C for 10 min. Aliquots of plasma were frozen in liquid nitrogen and stored at -80°C until analysis. Plasma glucose

concentrations were analyzed with the Cobas Fara semiautomatic analyzer (Uni Kit III, 07367204; Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Linco Human Insulin RIA kit; Linco Research, Inc., St. Charles, MO). Plasma (100 μ L) for amino acid analyses was deproteinized on ice with 10 mg of dry 5-sulfosalicylic acid and mixed, and the clear supernatant was collected after centrifugation. Plasma amino acid concentrations were determined by High Performance Liquid Chromatography, after precolumn derivatization with o-phthalaldehyde. For plasma [$1-^{13}\text{C}$]phenylalanine, [$1-^{13}\text{C}$]tyrosine, [ring- $^2\text{H}_5$]phenylalanine, [ring- $^2\text{H}_4$]tyrosine, and [ring- $^2\text{H}_2$]tyrosine enrichment measurements, plasma phenylalanine and tyrosine were derivatized to their t-butyl dimethylsilyl derivatives, and their ^{13}C and/or ^2H enrichments were determined by electron ionization gas chromatography–mass spectrometry (Agilent 6890N GC/5973N MSD; Little Falls, NJ) using selected ion monitoring of masses 336, 337, and 341 for unlabeled and [$1-^{13}\text{C}$] and [ring- $^2\text{H}_5$]–labeled phenylalanine, respectively. We applied standard regression curves in all isotopic enrichment analyses to assess linearity of the mass spectrometer and to control for the loss of tracer. Enrichments (MPE) were calculated according to Biolo et al. (7) to correct for the presence of both the [$1-^{13}\text{C}$] and [ring- $^2\text{H}_5$]phenylalanine isotopes.

Muscle analyses. For measurement of [ring- $^2\text{H}_5$]phenylalanine enrichment in mixed muscle protein, 55 mg of wet muscle was freeze dried. Collagen, blood, and other non-muscle fiber material were removed from the muscle fibers under a light microscope. The isolated muscle fiber mass (2–3 mg) was weighed, and eight volumes ($8 \times$ dry weight of isolated muscle fibers \times wet–dry ratio) of ice-cold 2% perchloric acid were added. The tissue was then homogenized and centrifuged. The PRO pellet was washed with three additional 1.5-mL washes of 2% perchloric acid, dried, and hydrolyzed in 6-M HCl at 120°C for 15–18 h. The hydrolyzed protein fraction was dried under a nitrogen stream while it was heated to 120°C; then, a 50% acetic acid solution was added, and the hydrolyzed protein was passed over a Dowex exchange resin (AG 50W-X8, 100–200 mesh hydrogen form; Biorad, Hercules, CA) using 2 M of NH_4OH . The eluate was divided over two vials for the measurement of [ring- $^2\text{H}_5$]phenylalanine enrichment in mixed muscle protein. The latter was determined by derivatizing [ring- $^2\text{H}_5$]phenylalanine to N-(tert-butyl dimethylsilyl)-N-methyltrifluoroacetamide-phenyl-ethylamine. Thereafter, the ratios of labeled/unlabeled derivatives were determined by gas chromatography–mass spectrometry. Standard regression curves were applied to assess linearity of the mass spectrometer and to control for loss of tracer. The coefficient of variance for the measurement of [ring- $^2\text{H}_5$]phenylalanine enrichment in mixed muscle protein averaged $1.0\% \pm 0.1\%$.

Calculations. Ingestion of [$1-^{13}\text{C}$]phenylalanine and intravenous infusion of [ring- $^2\text{H}_5$]phenylalanine and [ring- $^2\text{H}_2$]tyrosine with muscle and arterialized blood sampling were used to simultaneously assess whole-body amino acid

kinetics and the fractional synthetic rate (FSR) of mixed muscle protein. Total, exogenous, and endogenous phenylalanine rate of appearance (R_a) and plasma availability of dietary phenylalanine (i.e., the fraction of dietary phenylalanine that appeared in the systemic circulation ($\text{Phe}_{\text{plasma}}$)) were calculated using modified Steele equations adapted for stable isotope methodology (16,34). These parameters were calculated as follows:

$$R_a = \frac{F - pV[(C_2 + C_1) / 2] [(E_{iv2} - E_{iv1}) / (t_2 - t_1)]}{(E_{iv2} + E_{iv1}) / 2}$$

$$\text{Exo } R_a = \frac{\text{Total } R_a [(E_{po2} + E_{po1}) / 2] + V[(E_{po2} - E_{po1}) / (t_2 - t_1)]}{E_{prot}}$$

$$\text{Endo } R_a = \text{Total } R_a - \text{Exo } R_a - F$$

$$\text{Phe}_{\text{plasma}} = 100 \left(\frac{\text{AUC}_{\text{ExoPhe}R_a}}{\text{Phe}_{\text{prot}}} \right)$$

where F is the infusion rate ($\mu\text{mol} \cdot \text{kg}^{-1}$ body weight $\cdot \text{min}^{-1}$), pV is the distribution volume for phenylalanine ($125 \text{ mL} \cdot \text{kg}^{-1}$) (17), C_1 and C_2 are the phenylalanine concentrations ($\text{mmol} \cdot \text{L}^{-1}$), and E_{iv1} and E_{iv2} are the plasma [ring- $^2\text{H}_5$]phenylalanine enrichments (expressed in MPE) in arterialized plasma at times 1 (t_1) and 2 (t_2), respectively. $\text{Exo } R_a$ represents the plasma entry rate of dietary phenylalanine, E_{po} is the plasma phenylalanine enrichment for the oral tracer, and E_{prot} is the [$1-^{13}\text{C}$]phenylalanine enrichment in the ingested protein; Phe_{prot} is the amount of phenylalanine ingested, and $\text{AUC}_{\text{ExoPhe}R_a}$ represents the area under the curve of $\text{ExoPhe}R_a$, which corresponds to the amount of dietary phenylalanine that appeared in the blood during a 7.5-h period after protein ingestion.

The total rate of disappearance (R_d) of phenylalanine equals the rate of phenylalanine hydroxylation and utilization for protein synthesis. These parameters can be calculated as follows:

$$R_d = R_a - V \left(\frac{C_2 - C_1}{t_2 - t_1} \right)$$

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 [ring- $^2\text{H}_5$]phenylalanine to [ring- $^2\text{H}_4$]tyrosine. The rate of hydroxylation (Q_{pt}) was calculated by using the following formula (33):

$$Q_{pt} = Q_t \left(\frac{E_t}{E_p} \right) \left(\frac{Q_p}{F + Q_p} \right)$$

where Q_t and Q_p are the flux rates for [ring- $^2\text{H}_4$]tyrosine and [ring- $^2\text{H}_5$]phenylalanine, respectively; E_t and E_p are the [ring- $^2\text{H}_4$]tyrosine and [ring- $^2\text{H}_5$]phenylalanine enrichments in plasma, respectively; and F is the infusion rate of phenylalanine.

Whole-body protein synthesis was calculated using the following:

$$\text{PRO synthesis} = \text{Total } R_d - \text{Phe hydroxylation}$$

$$\text{Phe net balance} = \text{PRO synthesis} - \text{Endo } R_d$$

Fractional rate of mixed muscle protein synthesis (FSR) was calculated by dividing the increment in enrichment in the product, i.e., protein-bound [ring- $^2\text{H}_5$]phenylalanine, by the enrichment of the precursor. Muscle FSR ($\% \cdot \text{h}^{-1}$) was calculated as follows (23):

$$\text{FSR} = \frac{\Delta E_p}{E_{\text{precursor}} t} \times 100$$

where ΔE_p is the delta increment of PRO-bound [ring- $^2\text{H}_5$]phenylalanine during incorporation periods, $E_{\text{precursor}}$ is the average plasma [ring- $^2\text{H}_5$]phenylalanine enrichment during the period for determination of amino acid incorporation, and t indicates the time interval (h) between biopsies.

Statistics. All data are expressed as means \pm SEM. ANOVA was used to determine the effects of treatment as a between-subjects factor and time ($t = 0$ –7.5 h) as a within-subjects factor, as well as any interaction between the two.

In case of a significant interaction, group differences were analyzed for each time point separately (i.e., multiple t -tests using Bonferroni correction). For non-time-dependent variables, an unpaired Student's t -test was used to compare differences in between groups. All calculations were performed using SPSS 15.0 (SPSS, Inc., Chicago, IL). Statistical significance was set at $P < 0.05$.

RESULTS

Participants. Subjects' characteristics are presented in Table 1. No differences in age, weight, height, body fat, leg volume, 1RM leg press, 1RM leg extension, and habitual physical activity were observed between groups. All subjects were able to sleep during their overnight stay and reported no problems sleeping. The use of a catheter extension cable allowed us to draw blood samples throughout the night without waking the subjects. Average HR throughout the night did not differ between groups (54 ± 3 and 53 ± 1 bpm in the PRO and PLA experiments, respectively).

Plasma metabolites. Plasma glucose concentrations changed over time in both groups ($P < 0.01$). However, no treatment \times time interactions were observed for plasma glucose concentration. Plasma glucose concentrations throughout the

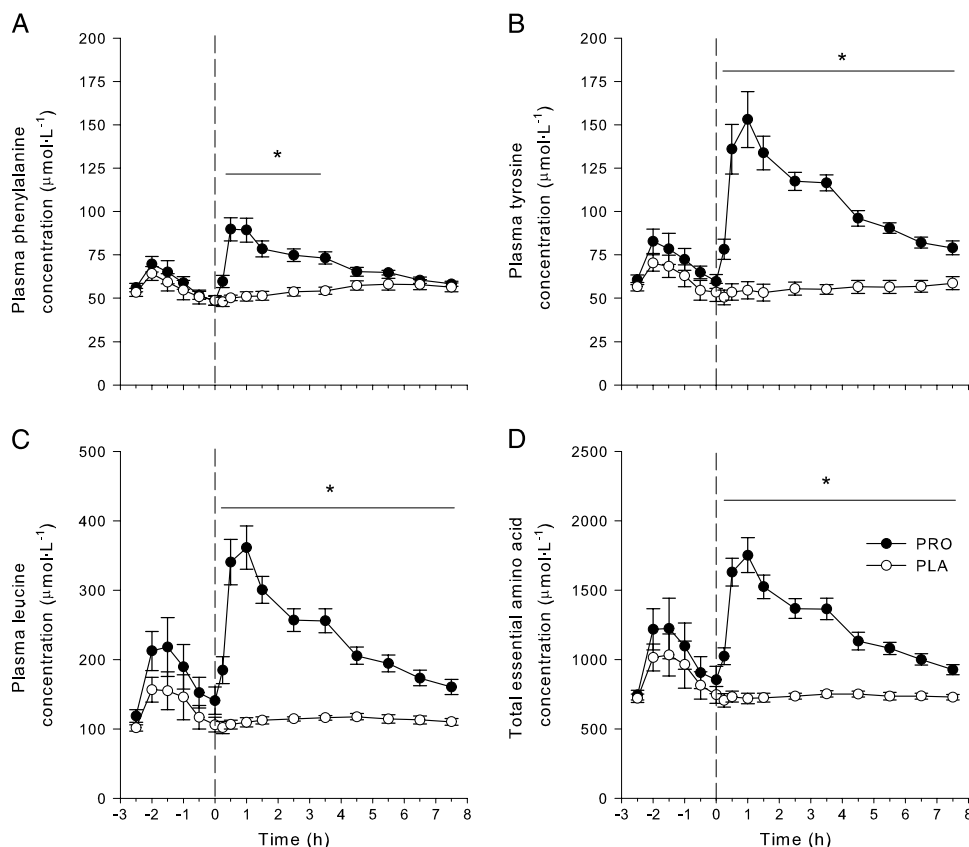


FIGURE 2—Plasma phenylalanine, tyrosine, leucine, and total essential amino acid concentrations. Values are expressed as means \pm SEM. Data were analyzed with repeated-measures ANOVA (treatment \times time). Plasma phenylalanine: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma tyrosine: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma leucine: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma total essential amino acids: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. *Significantly different from PLA ($P < 0.05$). PRO, protein experiment; PLA, placebo experiment.

night averaged 5.1 ± 0.1 and 5.2 ± 0.1 $\text{mmol}\cdot\text{L}^{-1}$ in the PRO and PLA experiments, respectively, with no significant differences between experiments. Plasma insulin levels showed a significant treatment \times time interaction ($P < 0.01$). In the PRO experiment, plasma insulin concentrations were higher at $t = 15$ and 30 min when compared with PLA ($P < 0.01$), with values reaching 20.8 ± 2.1 $\text{mU}\cdot\text{L}^{-1}$. Throughout the night, plasma insulin concentrations did not differ significantly between experiments (12.1 ± 0.9 and 10.8 ± 0.8 $\text{mU}\cdot\text{L}^{-1}$ in the PRO and PLA experiments, respectively). Plasma essential amino acid concentrations increased after ingestion of the recovery nutrition that was provided immediately after cessation of exercise in both groups ($P < 0.01$) after which they returned to baseline values within 150 min. After supplement ingestion before sleep, total plasma essential amino acid concentrations increased rapidly and remained elevated throughout the night in the PRO experiment when compared with the PLA experiment ($P < 0.01$). Plasma essential amino acid levels showed a significant treatment \times time interaction. Plasma phenylalanine, tyrosine, leucine, and total essential amino acid concentrations over time are presented in Figure 2.

Plasma tracer enrichments. Significant time \times treatment interactions were observed for all plasma tracer enrichment data. Plasma [$1\text{-}^{13}\text{C}$]phenylalanine enrichments increased after protein ingestion in the PRO group after which levels remained elevated in the PRO compared with the PLA group throughout the night ($P < 0.05$; Fig. 3A). Plasma [ring- $^2\text{H}_5$]phenylalanine, [ring- $^2\text{H}_4$]tyrosine, and [ring- $^2\text{H}_2$]tyrosine enrichments decreased after protein ingestion in the PRO group. The enrichments of these three tracers were significantly lower in the PRO group compared with the PLA group for 2.5, 3, and 4 h, respectively, after ingestion of the test drinks ($P < 0.05$; Figs. 3B, C, D).

Whole-body PRO metabolism. Whole-body PRO kinetics were calculated for the overnight recovery period. A significant treatment \times time interaction was observed for total phenylalanine rate of appearance, exogenous phenylalanine rate of appearance, endogenous phenylalanine rate of appearance, and total phenylalanine R_d . Total phenylalanine rate of appearance (R_a) was higher in the PRO compared with the PLA group between $t = 0.5$ h and $t = 3.5$ h ($P < 0.05$; Fig. 4A). Exogenous phenylalanine R_a was greater in the PRO compared with the PLA group throughout overnight

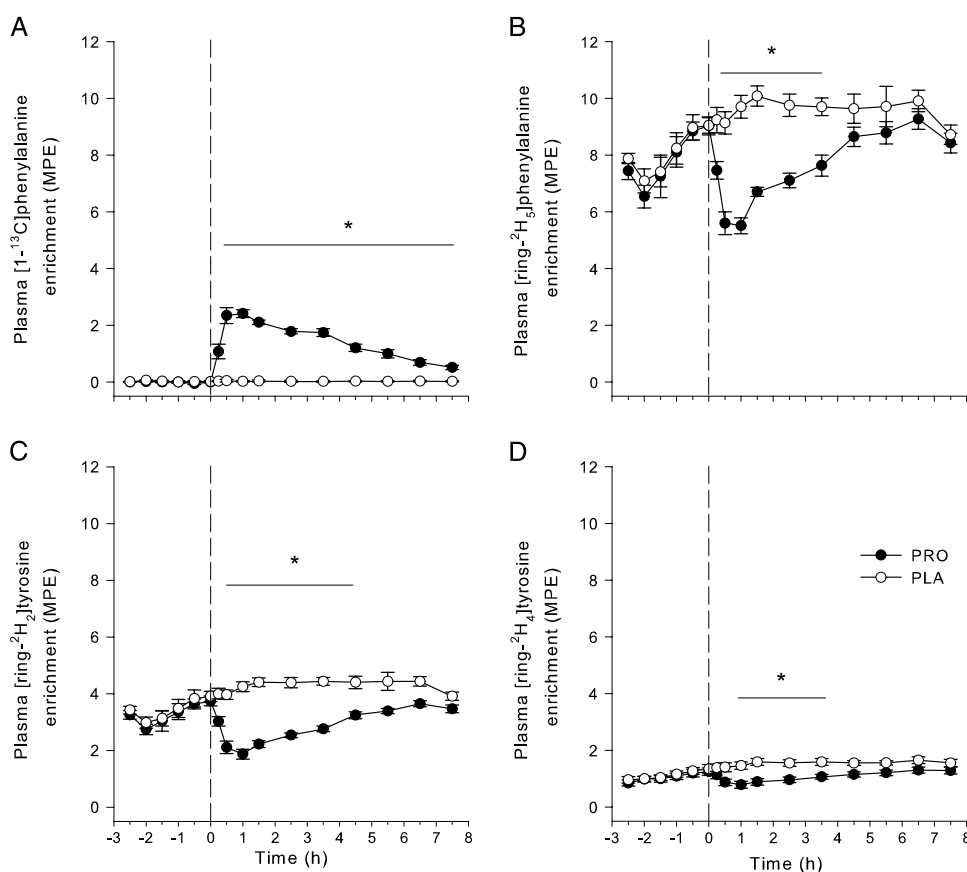


FIGURE 3—Plasma [$1\text{-}^{13}\text{C}$]phenylalanine (A), [ring- $^2\text{H}_5$]phenylalanine (B), [ring- $^2\text{H}_2$]tyrosine (C), and [ring- $^2\text{H}_4$]tyrosine (D) enrichments. Values are expressed as means \pm SEM. Data were analyzed with repeated-measures ANOVA (treatment \times time). Plasma [$1\text{-}^{13}\text{C}$]phenylalanine enrichment: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma [ring- $^2\text{H}_5$]phenylalanine enrichment: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma [ring- $^2\text{H}_2$]tyrosine enrichment: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma [ring- $^2\text{H}_4$]tyrosine enrichment: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. *Significantly different from PLA ($P < 0.05$). PRO, protein experiment; PLA, placebo experiment.

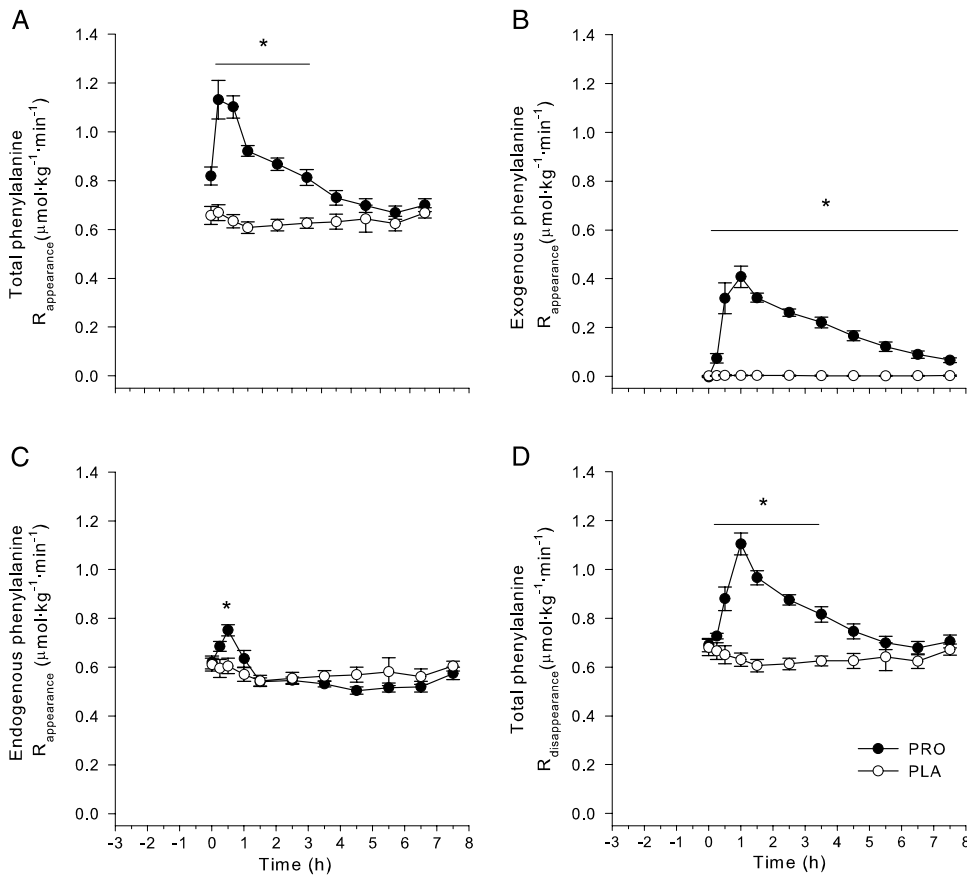


FIGURE 4—Total phenylalanine rate of appearance (R_a) (A), exogenous phenylalanine R_a (B), endogenous phenylalanine R_a (C), and total phenylalanine R_d (D). Values represent means \pm SEM. Data were analyzed with repeated-measures ANOVA (treatment \times time). Total plasma phenylalanine R_a : treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Exogenous plasma phenylalanine R_a : treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Endogenous plasma phenylalanine R_a : treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma phenylalanine R_d : treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. *Significantly different from PLA ($P < 0.05$). PRO, protein experiment; PLA, placebo experiment.

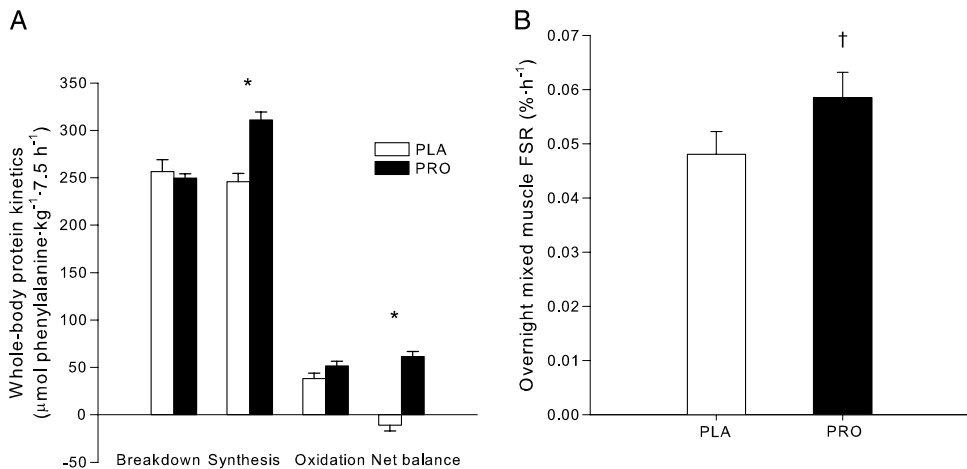


FIGURE 5—A, Rates of whole-body protein breakdown, synthesis, and oxidation rates and net protein balance (expressed as micromoles of phenylalanine per kilogram per 7.5 h) in the PRO and PLA experiments measured during the 7.5 h of overnight recovery. B, Mixed muscle protein FSR during overnight recovery (0–7.5 h) in the PRO and PLA experiments using average plasma [ring- $^2\text{H}_5$]phenylalanine enrichment as a precursor. A tendency toward higher FSR values in the PRO versus the PLA experiment was observed during overnight recovery. Values represent means \pm SEM. Data were analyzed with an unpaired Student's t -test. *Significantly different from PLA ($P < 0.05$); †different from PLA ($P = 0.05$). PRO, protein experiment; PLA, placebo experiment.

recovery ($P < 0.05$; Fig. 4B). In the PRO group, $50\% \pm 2\%$ of the ingested protein had become available as plasma-derived amino acids during the 7.5-h postprandial phase. Endogenous phenylalanine R_a was higher in the PRO compared with the PLA group at $t = 0.5$ h ($P < 0.05$; Fig. 4C). Total phenylalanine R_d was higher in the PRO compared with the PLA group between $t = 0.5$ h and $t = 3.5$ h ($P < 0.05$; Fig. 4D). Whole-body phenylalanine hydroxylation rates were similar between groups. Whole-body protein synthesis rates were higher in the PRO versus the PLA experiment resulting in a net PRO balance of $+61 \pm 5$ versus $-11 \pm 6 \mu\text{mol}\cdot\text{kg}^{-1}$ per 7.5 h, respectively ($P < 0.01$; Fig. 5A).

Muscle analyses. Mixed muscle PRO FSR, with mean plasma [ring- $^2\text{H}_5$]phenylalanine enrichment as the precursor, was calculated for the 7.5 h of overnight sleep (Fig. 5B). Muscle FSR during overnight recovery showed $\sim 22\%$ higher values in the PRO compared with the PLA experiment, which reached borderline significance ($0.059\%\cdot\text{h}^{-1} \pm 0.005\%\cdot\text{h}^{-1}$ vs $0.048\%\cdot\text{h}^{-1} \pm 0.004\%\cdot\text{h}^{-1}$, respectively, $P = 0.05$).

DISCUSSION

The present study examined the effect of dietary protein ingestion immediately before sleep on dietary protein digestion and absorption kinetics and subsequent protein metabolism during overnight recovery from a single bout of resistance-type exercise. The main findings are twofold: dietary protein ingested immediately before sleep 1) is effectively digested and absorbed, thereby increasing overnight plasma amino acid availability, and 2) stimulates muscle protein synthesis rates, thereby improving overnight protein balance.

We hypothesized that dietary protein ingested immediately before sleep is effectively digested and absorbed, thereby increasing plasma amino acid availability throughout the night. To assess this, we applied specifically produced intrinsically [$1\text{-}^{13}\text{C}$]phenylalanine-labeled casein (39). This allowed the assessment of dietary protein digestion and absorption kinetics *in vivo* in humans (24,39). Consistent with our hypothesis, we observed a substantial rise in circulating plasma amino acid concentrations after the ingestion of 40 g of intrinsically labeled casein that was sustained throughout overnight recovery (Fig. 2). These plasma amino acids were derived from the ingested protein as is evident from the increase in both plasma phenylalanine concentration (Fig. 2A) and plasma [$1\text{-}^{13}\text{C}$]phenylalanine enrichment level (Fig. 3A). In line with previous findings during daytime (30), we show that about approximately half of the ingested casein becomes available as plasma amino acids throughout overnight recovery. The present study is the first to provide data showing that dietary protein ingested immediately before sleep is digested and absorbed normally throughout the night and, therefore, represents an effective strategy to increase amino acid availability during overnight sleep.

An increase in plasma amino acid concentration has previously been shown to stimulate protein synthesis and in-

hibit protein breakdown, thereby improving net protein balance (12,28,29,36). In line with these observations, we observed substantially higher whole-body protein synthesis rates during the night after dietary protein ingestion before sleep (PRO) when compared with the control experiment (PLA). Because protein ingestion before sleep did not affect whole-body protein breakdown rates, net protein balance became positive in the PRO experiment ($+61 \pm 5$ vs $-11 \pm 6 \mu\text{mol}\cdot\text{kg}^{-1}$ per 7.5 h in the PRO vs the PLA experiment, respectively; Fig. 5A).

Because any change at the level of the skeletal muscle cannot be accurately determined from whole-body protein kinetics, we also assessed muscle protein FSR by collecting muscle biopsies before and after postexercise overnight recovery (Fig. 5B). Overnight FSR values were 22% higher in the PRO compared with the PLA experiment, reaching borderline statistical significance (0.059 ± 0.005 vs 0.048 ± 0.004 , respectively, $P = 0.05$). Because muscle protein synthesis is only stimulated for several hours after an increase in plasma amino acid availability (2,9), it could be speculated that differences in FSR values between the PRO and PLA experiments were greatest during the first 4 h of postprandial sleep. Muscle biopsy sampling between 0300 and 0400 h would have provided us with appropriate muscle free phenylalanine precursor enrichment data and a likely higher muscle protein synthesis rate during this specific time frame. However, muscle biopsy collection during this time frame would not have been possible without waking the subjects. Therefore, we assessed protein balance and muscle protein synthesis rates throughout the overnight period, with plasma phenylalanine enrichments as a reliable precursor pool. Furthermore, we chose a parallel study design, despite its obvious limitations, to prevent confounding from the appearance of [$1\text{-}^{13}\text{C}$]phenylalanine administered during the first experiment on the follow-up experiment in the same individual. Regardless, this study clearly shows that protein ingested immediately before sleep is normally digested and absorbed, thereby stimulating overnight muscle protein synthesis rates. Phillips et al. recently demonstrated that acute postexercise changes in muscle protein synthesis and breakdown rates can be predictive of the adaptive response to more prolonged intervention (19,40). In line with these findings, it will be of importance to assess whether the acute metabolic benefits conferred from the application of dietary protein supplementation immediately before sleep will translate to a greater skeletal muscle adaptive response to more prolonged resistance-type exercise training.

Besides the proposed relevance to optimize skeletal muscle reconditioning during prolonged resistance-type exercise training in a sport-specific application, nighttime protein supplementation might also be of substantial clinical relevance. For example, because the muscle protein synthetic response to food intake has been reported to be blunted in the elderly (14,15,22), it is speculated that nighttime protein provision could prove to be an effective nutritional strategy to combat age-related muscle loss by providing an

additional anabolic stimulus. In general, such a feeding strategy could be of important clinical relevance in many compromised clinical (sub)populations.

In conclusion, this is the first study to show that protein ingested immediately before sleep is effectively digested and absorbed, thereby stimulating muscle protein synthesis and improving whole-body protein balance during postexercise overnight recovery.

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