Intragastric protein administration stimulates overnight muscle protein synthesis in elderly men

Bart B. L. Groen,1 Peter T. Res,1 Bart Pennings,1 Elisabeth Hertle,1 Joan M. G. Senden,1
Wim H. M. Saris,2 and Luc J. C. van Loon1

Departments of 1Human Movement Sciences and 2Human Biology, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Center, Maastricht, The Netherlands

Submitted 1 July 2011; accepted in final form 9 September 2011

Groen BB, Res PT, Pennings B, Hertle E, Senden JM, Saris WH, van Loon LJ. Intragastric protein administration stimulates overnight muscle protein synthesis in elderly men. Am J Physiol Endocrinol Metab 302:E52–E60, 2012. First published September 13, 2011; doi:10.1152/ajpendo.00321.2011.—The loss of skeletal muscle mass with aging has been attributed to an impaired muscle protein synthetic response to food intake. Therefore, nutritional strategies are targeted to modulate postprandial muscle protein accretion in the elderly. The purpose of this study was to assess the impact of protein administration during sleep on in vivo protein digestion and absorption kinetics and subsequent muscle protein synthesis rates in elderly men. Sixteen healthy elderly men were randomly assigned to an experiment during which they were administered a single bolus of intrinsically labeled casein protein (PRO) or a placebo (PLA) during sleep. Continuous infusions with l-[ring-3H5]phenylalanine and l-[ring-2H2]tyrosine were applied to assess in vivo dietary protein digestion and absorption kinetics and subsequent muscle protein synthesis rates during sleep. We found that exogenous phenylalanine appearance rates increased following protein administration. The latter stimulated protein synthesis, resulting in a more positive overnight whole body protein balance (0.30 ± 0.1 vs. 11.8 ± 1.0 μmol phenylalanine kg^{-1}h^{-1} in PLA and PRO, respectively; P < 0.05). In agreement, overnight muscle protein fractional synthesis rates were much greater in the PRO experiment (0.045 ± 0.002 vs. 0.029 ± 0.002%/h, respectively; P < 0.05) and showed abundant incorporation of the amino acids ingested via the intrinsically labeled protein (0.058 ± 0.006%/h). This is the first study to show that dietary protein administration during sleep is followed by normal digestion and absorption kinetics, thereby stimulating overnight muscle protein synthesis. Dietary protein administration during sleep stimulates muscle protein synthesis and improves overnight whole body protein balance. These findings may provide a basis for novel interventional strategies to attenuate muscle mass loss.

Address for reprint requests and other correspondence: L. J. C. van Loon, Dept. of Human Movement Sciences, MUMC, P.O. Box 616, 6200 MD, Maastricht, The Netherlands (e-mail: L.vanloon@maastrichtUniv.nl).
efficacy of nighttime protein provision to stimulate muscle protein accretion in the elderly. This novel nutritional intervention concept may prove to be of important clinical relevance to attenuate muscle mass loss.

**MATERIALS AND METHODS**

**Subjects.** A total of 16 healthy, normoglycemic, elderly men (74 ± 1 yr) were selected to participate in the present study. Subjects’ characteristics are presented in Table 1. Subjects were randomly assigned to either the placebo (PLA) or protein (PRO) experiment. No differences were observed in subjects’ characteristics between experimental groups, P < 0.05.

<table>
<thead>
<tr>
<th>Table 1. <em>Subject characteristics</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo (n = 8)</strong></td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Body wt, kg</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>Body fat, %</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
</tr>
<tr>
<td>Leg volume, liters</td>
</tr>
<tr>
<td>HbA1c</td>
</tr>
<tr>
<td>Basal plasma glucose, mmol/l</td>
</tr>
<tr>
<td>Basal plasma insulin, mU/l</td>
</tr>
<tr>
<td>HOMA-IR</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n, no. of subjects. HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance. No differences between experimental groups, P < 0.05.

**Diet and physical activity in testing.** All volunteers were instructed to refrain from any exhaustive physical activity and to keep their diet as constant as possible 3 days before the trial. Food intake and physical activity records were collected for 2 days before the experiment and were analyzed by a certified dietician. Overall (n = 16), habitual daily energy intake was 8.6 ± 0.4 MJ, providing 57 ± 1 energy/100% energy (En%) carbohydrate, 31 ± 1 En% fat, and 12 ± 0.1 En% protein. Total carbohydrate, fat, and protein intake averaged 299 ± 14, 73 ± 3, and 63 ± 3 g, respectively. Dietary protein intake averaged 0.80 ± 0.04 g/kg body wt⁻¹ day⁻¹. On the day of the experiment, subjects consumed a standardized breakfast, midmorning snack, lunch, midafternoon snack, and dinner. The standardized diet (3 meals and 2 snacks) provided 9.8 MJ and provided 58 En% carbohydrate, 30 En% fat, and 12 En% protein. Dietary protein intake averaged 0.89 ± 0.09 g/kg body wt, with 30% of the protein provided with dinner. In the morning following the nocturnal intervention, hunger rating was assessed using the visual analog scale (36). Subsequently, subjects were invited to have breakfast, for which they were provided with a large range of products that could be consumed ad libitum. Food intake at breakfast was recorded for all subjects.

**Study design.** An outline of the study protocol is provided in Fig. 1. Each subject participated in one experiment, in which 400 ml water with (PRO) or without (PLA) 40 g intrinsically L-[1-13C]phenylalanine-labeled casein protein was administered via a nasogastric tube during overnight sleep from 0200 to 0205. Before and after protein administration, plasma and muscle samples were collected. These experiments were designed to simultaneously assess dietary protein digestion and absorption kinetics, splanchnic amino acid extraction, and mixed-muscle protein fractional synthetic rate (FSR) in vivo in humans during overnight sleep.

**Protocol.** At 1800, participants reported to the hospital ward where a nasogastric stomach feeding catheter (Bengmark; Flocare, Zoetermeer, the Netherlands) and two Teflon catheters were inserted. The first catheter was inserted in an antecubital vein, and the second catheter was inserted in an antecubital vein of the contralateral hand, which was placed in a hotbox to allow arterialized blood sampling (10a). After a background plasma sample was collected at 1855, the plasma phenylalanine pool was primed with a single intravenous dose of labeled phenylalanine and tyrosine (priming dose: 2.4 μmol/kg L-[ring-2H₅]phenylalanine, 0.925 μmol/kg L-[ring-2H₅]tyrosine). After the onset of the continuous stable isotope infusion (infusion rate: 0.06 μmol·kg⁻¹·min⁻¹ L-[ring-2H₅]phenylalanine, 0.023 μmol·kg⁻¹·min⁻¹ L-[ring-2H₅]tyrosine; Cambridge Isotopes Laboratories, Andover, MA) the blood sample and muscle biopsy were collected. Each subject participated in one experiment, in which 400 ml water with (PRO) or without (PLA) 40 g intrinsically L-[1-13C]phenylalanine-labeled casein protein was administered via a nasogastric tube during overnight sleep from 0200 to 0205. Before and after protein administration, plasma and muscle samples were collected. These experiments were designed to simultaneously assess dietary protein digestion and absorption kinetics, splanchnic amino acid extraction, and mixed-muscle protein fractional synthetic rate (FSR) in vivo in humans during overnight sleep.

**Diet and physical activity in testing.** All volunteers were instructed to refrain from any exhaustive physical activity and to keep their diet as constant as possible 3 days before the trial. Food intake and physical activity records were collected for 2 days before the experiment and were analyzed by a certified dietician. Overall (n = 16), habitual daily energy intake was 8.6 ± 0.4 MJ, providing 57 ± 1 energy/100% energy (En%) carbohydrate, 31 ± 1 En% fat, and 12 ± 0.1 En% protein. Total carbohydrate, fat, and protein intake averaged 299 ± 14, 73 ± 3, and 63 ± 3 g, respectively. Dietary protein intake averaged 0.80 ± 0.04 g/kg body wt⁻¹ day⁻¹. On the day of the experiment, subjects consumed a standardized breakfast, midmorning snack, lunch, midafternoon snack, and dinner. The standardized diet (3 meals and 2 snacks) provided 9.8 MJ and provided 58 En% carbohydrate, 30 En% fat, and 12 En% protein. Dietary protein intake averaged 0.89 ± 0.09 g/kg body wt, with 30% of the protein provided with dinner. In the morning following the nocturnal intervention, hunger rating was assessed using the visual analog scale (36). Subsequently, subjects were invited to have breakfast, for which they were provided with a large range of products that could be consumed ad libitum. Food intake at breakfast was recorded for all subjects.

**Study design.** An outline of the study protocol is provided in Fig. 1. Each subject participated in one experiment, in which 400 ml water with (PRO) or without (PLA) 40 g intrinsically L-[1-13C]phenylalanine-labeled casein protein was administered via a nasogastric tube during overnight sleep from 0200 to 0205. Before and after protein administration, plasma and muscle samples were collected. These experiments were designed to simultaneously assess dietary protein digestion and absorption kinetics, splanchnic amino acid extraction, and mixed-muscle protein fractional synthetic rate (FSR) in vivo in humans during overnight sleep.

**Protocol.** At 1800, participants reported to the hospital ward where a nasogastric stomach feeding catheter (Bengmark; Flocare, Zoetermeer, the Netherlands) and two Teflon catheters were inserted. The first catheter was inserted in an antecubital vein, and the second catheter was inserted in an antecubital vein of the contralateral hand, which was placed in a hotbox to allow arterialized blood sampling (10a). After a background plasma sample was collected at 1855, the plasma phenylalanine pool was primed with a single intravenous dose of labeled phenylalanine and tyrosine (priming dose: 2.4 μmol/kg L-[ring-2H₅]phenylalanine, 0.925 μmol/kg L-[ring-2H₅]tyrosine). After the onset of the continuous stable isotope infusion (infusion rate: 0.06 μmol·kg⁻¹·min⁻¹ L-[ring-2H₅]phenylalanine, 0.023 μmol·kg⁻¹·min⁻¹ L-[ring-2H₅]tyrosine; Cambridge Isotopes Laboratories, Andover, MA) the blood sample and muscle biopsy were collected.
MA), a standardized dinner was ingested at 1900. Participants rested in a supine position for 3 h. At 2300, the first muscle biopsy sample from the vastus lateralis muscle was taken. Subsequently, subjects went to sleep at 0000. During the night, at 0200, a bolus of 400 ml water with or without 40 g intrinsically labeled L-[1-13C]phenylalanine-labeled casein protein was administered via a nasogastric tube within 5 min without disturbing the subjects in their sleep. A second muscle biopsy was obtained from the vastus lateralis in the contralateral leg at 0700. Blood samples (8 ml) were taken from the arterialized hand vein at 1900, 2000, 2100, 2200, 2300, 0000, 0100, 0200, 0230, 0300, 0330, 0400, 0500, 0600 and 0700.

Blood samples were collected in EDTA-containing tubes and centrifuged at 1,000 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 vastus lateralis, 15 cm above the patella and −4 cm below entry through the fascia, using the percutaneous needle biopsy technique (4). 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.

Protein administration and tracer infusion. Intrinsically labeled L-[1-13C]phenylalanine-labeled casein protein was obtained by infusing a Holstein cow with large quantities of L-[1-13C]phenylalanine, collecting milk, and purifying the casein fraction as described previously (44). The L-[1-13C]phenylalanine enrichment in the casein fraction averaged 37.4 mole percent enrichment (MPE). The casein protein met all chemical and bacteriological specifications for human consumption. Subjects were administered either 40 g intrinsically labeled L-[1-13C]phenylalanine-labeled casein protein or a placebo drink (water only) via a nasogastric tube. The stable isotope tracers L-[ring-2H5]phenylalanine and L-[ring-2H5]tyrosine were purchased from Cambridge Isotopes and dissolved in 0.9% saline before infusion. Continuous intravenous infusions were performed using a calibrated IVAC 598 pump (San Diego, CA). Tracers were prepared by the pharmacy of the Maastricht University Medical Center.

Plasma analysis. Plasma glucose (Uni Kit III, 07367204; Roche, Basel, Switzerland) concentrations were analyzed with the COBAS FARA semiautomatic analyzer (Roche). Insulin was analyzed by radioimmunoassay (Insulin RIA kit; LINCO Research, St. Charles, MO). Plasma (100 µl) for amino acid analyses was deproteinized on ice with 4 mg/ml of 24% (wt/vol) 5-sulfosalicylic acid and mixed, and the clear supernatant was collected after centrifugation. Plasma amino acid concentrations were analyzed by an automated RP-HPLC-based amino acid analyzer using an automated precolumn derivatization procedure and a ternary solvent system (43). For plasma L-[1-13C]phenylalanine, L-[1-13C]tyrosine, L-[ring-2H5]phenylalanine, L-[ring-2H5]tyrosine, and L-[ring-2H5]tyrosine enrichment measurements, plasma phenylalanine and tyrosine were derivatized to their t-butyldimethylsilyl (TBDMS) derivatives, and their 13C and/or 2H enrichments were determined by electron ionization gas chromatography-mass spectrometry (GC-MS; Agilent 6890N GC/5973N MSD) using selected ion monitoring of masses 336, 337, and 341 for labeled and 1-13C[-] and L-[ring-2H5]phenylalanine, respectively. Masses 466, 467, 468, and 470 were assessed for labeled and unlabeled (1-13C, ring-2H5, and ring-2H5) tyrosine (48). We applied standard regression curves in all isotopic enrichment analyses to assess linearity of the mass spectrometer and to control for loss of tracer.

Calculations. Ingestion of L-[1-13C]phenylalanine-labeled protein, intravenous infusion of L-[ring-2H5]phenylalanine, and arterialized blood sampling were used to assess whole body amino acid kinetics in non-steady-state conditions. Total, exogenous, and endogenous rate of appearance (Re), and splanchic extraction (i.e., fraction of dietary amino acid taken up by all other organs except from muscle tissue and plasma volume, Sp) for phenylalanine were calculated using modified Steele’s equation (10, 13). These parameters were calculated as follows:

\[
F - pV \cdot C(t) = \frac{\Delta E_{iv}}{\Delta t} \tag{1}
\]

\[
\text{TotalRa}_A = \frac{\text{ExoR}_A}{E_{prot}} = \frac{\Delta E_{po}}{\Delta t} \tag{2}
\]

\[
\text{EndoR}_A = \text{TotalRa}_A - \text{ExoR}_A = F \tag{3}
\]

\[
\text{Phe}_{\text{plasma}} = \frac{\text{AUC}_{\text{ExoR}_A}}{\text{Phe}_{\text{prot}}} \cdot 100 \tag{4}
\]

where F is the intravenous tracer infusion rate (µmol·kg⁻¹·min⁻¹) and pV (0.125) is the distribution volume for phenylalanine (10). C(t) is the mean plasma phenylalanine concentration between two time points. ∆Eiv/∆t represents the time-dependent variations of plasma phenylalanine enrichment derived from the intravenous tracer, and ∆Epo/∆t represents the time-dependent variations of plasma phenylalanine enrichment derived from the oral tracer, and Eprot is the L-[1-13C]phenylalanine enrichment in the dietary protein. Pheplasma calculates the percentage administered phenylalanine that becomes available in the plasma and is defined by Phe_{prot} and AUC_{ExoR}_A. Phesp, the amount of dietary phenylalanine ingested and AUC_{ExoR}_A, represents the area under the curve (AUC) of Exo Phe Ra, which corresponds to the amount of dietary phenylalanine that appeared in the blood over a 5-h period following administration by a nasogastric tube.

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

\[
\text{Phe}_{\text{prot}} = \frac{\text{AUC}_{\text{ExoR}_A}}{\text{Phe}_{\text{prot}}} \cdot 100 \tag{4}
\]
Because whole body R₄ comprises the rate of phenylalanine disappearance from the free amino acid pool in the blood due to protein synthesis (S) and oxidation, whole body protein synthesis can be calculated as R₄ minus oxidation. Whole body phenylalanine oxidation can be determined from the conversion (hydroxylation) of L-[^2H₅]phenylalanine to L-[^2H₅]tyrosine. The rate of hydroxylation was calculated by using the following formula (40):

\[
Phe_{hydroxylation} = \frac{\text{TyrR₄}}{\text{E₄(t)}} \cdot \frac{\text{PheR₄}}{\text{E₄(t)}} \cdot \frac{\text{PheR₄}}{\text{PheR₄} + \text{PheR₄}}
\]

where TyrR₄ represents the rate of appearance of L-[^2H₅]tyrosine, E₄(t) and E₄(t) are the L-[^2H₅]tyrosine and L-[^2H₅]phenylalanine enrichments in plasma between two consecutive time points, respectively, PheR₄ is the rate of disappearance of L-[^2H₅]phenylalanine, and F₄phe is the infusion rate of phenylalanine. Whole body protein synthesis was calculated using:

\[
S = R₄ - Phe_{hydroxylation}
\]

The fractional rate of mixed-muscle protein synthesis (FSR) was calculated by dividing the increment in enrichment in the product, i.e., protein-bound L-[^1-13C]phenylalanine or L-[^2H₅]phenylalanine, by the enrichment of the precursor. Non-steady-state plasma L-[^1-13C]phenylalanine and plasma L-[^2H₅]phenylalanine enrichments were used as the preferred precursor pools to estimate mixed-muscle protein fractional synthesis rates from both the ingested (dietary protein-bound L-[^1-13C]phenylalanine) or continuously infused (L-[^2H₅]phenylalanine) tracer. Consequently, muscle FSR values were calculated as follows (50):

\[
\text{FSR} = \frac{\text{E₄(t)} - \text{E₄(0)}}{\text{E₄(t)}} \cdot \frac{\Delta C}{\Delta t} \cdot 100
\]

where E₄(t) represents muscle protein-bound L-[^1-13C]phenylalanine or L-[^2H₅]phenylalanine, E₄ represent the average plasma L-[^1-13C]phenylalanine or L-[^2H₅]phenylalanine enrichment during the during the tracer incorporation period and t indicates the time interval (h) between biopsies.

**Statistics.** All data are displayed as arithmetic means ± SE (= SD/√no. of subjects). Time-dependent variables like plasma amino acid concentrations, plasma enrichments, and whole body protein kinetics were analyzed by two-factor repeated-measures ANOVA and by calculating the AUC in both groups. Group-dependent variables such as AUC and FSR were analyzed by Student’s t-test. Two-factor repeated-measures ANOVA was conducted with group as the between-subjects factor and time as the within-subjects factor. To differentiate the protein effect and to control group homogeneity before the intervention, two-factor repeated-measures ANOVA was carried out separately for the period from t = −6 h to t = 0 h and for the period after the protein administration between t = 0 h and t = 5 h. Statistical significance was set at P < 0.05. All calculations were performed using SPSS 15.0 (SPSS, Chicago, IL).

**RESULTS**

**Plasma analysis.** Plasma glucose and insulin concentrations increased rapidly after dinner and normalized to baseline levels before the first biopsy that was obtained at 2300. Plasma glucose concentrations averaged 5.2 ± 0.0 and 5.5 ± 0.1 mmol/l between 2300 and 0700 in the PLA and PRO experiment, respectively. Casein administration at 0200 did not modulate circulating plasma glucose concentrations. Plasma insulin concentrations between 2300 and 0700 averaged 10.7 ± 0.3 and 19.1 ± 2.1 mU/l in the PLA and PRO experiment, respectively (P < 0.05). Provision of intrinsically labeled casein resulted in a small but significant increase in circulating plasma insulin concentrations. Plasma insulin levels peaked 60 min after protein administration at 33.8 ± 4.9 mU/l in the PRO experiment compared with 11.6 ± 2.1 mU/l in the PLA experiment (P < 0.05). Thereafter, plasma insulin levels returned to values that were no longer different between experiments (data not shown).

Plasma phenylalanine, leucine, and essential amino acid concentrations over time are shown in Fig. 2. No differences in basal plasma amino acid concentrations were observed between experimental groups. After administration of intrinsically labeled casein, plasma amino acid concentrations increased rapidly and remained significantly elevated for the remaining 5 h. Plasma amino acid concentrations were significantly higher in the PRO compared with the PLA experiment. Peak plasma phenylalanine (154 ± 14 μmol/l) and leucine (449 ± 31 μmol/l) concentrations were reached within 30 min after protein administration.

Continuous infusions with L-[^2H₅]phenylalanine and L-[^1-13C]tyrosine were applied in both experiments from 1900 until 0700. L-[^1-13C]phenylalanine-labeled casein was administered via a nasogastric tube at 0200 in the PRO experiment. Plasma tracer enrichments from infused and ingested amino acid tracers are shown in Fig. 3, A–D. At baseline, plasma tracer enrichments were similar in the PLA and PRO experiments. Directly after L-[^1-13C]phenylalanine-labeled casein administration, plasma L-[^1-13C]phenylalanine and L-[^1-13C]tyrosine enrichments increased rapidly, reaching peak levels of 17.3 ± 1.2 and 6.2 ± 0.3 MPE after 30 and 60 min, respectively (Fig. 3, A and B). Plasma L-[^1-13C]phenylalanine enrichments subsequently declined to 8.30 ± 0.27 MPE 5 h after administration of the protein drink. No L-[^1-13C]phenylalanine-labeled protein was provided in the PLA experiment, and, as such, enrichments levels were nil. Plasma L-[^2H₅]phenylalanine and L-[^2H₅]tyrosine enrichments declined following protein administration in the PRO experiment and remained well below the levels observed in the PLA experiment throughout the remainder of the night (Fig. 3, C and D).

**Plasma amino acids kinetics.** Whole body plasma amino acid kinetics are presented in Fig. 4, A–E. Immediately after administration of the intrinsically labeled casein, exogenous L-[^1-13C]phenylalanine entered the circulation. Exogenous phenylalanine appearance rates reached peak values at (0.43 ± 0.03 μmol·kg⁻¹·min⁻¹) within 60 min after protein administration (Fig. 4A). In response, endogenous plasma phenylalanine appearance rates declined in the PRO experiment and remained well below levels observed in the PLA experiment. Whole body plasma phenylalanine disappearance rate followed the rate of appearance and showed significant postprandial phenylalanine disposal in the PRO experiment (Fig. 4C). In the PRO experiment, postprandial L-[^1-13C]phenylalanine extraction in the splanchic area between 0200 and 0700 averaged 42 ± 4%. Conversion of L-[^2H₅]phenylalanine to L-[^2H₅]tyrosine was higher in the PRO compared with the PLA experiment (Fig. 4D).

Calculated whole body protein synthesis and oxidation rates were significantly higher in the PRO vs. the PLA experiment. In agreement, net protein balance following protein adminis-
tration was significantly higher in the PRO compared with the PLA experiment (11.8 ± 1.0 and 0.3 ± 0.1 μmol phenylalanine·kg\(^{-1}·h^{-1}\), respectively; \(P < 0.05\); Fig. 5).

**Muscle tissue analysis.** Muscle tissue analyses were performed on muscle biopsy samples collected before and 5 h after protein or placebo administration. Muscle free and muscle protein-bound \(\text{l-}[\text{ring-}^{2}\text{H}\text{}_5]\)phenylalanine enrichments increased equally in the PLA and PRO experiment. Continuous infusion of \(\text{l-}[\text{ring-}^{2}\text{H}\text{}_5]\)phenylalanine resulted in muscle free \(\text{l-}[\text{ring-}^{2}\text{H}\text{}_5]\)phenylalanine enrichments of 7.16 ± 0.32 and 6.32 ± 0.35 MPE in the PLA and PRO experiments, respectively (\(P < 0.05\)). The increase in muscle protein-bound \(\text{l-}[\text{ring-}^{2}\text{H}\text{}_5]\)phenylalanine enrichments averaged 0.000254 ± 0.0000145 and 0.000300 ± 0.0000161 MPE in the PLA and PRO experiments, respectively (\(P = 0.04\)). Following the gastric administration of intrinsically \(\text{l-}[\text{1-}^{13}\text{C}\text{]}\)phenylalanine-labeled protein, muscle free \(\text{l-}[\text{1-}^{13}\text{C}\text{]}\)phenylalanine enrichment had increased up to 6.20 ± 0.65 MPE in the PRO experiment. Muscle protein-bound \(\text{l-}[\text{1-}^{13}\text{C}\text{]}\)phenylalanine enrichment had increased to 0.000322 ± 0.0000286 MPE during overnight sleep.

**Mixed-muscle fractional synthesis rates.** Mixed-muscle protein synthesis rates, expressed as FSR, are presented in Fig. 6, A and B. Muscle biopsies were taken at 2300 and 0700. Mixed-muscle protein FSR, with the mean plasma \(\text{l-}[\text{ring-}^{2}\text{H}\text{}_5]\)phenylalanine enrichment as precursor, was significantly higher in the PRO compared with the PLA experiment (0.045 ± 0.002 and 0.029 ± 0.002%/h, respectively; \(P < 0.05\)). In agreement, a substantial net increase in muscle protein-bound \(\text{l-}[\text{1-}^{13}\text{C}\text{]}\)phenylalanine was observed in the PRO experiment. With the mean plasma \(\text{l-}[\text{1-}^{13}\text{C}\text{]}\)phenylalanine enrichment as precursor, mixed-muscle protein FSR from the ingested protein averaged 0.058 ± 0.006%/h (Fig. 6B).

**Satiety after intervention.** In the PRO experiment, subjects were provided with 40 g casein at night, representing 62.0 ± 2.0% of their habitual daily protein intake. The following morning, hunger ratings were assessed by using the Visual Analog Scale with scores of 47 vs. 24 ± 4% (\(P < 0.05\)) in the PLA and PRO experiments, respectively (36). Energy intake (2.5 ± 0.5 and 2.1 ± 0.4 MJ; \(P = 0.48\)) and total protein intake (20.9 ± 4.9 and 18.2 ± 3.3; \(P = 0.65\)) with breakfast did not differ between the PLA and PRO experiments, respectively.

**DISCUSSION**

In the present study, we show that dietary protein administration by nasogastric tube feeding during overnight sleep is accompanied by normal dietary protein digestion and absorption kinetics in healthy, elderly men. Protein provision during sleep increases plasma amino acid availability and stimulates skeletal muscle protein synthesis, improving overnight whole body protein balance.

The loss of muscle mass with aging has been associated with a blunted skeletal muscle protein synthetic response to food intake (11, 21). Consequently, many research groups have been trying to define effective dietary strategies to augment the postprandial muscle protein synthetic response to meal ingestion in the elderly (26, 27, 33, 45). In contrast, we speculated on the potential to stimulate overnight muscle protein synthesis by providing dietary protein during sleep. The provision of
protein during overnight sleep might enlarge the window of opportunity to improve 24-h protein balance. However, it is generally assumed that food digestion and absorption is not efficient during overnight sleep. It has been reported that human intestinal motility follows a circadian rhythm with reduced nocturnal activity (16, 23, 29). Therefore, the first question that needs to be addressed is whether dietary protein administration during sleep is followed by normal protein

Fig. 3. Plasma l-[1-13C]phenylalanine (A), l-[1-13C]tyrosine (B), l-[ring-2H5]phenylalanine (C), and l-[ring-2H2]tyrosine (D) enrichments. MPE, mole % excess. Values are expressed as means ± SE. Data were analyzed with repeated-measures (treatment × time) ANOVA. Plasma l-[1-13C]phenylalanine: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. l-[1-13C]tyrosine enrichment: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. l-[ring-2H5]phenylalanine enrichment: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. l-[ring-2H2]tyrosine enrichment: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. *Significantly different from PLA (Scheffé’s post hoc test, P < 0.05).

Fig. 4. Exogenous phenylalanine rate of appearance (Ra) (A), total phenylalanine Ra (B), total phenylalanine rate of disappearance (Rd) (C), and phenylalanine-to-tyrosine conversion (D). Values represent means ± SE. Data were analyzed with repeated-measures (treatment × time) ANOVA. Exogenous plasma phenylalanine Ra: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. Total plasma phenylalanine Ra: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. Total plasma phenylalanine Rd: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. Plasma phenylalanine-to-tyrosine conversion: treatment effect, P < 0.01; time effect, P < 0.01; interaction of treatment and time, P < 0.01. *Significantly different from PLA (Scheffé’s post hoc test, P < 0.05).
digestion and absorption kinetics. Subsequently, it needs to be established whether this will effectively increase plasma amino acid availability, which is required to stimulate muscle protein synthesis (7, 12). To address these questions, we administered 40 g intrinsically L-[1-13C]phenylalanine-labeled protein via a nasogastric tube in elderly subjects during their sleep (Fig. 1).


Directly after intragastric protein administration, plasma amino acid concentrations rapidly increased and remained elevated throughout the remainder of the night (Fig. 2). Peak essential amino acid concentrations were observed within 30 min after protein administration (Fig. 2C). The latter was entirely attributed to the digestion and absorption of the administered protein, since plasma L-[1-13C]phenylalanine enrichments increased immediately after protein administration, reaching peak values within 60 min (Fig. 3). In accordance, calculated exogenous phenylalanine appearance rates rapidly increased following protein administration, showing that dietary protein provision during sleep is followed by normal dietary protein digestion and absorption (Fig. 4). The latter does not seem to be different from digestion and absorption kinetics when protein is ingested during daytime. The amount of dietary protein-derived phenylalanine that became available in the circulation during the 5-h postprandial period averaged 58 ± 4%. The latter is in line with previous work reporting similar findings after ingesting 20–35 g casein or whey protein in the morning following an overnight fast (10, 28, 35). In fact, when comparing the increase in exogenous amino acid appearance rates following casein ingestion reported in Koopman et al. (28) with the present findings, it might even be speculated that casein digestion and absorption was even more rapid when provided during overnight sleep. The present results demonstrate that the gastrointestinal tract is fully functional during overnight sleep when food is made available, allowing proper dietary protein digestion and absorption in healthy, elderly men.

We combined the use of specifically produced, high-level intrinsically L-[1-13C]phenylalanine-labeled dietary protein with continuous intravenous L-[ring-2H5]phenylalanine infusion to allow accurate assessment of exogenous protein digestion and absorption kinetics but also to study its subsequent impact on whole body and muscle protein metabolism. Whole body muscle protein synthesis rates increased rapidly following protein administration, resulting in much higher whole body protein synthesis rates in the PLA vs. PRO experiment (Fig. 5). In agreement, overnight whole body protein balance was more positive in the PRO compared with the PLA experiment. The latter shows that dietary protein provided during sleep is absorbed and retained, thereby allowing whole body protein accretion. However, it should be noted that changes in whole body protein kinetics do not necessarily represent changes on a muscle tissue level (30).

Therefore, we also determined mixed-muscle protein fractional synthesis rates by measuring both the incorporation of L-[ring-2H5]phenylalanine and L-[1-13C]phenylalanine in skeletal muscle protein from biopsies collected before sleep and after waking up at 0700 in the morning. Muscle protein fractional synthesis rates derived from the incorporation of L-[ring-2H5]phenylalanine showed much higher synthesis rates...
when protein was provided during sleep. These results show that protein administration during sleep strongly stimulates mixed-muscle protein synthesis (Fig. 6A). These findings were obtained despite the fact that our subjects had consumed a standardized diet that provided ample dietary protein (~70 g protein) throughout the day. The latter underlines the efficacy of nighttime protein provision to modulate muscle protein synthesis. The application of intrinsically L-[1-13C]phenylalanine-labeled casein, with an enrichment level of up to 40 MPE (44), allows us to also assess the incorporation of the absorbed L-[1-13C]phenylalanine into skeletal muscle protein. In the PRO experiment, muscle protein-bound L-[1-13C]phenylalanine had increased substantially, which translated into a fractional mixed-muscle protein synthesis rate of 0.058 ± 0.006%/h (Fig. 6B). The latter provides direct evidence that dietary protein administered during sleep is not only adequately digested and absorbed but is also effectively used for de novo muscle protein synthesis.

Evidence for proper nocturnal protein digestion and absorption as well as subsequent de novo muscle protein synthesis in vivo may be of clinical relevance. The concept of overnight protein provision can be translated to more practical dietary interventions. For example, specifically designed proteins or prebedtime protein meals might be applied to allow dietary protein provision throughout overnight sleep. In the present study, the large amounts of dietary protein (40 g) administered during sleep were well received and did not cause any gastrointestinal disturbances or nausea. In fact, none of the subjects woke up during or immediately after administration of the protein (or placebo) solution. It could be speculated that overnight protein provision might modulate food intake during the subsequent day(s). Although beyond the scope of the study, we assessed hunger levels before breakfast (41). Lower hunger ratings were reported when protein was provided during sleep. However, the latter did not seem to affect energy and/or protein intake during subsequent breakfast. So far, the present study suggests that it is possible to expand the window for nutritional support by protein provision during the night. Consequently, future research should investigate whether nighttime protein provision could represent a feasible concept to attenuate muscle mass loss in various clinical conditions (24, 42), such as posturgical muscle wasting (17, 22, 38) and cachexia in elderly males. Nocturnal dietary protein provision can be translated to more practical dietary intervention strategy to support muscle mass maintenance in both health and disease.

ACKNOWLEDGMENTS

We acknowledge the support of the subjects who volunteered to participate in these experiments.

AUTHOR CONTRIBUTIONS

B. B. L. Groen: designed the study, organized and carried out the clinical experiments, performed the (statistical) analysis of the data, and wrote the manuscript. E. Herle: helped organize and carry out the clinical experiments. I. M. G. Senden: performed the stable isotope analyses. L. J. C. van Loon: designed the study and helped write the manuscript.

DISCLOSURES

None of the authors had any personal or financial conflict of interest.

REFERENCES


