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Protein coingestion stimulates muscle protein synthesis during resistance-type exercise

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Beelen M, Koopman R, Gijsen AP, Vandereyt H, Kies AK, Kuipers H, Saris WH, van Loon LJ. Protein coingestion stimulates muscle protein synthesis during resistance-type exercise. *Am J Physiol Endocrinol Metab* 295: E70–E77, 2008. First published April 22, 2008; doi:10.1152/ajpendo.00774.2007.—In contrast to the effect of nutritional intervention on postexercise muscle protein synthesis, little is known about the potential to modulate protein synthesis during exercise. This study investigates the effect of protein coingestion with carbohydrate on muscle protein synthesis during resistance-type exercise. Ten healthy males were studied in the evening after they consumed a standardized diet throughout the day. Subjects participated in two experiments in which they ingested either carbohydrate or carbohydrate with protein during a 2-h resistance exercise session. Subjects received a bolus of test drink before and every 15 min during exercise, providing 0.15 $g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate with (CHO + PRO) or without (CHO) 0.15 $g \cdot kg^{-1} \cdot h^{-1}$ protein hydrolysate. Continuous intravenous infusions with L-[*ring*-$^{13}C_6$]phenylalanine and L-[*ring*-$^2H_2$]tyrosine were applied, and blood and muscle biopsies were collected to assess whole body and muscle protein synthesis rates during exercise. Protein coingestion lowered whole body protein breakdown rates by 8.4 ± 3.6% ($P = 0.066$), compared with the ingestion of carbohydrate only, and augmented protein oxidation and synthesis rates by 77 ± 17 and 33 ± 3%, respectively ($P < 0.01$). As a consequence, whole body net protein balance was negative in CHO, whereas a positive net balance was achieved after the CHO + PRO treatment (−4.4 ± 0.3 vs. 16.3 ± 0.4 μmol phenylalanine$\cdot kg^{-1} \cdot h^{-1}$, respectively; $P < 0.01$). In accordance, mixed muscle protein fractional synthetic rate was 49 ± 22% higher after protein coingestion (0.088 ± 0.012 and 0.060 ± 0.004%/h in CHO + PRO vs. CHO treatment, respectively; $P < 0.05$). We conclude that, even in a fed state, protein coingestion stimulates whole body and muscle protein synthesis rates during resistance-type exercise.

nutrition; amino acids; muscle anabolism

MANY STUDIES HAVE ASSESSED the effect of nutritional modulation on muscle protein metabolism during postexercise recovery. Exercise has been shown to stimulate both muscle protein synthesis (3, 26, 29, 34) and protein breakdown (3, 26, 29), but in the absence of food intake net protein balance remains negative (3, 26). The ingestion of carbohydrate after resistance-type exercise attenuates the exercise-induced increase in protein breakdown, thereby improving net muscle protein balance (9, 25, 28). However, the ingestion of protein and/or amino acids is essential to stimulate postexercise muscle protein synthesis and, as such, to achieve a positive net muscle protein balance during recovery from exercise (4, 10, 20, 25, 27, 33).

Previous studies have reported that protein turnover is either decreased or unchanged during resistance (14, 15)- and endurance (11, 12, 18, 40, 41)-type exercise activities. In contrast to protein metabolism during postexercise recovery, little is known about the effects of nutritional intervention on muscle protein synthesis during exercise. Thus far, only two studies (18, 35) have examined the role of nutrition on whole body protein turnover during exercise. These studies report an improvement in whole body protein balance after protein coingestion during either prolonged endurance (18)- or resistance-type exercise activities (35). In the latter study, Tipton et al. (35) suggest that protein ingestion before, as opposed to after, exercise could further augment net muscle protein accretion during recovery. This has been attributed to a more rapid supply of amino acids to the muscle during the acute stages of postexercise recovery. However, it could also be speculated that protein ingestion before and/or during resistance-type exercise already stimulates muscle protein synthesis during exercise, thereby creating a larger time frame for muscle protein synthesis to be elevated. However, the effect of protein coingestion on muscle protein synthesis during exercise has not yet been established.

Studies (4, 10, 20, 25, 27, 33, 35) that have reported on the benefits of protein ingestion on postexercise recovery generally investigate subjects in the overnight fasted state. Under these conditions, it might be speculated that endogenous amino acid availability from the gut (13) and/or the intramuscular free amino acid pool might be limiting. Such postabsorptive conditions differ substantially from normal everyday practice in which recreational sports activities are generally performed in the evening in a fed state. Thus far, no data are available on the effect of protein and carbohydrate coingestion on muscle protein synthesis during resistance-type exercise under normal, fed conditions.

In the present study, we assessed the surplus value of protein coingestion with carbohydrate during resistance-type exercise on whole body protein balance and skeletal muscle protein synthesis rate under normal, practical conditions, during which exercise is being performed in the evening after the consumption of a standardized diet throughout the day.

METHODS

Subjects. Ten healthy, male volunteers participated in this study (age: 20 ± 1 yr, body wt: 69.1 ± 2.4 kg; height: 1.79 ± 0.03 m; body

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Table 1. *Subjects' characteristics*

Characteristics	
Age, yr	20±1
Height, cm	179±3
Weight, kg	69.1±2.4
BMI, kg/m^2	21.6±0.7
Body fat, % (Siri)	12.0±1.3
Leg volume, liters	7.2±0.8
1RM leg press, kg	217±10
1RM leg extension, kg	122±4
W_{max}, W	305±11
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	50.5±2.5

Values are means ± SE; n = 10. 1RM, subject's one repetition maximum; BMI, body mass index.

mass index: 21.6 ± 0.7 kg/m^2; and percentage body fat: 12.0 ± 1.3%; Table 1). All subjects were recreationally active but were not enrolled in any regular exercise program. Subjects were fully informed of 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 Academic Hospital Maastricht (Maastricht, The Netherlands).

Pretesting. All subjects participated in two screening sessions, separated by at least 5 days. In the morning after an overnight fast, body composition was determined by the hydrostatic weighing method. Body fat percentage was calculated using Siri's equation (31). Leg volume was measured by anthropometry (16) and averaged 8.2 ± 0.4 liters. Subsequently, subjects were familiarized with the exercise equipment and exercise procedure. Proper lifting technique was demonstrated and practiced for each of the upper body exercises (chest press, shoulder press, and lat pulldown) and for the two lower limb exercises (leg press and leg extension). Thereafter, maximum strength for the two leg exercises was estimated, using the multiple repetition testing procedure (24).

In the second screening session, the subject's one repetition maximum (1RM) was determined for the two leg exercises (22). After the subject warmed up, the load was set at 97.5% of the estimated 1RM and increased after each successful lift until failure. Between successive attempts, 5-min rest periods were allowed. A repetition was valid if the subject used proper form and was able to complete the entire lift in a controlled manner without assistance. In addition, subjects performed an incremental exhaustive exercise test on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) to measure their maximal oxygen uptake capacity ($\dot{V}O_{2\,max}$) and workload capacity (W_{max}; Ref. 23).

Diet and activity before the experiments. All subjects received a standardized diet the evening before each experimental day [54.3 ± 1.8 kJ/kg body wt, consisting of 62 energy% (En%) carbohydrate, 22 En% fat, and 16 En% protein] and during the entire experimental day (0.16 ± 0.01 MJ·kg body wt^{-1}·day^{-1}, consisting of 62 ± 1 En% carbohydrate, 13 ± 0.4 En% protein, and 26 ± 1 En% fat). Subjects were provided with measured amounts of all food products and ingested all meals/snacks at predetermined time intervals. In both experiments, subjects ingested 78 ± 3 g protein via the standardized diet, with an additional 21 ± 1 g supplemented in the CHO + PRO treatment. All volunteers were instructed to refrain from any sort of heavy physical labor and to keep their diet as constant as possible 2 days before the experimental day. In addition, subjects filled in food intake and physical activity questionnaires for 2 days before the start of the first experiment, which were used to standardize food intake and physical activity before the second experiment.

Design. Each subject participated in two treatments separated by at least 2 wk. During the experimental day, all subjects received a standardized diet (breakfast, lunch, dinner, and snacks), participated in their normal daily activities, and reported to the laboratory in the evening. Subjects performed a 2-h resistance-type exercise session, during which either carbohydrate (CHO) or carbohydrate and protein (CHO + PRO) were ingested. Both treatments were performed in a double-blind, randomized order. Plasma samples were collected every 15 min, and muscle biopsies were taken before and immediately after the cessation of exercise. Tests were designed to simultaneously assess whole body amino acid kinetics and fractional synthetic rate (FSR) of mixed muscle protein by the incorporation of L-[*ring*-$^{13}C_6$]phenylalanine in the mixed protein pool of muscle tissue samples collected from the vastus lateralis muscle.

Experimental protocol. At 7:00 PM, 2 h after ingesting a standard dinner, subjects reported to the laboratory, where a Teflon catheter was inserted into an antecubital vein for the primed, continuous infusion of isotopically labeled phenylalanine and tyrosine. A second Teflon catheter was inserted into a contralateral hand vein, which was placed in a hot-box to allow arterialized blood sampling. After a background blood sample was collected (t = −60), tracer infusion was started and subjects rested in a supine position for 1 h. Before the subjects engaged in the exercise protocol (t = 0 min), the first muscle biopsy was collected after which the first bolus of test drink was ingested (4.5 ml/kg). During exercise, subjects received subsequent boluses (1.5 ml/kg) of the test drink every 15 min. The exercise protocol consisted of an interval-cycling program followed by whole body resistance-type exercise training. After a 10-min warm-up on a cycle ergometer (50% W_{max}), subjects cycled 4 × 5 min at 65% W_{max}, alternated by 4 × 2.5 min at 45% W_{max}. No rest periods were allowed between cycling intervals. After a 5-min resting period, subjects started with the resistance-type exercise protocol, consisting of an upper and a lower body workout. The upper body workout was performed with a workload set at 40% of the total bodyweight in which subjects completed 5 sets of 10 repetitions on 3 upper body machines (chest press, shoulder press, and lat pulldown). A resting period of 1 min between sets was allowed. This was followed by a lower limb workout, which consisted of 9 sets of 10 repetitions on the horizontal leg press machine (Technogym BV, Rotterdam, The Netherlands) and 9 sets of 10 repetitions on the leg extension machine (Technogym). On both machines, 3 sets were completed at 55% of subject's 1RM, 3 at 65% 1RM, and 3 at 75% 1RM, with a 2-min rest between sets. Finally, subjects performed two sets of 30 abdominal crunches. During the test, all subjects were verbally encouraged to complete the entire protocol within ~120 min. Immediately after the end of the exercise protocol (t = 120 min), an arterialized blood sample from the heated hand vein and a second muscle biopsy from the vastus lateralis muscle were obtained. Arterialized blood samples (8 ml) were taken at t = −60, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. Muscle biopsies were taken at t = 0 and 120 min.

Beverages. Subjects received a beverage volume of 1.5 ml/kg every 15 min during exercise to ensure a given dose of 0.15 g·kg^{-1}·h^{-1} carbohydrate (50% glucose and 50% maltodextrin), with or without 0.15 g·kg^{-1}·h^{-1} protein hydrolysate. The first bolus was provided in a volume of 4.5 ml/kg to stimulate gastric emptying. This supplementation regimen has been shown to allow a continuous supply of glucose and amino acids from the gut and, as such, minimizes perturbations in plasma glucose, amino acid, and circulating insulin concentrations during exercise (18, 37). Glucose and maltodextrin were obtained form AVEBE (Veendam, The Netherlands). The casein protein hydrolysate (PeptoPro, 85.3% protein) was prepared by DSM Food Specialties (Delft) and involved the enzymatic hydrolysis of casein protein by specific endopeptidases and proline-specific endoprotease. To make the taste comparable, all solutions were flavored by addition of 0.05 g/l sodium saccharinate, 0.9 g/l citric acid, and 5.0 g/l cream vanilla flavor (Quest International, Naarden, The Netherlands). Treatments were performed in a randomized order, with test-drinks provided in a double-blind fashion.

Tracer. The stable isotope tracers L-[*ring*-$^{13}C_6$]phenylalanine and L-[*ring*-$^{2}H_2$]tyrosine were purchased from Cambridge Isotopes (Andover, MA) and dissolved in 0.9% saline before infusion. Continuous

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intravenous infusion (over a period of 3 h, 0.05 μmol·kg^{-1}·min^{-1} L-[*ring*-$^{13}C_6$]phenylalanine; 0.02 μmol·kg^{-1}·min^{-1} L-[*ring*-$^2H_2$]tyrosine) of the labeled isotopes was performed using a calibrated IVAC 560 pump (San Diego, CA). Both the phenylalanine and tyrosine pools were primed (2 μmol/kg L-[*ring*-$^{13}C_6$]phenylalanine; 0.775 μmol/kg [$^2H_2$]tyrosine) to enable the calculation of whole body phenylalanine kinetics using established tracer models (30, 32).

Muscle biopsies. Muscle biopsies were obtained from the middle region of the vastus lateralis muscle (15 cm above the patella) and ~2 cm below the entry through the fascia by means of the percutaneous needle biopsy technique described by Bergström (2). The pre- and postexercise biopsies were taken through the same incision, with the needle pointing in distal and proximal direction, respectively. As such, the biopsies were taken ~10 cm apart to prevent any influence of the preexercise biopsy on protein turnover in the postexercise biopsy (21). All samples were carefully freed from any visible fat and blood, immediately frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

Plasma analysis. Blood samples (8 ml) were collected in EDTA containing tubes and centrifuged at 1,000 *g* 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 semi-automatic analyzer (Uni Kit III, 07367204, La Roche, Basel, Switzerland). Insulin was analyzed by RIA (Human Insulin RIA Kit, Linco Research, St. Charles, MO). Plasma (500 μl) for amino acid analyses was deproteinized on ice with 100 μl of 24% (w/v) 5-sulphosalicylic acid and mixed, and the clear supernatant was collected after centrifugation. Plasma amino acids concentrations were analyzed on an automated dedicated amino acid analyzer (LC-A10, Shimadzu Benelux, Den Bosch, The Netherlands), using an automated precolumn derivatization procedure and a ternary solvent system. For plasma phenylalanine and tyrosine enrichment measurements, plasma phenylalanine and tyrosine were derivatized to their *t*-butyldimethylsilyl derivatives and their ^{13}C and/or ^2H enrichments were determined by electron ionization GC-MS (Agilent 6890N GC/5973N MSD Little Falls) using selected ion monitoring of masses 336 and 342 for unlabeled and labeled phenylalanine, respectively, and masses 466, 468, and 472 for unlabeled and ^2H- and ^{13}C-labeled tyrosine, respectively.

Muscle analyses. For measurement of L-[*ring*-$^{13}C_6$]phenylalanine enrichment in the free amino acid pool and mixed muscle protein, 55 mg of wet muscle were freeze dried. Collagen, blood, and other nonmuscle 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 × dry weight of isolated muscle fibers × wet/dry ratio) of ice-cold 2% PCA were added. The tissue was then homogenized and centrifuged. The supernatant was collected and processed in the same manner as the plasma samples, such that intracellular free L-[*ring*-$^{13}C_6$]phenylalanine, L-[*ring*-$^2H_2$]tyrosine, and L-[*ring*-$^{13}C_6$]tyrosine enrichments could be measured using their *t*-butyldimethylsilyl derivatives on a GC-MS. The free amino acid concentration in the supernatant was measured using an HPLC technique after precolumn derivatization with *o*-phthaldialdehyde (36). The protein pellet was washed with three additional 1.5-ml washes of 2% PCA and dried, and the proteins were hydrolyzed in 6 M HCl at 120°C for 15–18 h. The hydrolyzed protein fraction was dried under a nitrogen stream while being heated to 120°C, then dissolved in a 50% acetic acid solution, and passed over a Dowex exchange resin (AG 50W-X8, 100–200 mesh hydrogen form; Bio-Rad, Hercules, CA) using 2 M NH_4OH. Thereafter, the eluate was dried and the purified amino acid fraction was derivatized into the ethoxycarbonyl-ethylesters to determine the ^{13}C enrichment of protein bound phenylalanine using gas chromatography-isotope ratio mass spectrometry (Finnigan, MAT 252).

Calculations. Infusion of L-[*ring*-$^{13}C_6$]phenylalanine and L-[*ring*-$^2H_2$]tyrosine with muscle and arterialized blood sampling was used to simultaneously assess whole body amino acid kinetics and FSR of mixed muscle protein. Whole body kinetics for phenylalanine and tyrosine were calculated using the equations described by Thompson et al. (32) and Short et al. (30). Briefly, phenylalanine and tyrosine turnover [flux (Q)] were measured from the isotope dilution at isotopic steady state:

$$Q = i \cdot \left(\frac{E_i}{E_p} - 1\right) \tag{1}$$

where i is the isotope infusion rate (μmol·kg body wt^{-1}·h^{-1}) and E_i and E_p correspond to the enrichments of infusate and plasma amino acids, respectively. At isotopic steady state, protein flux (Q) equals the sum of protein synthesis (S) and oxidation (O) as well as the sum of the rate of appearance of meal protein from the gut (I) and protein breakdown (B). The rate of appearance of dietary protein was calculated as total dietary protein intake corrected for a 30% splanchnic extraction rate (38). Whole body protein synthesis rate was calculated as flux minus oxidation.

$$Q = S + O = B + I \tag{2}$$

$$S = Q - O \tag{3}$$

At isotopic steady state, whole body phenylalanine oxidation can be determined from the conversion (hydroxylation) of L-[*ring*-$^{13}C_6$]phenylalanine to L-[*ring*-$^{13}C_6$]tyrosine. The rate of hydroxylation (Q_{pt}) was calculated (32) using the formula

$$Q_{pt} = Q_t \cdot \frac{E_t}{E_p} \cdot \frac{Q_p}{(i_p + Q_p)} \tag{4}$$

where Q_t and Q_p are the flux rates for L-[*ring*-$^2H_2$]tyrosine and labeled phenylalanine, respectively, E_t and E_p are the L-[*ring*-$^{13}C_6$]tyrosine and L-[*ring*-$^{13}C_6$]phenylalanine enrichments in plasma, respectively, and i_p is the infusion rate of the phenylalanine tracer.

FSR was calculated by dividing the increment in enrichment in the product, i.e., protein-bound L-[*ring*-$^{13}C_6$]phenylalanine, by the enrichment of the precursor, i.e., plasma L-[*ring*-$^{13}C_6$]phenylalanine. Muscle FSRs were calculated as follows (20):

$$FSR = \frac{\Delta E_p}{E_{precursor} \cdot t} \cdot 100 \tag{5}$$

where ΔE_p is the delta increment of protein bound L-[*ring*-$^{13}C_6$]phenylalanine during incorporation periods, $E_{precursor}$ is the average plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment during the time period for determination of amino acid incorporation or muscle free L-[ring-$^{13}C_6$]phenylalanine enrichment (corrected for contribution of extracellular water; Ref. 39) determined in the biopsy taken after exercise, t indicates the time interval (h) between biopsies, and the factor 100 is needed to express the FSR in percent per hour (%/h).

Statistics. All data are means ± SE. The plasma insulin, glucose, and amino acid responses were calculated as area under the curve. A two-factor repeated-measures ANOVA with time and treatment as factors was used to compare differences between treatments over time. In case of significant F-ratios, Scheffé's post hoc tests were applied to locate the differences. For non-time-dependent variables, a paired Student's *t*-test was used to compare differences in treatment effect. Statistical significance was set at $P < 0.05$. All calculations were performed using StatView 5.0 (SAS Institute, Cary, NC).

RESULTS

Exercise session. The average workload that was applied in the cycling protocol was 152 ± 6 W for the first 10 min and 198 ± 7 and 137 ± 5 W for the interval protocol, respectively. For the upper body workout, the resistance was set at 40% body wt, which averaged 27.5 ± 0.8 kg. For the leg workout, subjects performed 3 sets of 10 repetitions at 55% 1RM, 3 sets

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at 65% 1RM, and 3 sets at 75% 1RM. This resulted in 119 ± 6, 141 ± 7, and 163 ± 8 kg lifted in the leg press, respectively. On the leg extension 67 ± 2, 79 ± 3, and 90 ± 4 kg were lifted, respectively. Two subjects were unable to complete the last three sets of the leg exercises at 75% 1RM and continued exercise at 65% 1RM. This change in protocol was recorded and repeated during the second experimental day.

Plasma analyses. Plasma glucose and insulin concentrations tended to decrease during the first 30 min of exercise. Thereafter, concentrations increased to 5.9 ± 0.2 and 6.2 ± 0.4 mmol/l (glucose), and 20.9 ± 6.7 mU/l and 30.5 ± 11.7 mU/l (insulin) after 60 min of exercise in the CHO and CHO + PRO treatment, respectively. Total plasma glucose and insulin responses, measured as area under the curve averaged 10.7 ± 0.2 and 11.0 ± 0.3 $\text{mmol}\cdot\text{l}^{-1}\cdot 2\ \text{h}^{-1}$ (glucose), and 28.0 ± 8.3 and 37.7 ± 12.7 $\text{mU}\cdot\text{l}^{-1}\cdot 2\ \text{h}^{-1}$ (insulin), respectively. No significant differences were observed between treatments ($P > 0.05$).

Plasma phenylalanine, tyrosine, leucine, valine, and isoleucine concentrations over time are shown in Fig. 1. Plasma amino acid concentrations were higher during the entire exercise period in CHO + PRO compared with CHO treatment ($P < 0.05$). Plasma amino acid responses, measured as area under the curve for all amino acids are provided in Table 2. Plasma amino acid responses were higher in the CHO + PRO compared with the CHO experiment for all amino

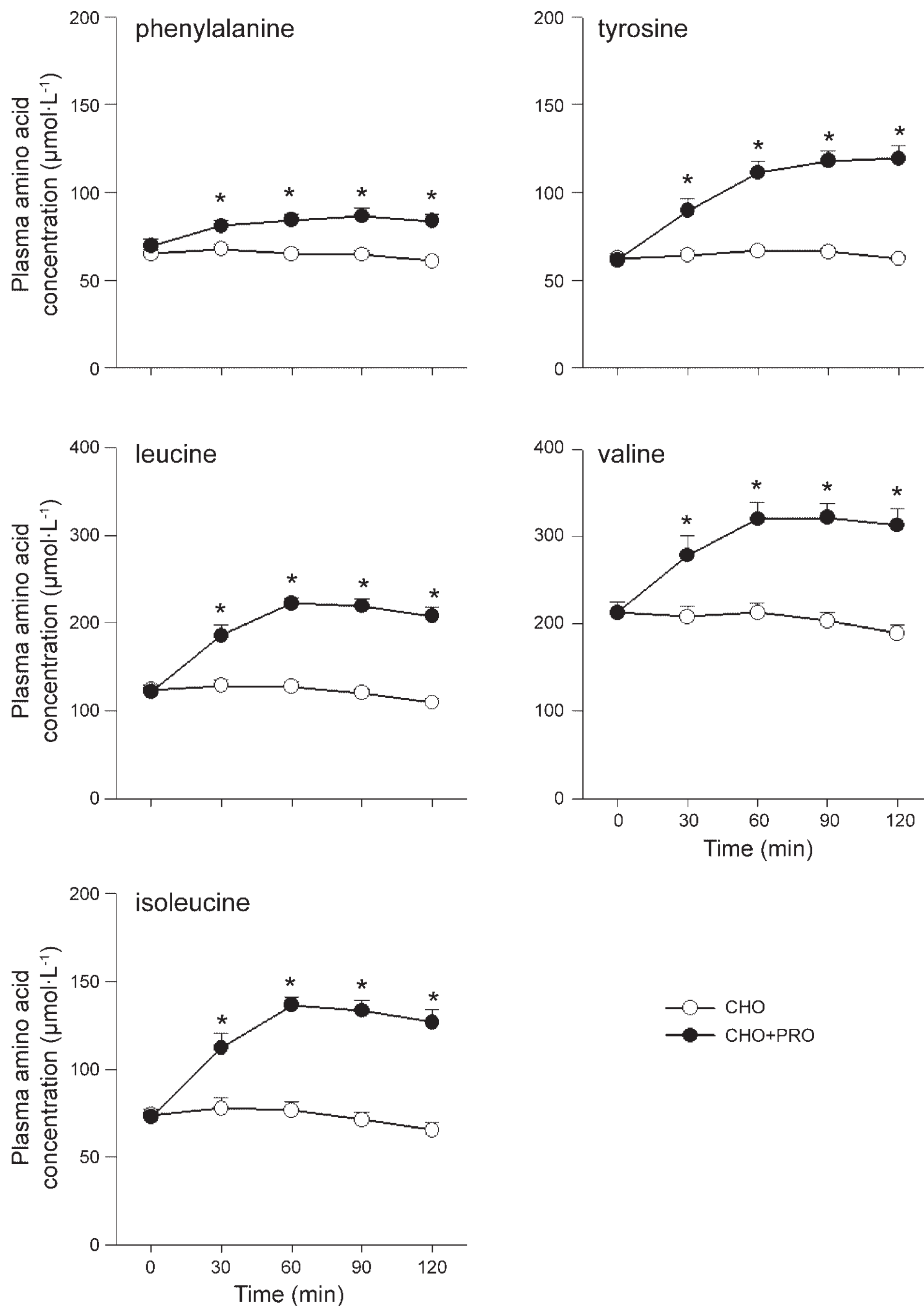

Fig. 1. Plasma phenylalanine, tyrosine, and branched chain amino acid (leucine, isoleucine, and valine) concentrations (μmol/l) in carbohydrate (CHO; $n = 10$) and carbohydrate and protein hydrolysate (CHO + PRO; $n = 10$) treatments. Values are means ± SE. Data were analyzed with repeated-measures ANOVA (treatment × 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 isoleucine: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. Plasma valine: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. *Significantly different from CHO (Scheffé's post hoc test, $P < 0.05$).

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Table 2. *Plasma amino acid responses*

Amino Acid	CHO (n = 10)	CHO + PRO (n = 10)
Phenylalanine	0.130±0.005	0.164±0.007*
Tyrosine	0.130±0.004	0.204±0.011*
Leucine	0.246±0.010	0.396±0.013*
Valine	0.412±0.020	0.591±0.034*
Isoleucine	0.148±0.010	0.241±0.010*
Glutamic acid	0.262±0.021	0.296±0.022
Asparagine	0.124±0.006	0.167±0.009*
Serine	0.229±0.012	0.294±0.017*
Glutamine	1.268±0.028	1.413±0.039*
Hystidine	0.175±0.010	0.209±0.008*
Glycine	0.463±0.015	0.497±0.023
Threonine	0.249±0.014	0.344±0.032*
Citrulline	0.079±0.010	0.090±0.004
Arginine	0.186±0.011	0.242±0.009*
Alanine	0.984±0.065	1.232±0.055*
Taurine	0.276±0.017	0.280±0.025
α-Aminobutyrate	0.025±0.002	0.033±0.004*
Methionine	0.046±0.003	0.075±0.003*
Tryptophan	0.104±0.006	0.123±0.004*
Ornithine	0.112±0.007	0.132±0.007*
Lysine	0.298±0.010	0.404±0.020*

Values are means ± SE. CHO, carbohydrate; PRO, protein hydrolysate. Plasma amino acid responses expressed as area under the curve (in $mmol \cdot l^{-1} \cdot 2\ h^{-1}$). *Significantly different from CHO ($P < 0.05$).

acids ($P < 0.05$), except for glutamic acid, glycine, citrulline, and taurine. The time course of the changes in plasma L-[*ring*-$^{13}C_6$]phenylalanine, L-[*ring*-$^{2}H_2$]tyrosine, and L-[*ring*-$^{13}C_6$]tyrosine enrichments is presented in Fig. 2. Overall, enrichments were significantly lower during the entire exercise trial in the CHO + PRO compared with the CHO treatment ($P < 0.01$).

Whole body protein metabolism. Phenylalanine flux was higher in the CHO + PRO compared with the CHO experiment and averaged 70 ± 3 and 54 ± 2 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, respectively ($P < 0.01$). Tyrosine flux was also higher in the CHO + PRO experiment, 115 ± 8 vs. 58 ± 3 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, respectively ($P < 0.01$). Whole body protein breakdown was 8.4 ± 3.6% lower ($P = 0.066$), and protein oxidation and synthesis rates were 77 ± 17 and 33 ± 3% higher ($P < 0.01$) in the CHO + PRO compared with the CHO experiment. As a consequence, whole body net protein balance was negative in CHO, whereas a positive net balance was achieved in the CHO + PRO treatment (−4.4 ± 0.3 vs. 16.3 ± 0.4 μmol $phenylalanine \cdot kg^{-1} \cdot h^{-1}$, respectively; $P < 0.01$; Fig. 3*A*). Without correction for splanchnic extraction of dietary protein (38), whole body protein breakdown rates would be 26 ± 4% lower in the CHO + PRO compared with CHO experiment. Furthermore, whole body protein balance would average 25.4 ± 0.4 vs. −4.4 ± 0.3 μmol

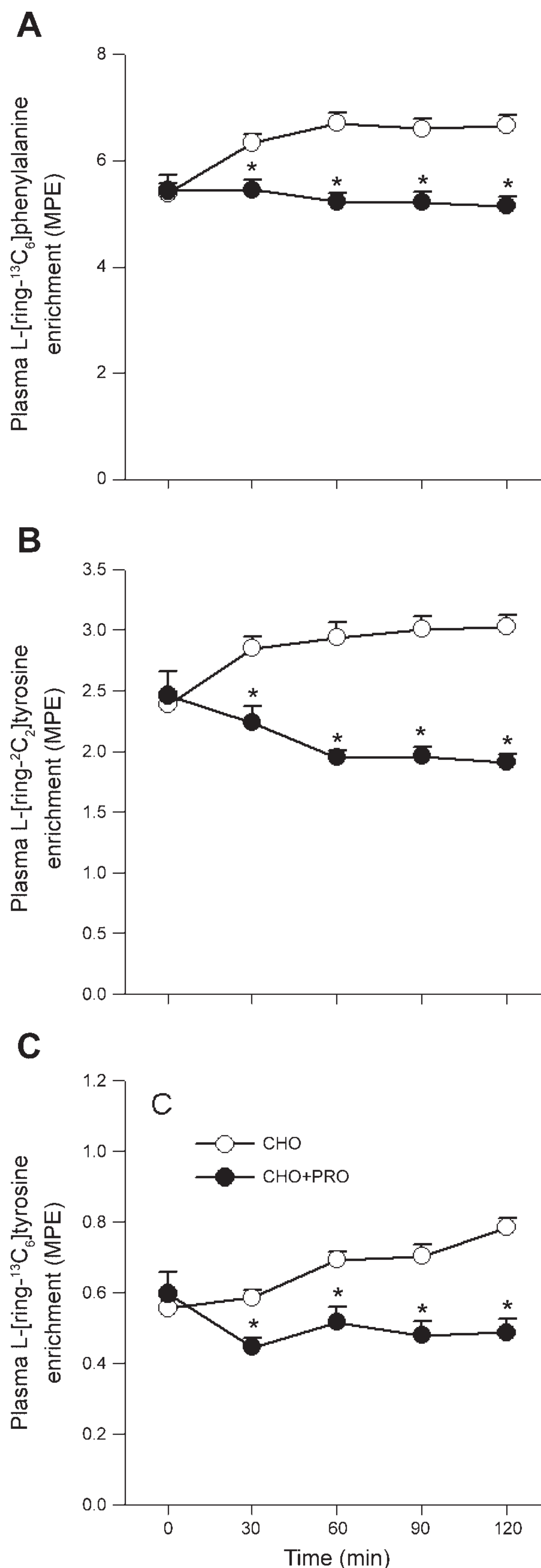

Fig. 2. Plasma L-[ring-$^{13}C_6$]phenylalanine (*A*), L-[*ring*-$^{2}H_2$]tyrosine (*B*), and L-[*ring*-$^{13}C_6$]tyrosine (*C*) enrichment during the CHO (n = 10) and CHO + + PRO (n = 10) experiment. MPE, mole percent excess. Values are means ± SE. Data were analyzed with repeated-measures ANOVA (treatment × time). Plasma L-[ring-$^{13}C_6$]phenylalanine enrichment: treatment effect, $P < 0.01$; time effect, $P < 0.05$; interaction of treatment and time, $P < 0.01$. Plasma L-[*ring*-$^{2}H_2$]tyrosine enrichment: treatment effect, $P < 0.01$; time effect, $P > 0.05$; interaction of treatment and time, $P < 0.01$. Plasma L-[*ring*-$^{13}C_6$]tyrosine enrichment: treatment effect, $P < 0.01$; time effect, $P < 0.01$; interaction of treatment and time, $P < 0.01$. *Significantly different from CHO (Scheffé's post hoc test, $P < 0.05$).

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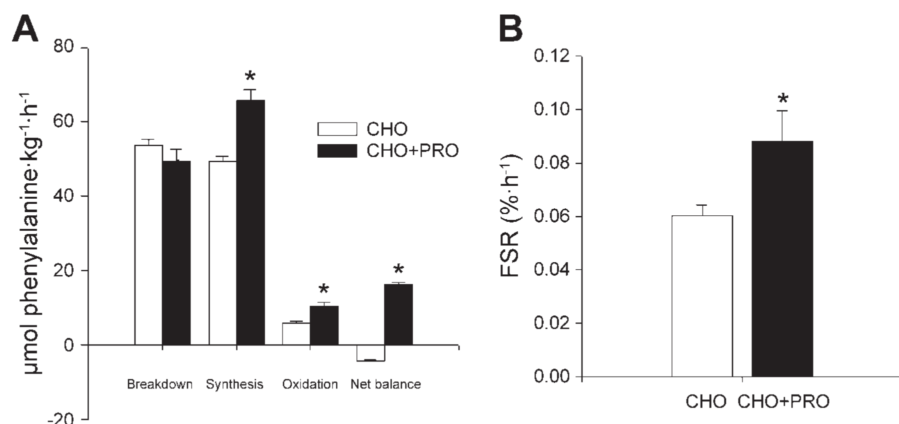

Fig. 3. Whole body protein breakdown, synthesis, oxidation rates, and net protein balance (*A*; expressed as μmol phenylalanine·kg^{-1}·h^{-1}; $n = 10$) and fractional synthesis rate (FSR) of mixed muscle protein (*B*; $n = 9$) in the CHO and CHO + PRO experiment. Values are means ± SE. Data were analyzed with the Student's *t*-test for paired samples. *Significantly different from CHO (Scheffé's post hoc test, $P < 0.05$).

phenylalanine·kg^{-1}·h^{-1} in the CHO + PRO and CHO experiment, respectively ($P < 0.01$). The latter provides the upper boundary value for net protein balance.

Mixed muscle protein synthesis rates. Mixed muscle protein FSR, using mean plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment as the precursor, were 49 ± 22% higher in the CHO + PRO vs. CHO experiment and averaged 0.088 ± 0.012 vs. 0.060 ± 0.004%/h, respectively (Fig. 3*B*; $P < 0.05$). When the intramuscular free intracellular L-[*ring*-$^{13}C_6$]phenylalanine enrichments (corrected for contribution of extracellular water; Ref. 39) were applied as the precursor pool, similar results were obtained. FSR values averaged 0.072 ± 0.004 vs. 0.104 ± 0.015%/h in the CHO and CHO + PRO experiment, respectively ($P = 0.088$). Plasma and muscle amino acid enrichments are presented in Table 3. In one subject, a postexercise muscle biopsy could not be obtained. Therefore, FSR and free intracellular tracer enrichments are presented for nine subjects only.

DISCUSSION

In the present study, we prove that, even in the fed state, protein coingestion (0.15 g·kg^{-1}·h^{-1}) with carbohydrate (0.15 g·kg^{-1}·h^{-1}) improves whole body protein balance and increases mixed muscle protein synthesis rate during resistance-type exercise. Previous studies (3, 26, 34) have reported that an acute bout of resistance-type exercise stimulates both muscle protein synthesis and breakdown rates. However, in the absence of subsequent food intake net protein balance remains negative (3, 26, 34). Carbohydrate ingestion after exercise has been reported to attenuate the exercise-induced increase in the protein breakdown rate (9, 25, 28), but protein and/or amino acid administration is prerequisite to stimulate muscle protein synthesis and, as such, to achieve a positive net muscle protein balance after exercise (4, 10, 20, 25, 27, 33).

In contrast to the wealth of data on the effect of exercise on muscle protein metabolism during postexercise recovery (3–10, 17, 20, 25–29, 33, 34), only a little information is available on muscle protein turnover during exercise. Few studies have examined the effect of endurance (12, 18, 40, 41)- and resistance (14, 15, 35)-type exercise on whole body and muscle protein synthesis rates during exercise. These studies have reported either a decrease (14, 40, 41) or no effect (12, 15, 41) of exercise on muscle protein synthesis rates. Only two studies (18, 35) have addressed the effect of nutrition on muscle protein synthesis during exercise. Their results show that the combined ingestion of carbohydrate and protein stimulates whole body protein synthesis during endurance (18)- and resistance (35)-type exercise. Koopman et al. (18) determined whole body protein turnover during 5 h of endurance exercise, during which subjects ingested carbohydrate drinks with or without added protein. Protein coingestion during exercise was shown to stimulate whole body protein synthesis rate and improve net protein balance. Furthermore, Tipton et al. (35) reported that protein ingestion before resistance-type exercise stimulates postexercise muscle protein synthesis more effectively than protein ingestion after exercise. The latter was attributed to a more rapid provision of amino acids to the muscle during the acute stages of postexercise recovery (35).

Table 3. *Plasma and muscle AA tracer enrichments*

	CHO	CHO + PRO	*P* Value
Plasma AA enrichments ($n = 10$)			
L-[*ring*-$^{13}C_6$]phenylalanine	0.0678±0.0020	0.0565±0.0021	<0.01
L-[*ring*-$^{2}H_2$]tyrosine	0.0293±0.0011	0.0219±0.0010	<0.01
L-[*ring*-$^{13}C_6$]tyrosine	0.0066±0.0002	0.0050±0.0002	<0.01
Muscle AA enrichments ($n = 9$)			
L-[*ring*-$^{13}C_6$]phenylalanine	0.0536±0.0028	0.0467±0.0019	0.056
L-[*ring*-$^{2}H_2$]tyrosine	0.0171±0.0024	0.0144±0.0009	0.337
L-[*ring*-$^{13}C_6$]tyrosine	0.0336±0.0121	0.0138±0.0044	0.102
δ-Enrichment muscle protein ($n = 9$)			
L-[*ring*-$^{13}C_6$]phenylalanine	0.000092±0.000013	0.000076±0.000006	0.230

Values are means ± SE. AA, amino acid. Data were analyzed with a paired sample *t*-test. Plasma AA enrichments represent the average plasma AA enrichment during exercise. Muscle AA enrichments represent the AA enrichment in the muscle biopsy taken after 2 h of exercise. δ-Enrichment muscle protein represents the increment in muscle protein enrichment from 0 to 2 h of exercise.

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However, based on their data, it could also be speculated that protein ingestion before and/or during resistance-type exercise already stimulates muscle protein synthesis during exercise activities.

In the present study, we observed that protein coingestion elevates whole body protein synthesis rates and, as such, improves whole body net protein balance during resistance-type exercise (Fig. 3*A*). Of course, estimates of whole body protein synthesis and/or net protein balance do not necessarily reflect skeletal muscle protein synthesis (13). Therefore, we also assessed muscle protein synthetic rates directly by determining the incorporation rate of ^{13}C-labeled phenylalanine in the mixed muscle protein pool. Our results show that protein coingestion substantially increases muscle protein synthesis during exercise, with mixed muscle protein synthesis rates averaging 0.088 ± 0.012 vs. 0.060 ± 0.004%/h in the CHO + PRO and CHO experiment, respectively ($P < 0.05$; Fig. 3*B*). It should be noted that the choice of the precursor pool, i.e., plasma or muscle free phenylalanine enrichment, can have a substantial effect on the calculated muscle protein synthesis rates. Therefore, we also calculated muscle protein synthesis rates based on the muscle free phenylalanine enrichment (Table 3). The latter resulted in similar findings (0.104 ± 0.015 vs. 0.072 ± 0.004%/h, respectively), which did not reach statistical significance ($P = 0.088$). We generally prefer the use of plasma enrichment data for precursor pool enrichment because we feel that multiple plasma samples collected throughout the exercise session give a better estimate of the changes in precursor enrichment over time than the enrichment in the muscle free amino acid pool, which is only measured before and immediately after exercise.

In contrast to previous studies investigating subjects in the overnight fasted state, subjects in the present study were investigated in a postprandial state. The latter might explain why the fractional muscle protein synthesis rates during exercise are quite similar to the protein synthesis rates observed previously during postexercise recovery (12, 20, 34). Dreyer et al. (14) reported lower muscle protein synthesis rates during resistance-type exercise, compared with rest and postexercise recovery. The apparent discrepancy might be explained by the fact that subjects in the present study were investigated in the fed state and ingested carbohydrate with or without additional protein during exercise. In accordance, previous work (19) from our laboratory has shown that coingestion of protein before resistance-type exercise enables the mammalian target of rapamycin signal transduction pathway to be activated during exercise. Furthermore, reduced endogenous amino acid availability from the gut and/or intramuscular free amino acid pool in the overnight-fasted state could likely attenuate the exercise-induced increase in muscle protein synthesis rate.

To increase the practical relevance of the study, we investigated our subjects in a fed state, i.e., during exercise performed in the evening after a full day of standardized dietary practice. To prevent potential flaws in the applied methodology due to large perturbations in circulating plasma amino acid concentrations, we started the exercise session 3 h after the last meal was ingested. Furthermore, during the exercise protocol we administered carbohydrate or carbohydrate-protein beverages every 15 min to allow a more continuous supply of glucose and amino acids from the gut. The latter prevented perturbations in plasma amino acid concentrations (Fig. 1) and enrichments (Fig. 2) throughout the latter stages of exercise.

Protein coingestion during exercise substantially augmented muscle protein synthesis rates (Fig. 3*B*). Consequently, our findings suggest that protein coingestion during exercise could represent an effective dietary strategy to further augment muscle protein accretion by creating a larger time frame for muscle protein synthesis to be elevated. However, as the progress in metabolomics and proteomics does not yet allow us to assess FSRs of individual proteins in vivo in human skeletal muscle, we cannot specify which proteins are being synthesized to a greater extent. Furthermore, it remains to be determined whether the observed effect of protein coingestion on mixed muscle protein synthesis during exercise is restricted to intermittent, resistance-type exercise activities. It is attractive to assume that AMPK is not continually activated throughout intermittent-type exercise activities when the exercise is performed in the fed state. The latter could prevent its proposed inhibitory effect on muscle protein synthesis (1, 14, 21) and allow protein synthesis rates to be increased during the resting periods between sets. It would be of interest to address the potential of protein coingestion to stimulate muscle protein synthesis during more continuous, endurance-type exercise activities.

In conclusion, even in a fed state, protein coingestion before and during resistance-type exercise improves whole body protein balance and stimulates muscle protein synthesis during exercise. Protein coingestion before and/or during resistance-type exercise might be advocated to further improve skeletal muscle reconditioning during resistance-type exercise training.

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DISCLOSURES

A. K. Kies is a researcher with DSM Food Specialties.

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