Am J Physiol Endocrinol Metab 288: E645–E653, 2005.
First published November 23, 2004; doi:10.1152/ajpendo.00413.2004.

Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects

René Koopman,[1] Anton J. M. Wagenmakers,[4] Ralph J. F. Manders,[1] Antoine H. G. Zorenc,[1] Joan M. G. Senden,[1] Marchel Gorselink,[3] Hans A. Keizer,[2] and Luc J. C. van Loon[1,2]

Departments of [1]Human Biology and [2]Movement Sciences, Nutrition Research Institute Maastricht, Maastricht University, Maastricht; [3]Numico Research BV, Wageningen, The Netherlands; and [4]School of Sport and Exercise Sciences, University of Birmingham, Birmingham, United Kingdom

Submitted 1 September 2004; accepted in final form 22 November 2004

Koopman, René, Anton J. M. Wagenmakers, Ralph J. F. Manders, Antoine H. G. Zorenc, Joan M. G. Senden, Marchel Gorselink, Hans A. Keizer, and Luc J. C. van Loon. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab* 288: E645–E653, 2005. First published November 23, 2004; doi:10.1152/ajpendo.00413.2004.—The present study was designed to determine postexercise muscle protein synthesis and whole body protein balance following the combined ingestion of carbohydrate with or without protein and/or free leucine. Eight male subjects were randomly assigned to three trials in which they consumed drinks containing either carbohydrate (CHO), carbohydrate and protein (CHO+PRO), or carbohydrate, protein, and free leucine (CHO+PRO+Leu) following 45 min of resistance exercise. A primed, continuous infusion of L-[*ring*-$^{13}C_6$]phenylalanine was applied, with blood samples and muscle biopsies collected to assess fractional synthetic rate (FSR) in the vastus lateralis muscle as well as whole body protein turnover during 6 h of postexercise recovery. Plasma insulin response was higher in the CHO+PRO+Leu compared with the CHO and CHO+PRO trials (+240 ± 19% and +77 ± 11%, respectively, $P < 0.05$). Whole body protein breakdown rates were lower, and whole body protein synthesis rates were higher, in the CHO+PRO and CHO+PRO+Leu trials compared with the CHO trial ($P < 0.05$). Addition of leucine in the CHO+PRO+Leu trial resulted in a lower protein oxidation rate compared with the CHO+PRO trial. Protein balance was negative during recovery in the CHO trial but positive in the CHO+PRO and CHO+PRO+Leu trials. In the CHO+PRO+Leu trial, whole body net protein balance was significantly greater compared with values observed in the CHO+PRO and CHO trials ($P < 0.05$). Mixed muscle FSR, measured over a 6-h period of postexercise recovery, was significantly greater in the CHO+PRO+Leu trial compared with the CHO trial (0.095 ± 0.006 vs. 0.061 ± 0.008%/h, respectively, $P < 0.05$), with intermediate values observed in the CHO+PRO trial (0.0820 ± 0.0104%/h). We conclude that coingestion of protein and leucine stimulates muscle protein synthesis and optimizes whole body protein balance compared with the intake of carbohydrate only.

resistance exercise; protein metabolism; supplements; protein intake

RESISTANCE TRAINING CAN SUBSTANTIALLY AUGMENT skeletal muscle size, and it has been shown that, after resistance exercise, mixed muscle protein synthesis is stimulated for up to 48 h (12, 35). Simultaneously, the rate of muscle protein degradation is also increased after exercise (7, 35). However, in the absence of food intake, net muscle protein balance remains negative (7, 35, 36). Carbohydrate supplementation in the postexercise recovery phase has been shown to result in a decrease in urinary 3-methylhistidine and urea excretion, suggesting that protein degradation is reduced (40). However, muscle protein synthesis does not seem to be affected after carbohydrate ingestion (10, 40). Although carbohydrate ingestion improves net leg amino acid balance compared with water or placebo intake, the net balance remains negative (10). It has been shown by use of different tracer methods/models (e.g., tracer incorporation measurements in muscle, leg amino acid exchange methods, whole body measurements) that the combined infusion or ingestion of carbohydrate and protein/amino acids is needed to increase protein synthesis rate, to reduce protein degradation, and thus to elicit a positive net protein balance under resting conditions (13, 33) and during recovery from resistance exercise (8, 11, 39, 43, 44). Moreover, in a recent study (25), we have shown that the combined ingestion of protein and carbohydrate leads to a positive protein balance during ultraendurance exercise and subsequent recovery. The combined ingestion of carbohydrate and protein/amino acids in the postexercise recovery phase can stimulate protein metabolism in several ways. Besides providing amino acids as precursors for protein synthesis, combined ingestion of carbohydrate and protein/amino acids can elicit a strong insulinotropic response (34, 38, 49).

Combined ingestion of carbohydrate with protein and/or free amino acids (34, 38, 47–49), as well as intravenous infusion of free amino acids (16, 17), has been shown to further elevate plasma insulin levels. It has been speculated that such elevated insulin concentrations can stimulate the uptake of selected amino acids (6) and muscle protein synthesis rate (6, 20). In addition, insulin has been reported to effectively inhibit proteolysis (9, 18, 19). Nonetheless, the exact mechanisms responsible for the stimulatory effects of carbohydrate and protein/amino acid ingestion on muscle protein synthesis during recovery from resistance exercise have not yet been established in detail. For example, in animal models, free leucine has been shown to stimulate protein synthesis by an insulin-independent mechanism (2, 3). Consequently, it has been speculated that a nutritional supplement containing carbohydrate, protein, and free leucine could represent an effective tool to further increase postexercise muscle protein synthesis and/or to inhibit protein degradation.

The main aim of the present study was to investigate the effect of carbohydrate (CHO), carbohydrate and protein

Address for reprint requests and other correspondence: R. Koopman, Dept. of Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands (E-mail: R.Koopman@HB.unimaas.nl).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

(CHO+PRO), and carbohydrate, protein, and leucine (CHO+PRO+Leu) ingestion on the anabolic response to resistance exercise. By measuring the incorporation rate of labeled amino acids in the skeletal muscle protein pool, mixed muscle protein synthesis rates were directly measured during 6 h of recovery from strenuous resistance exercise. A primed constant infusion of L-[*ring*-$^{13}C_6$]phenylalanine was combined with plasma and muscle tissue sampling to simultaneously measure mixed muscle fractional protein synthetic rate and whole body protein turnover.

METHODS

Subjects. Eight healthy, untrained male volunteers (age: 22.3 ± 0.9 yr; weight: 74.1 ± 3.5 kg; height: 1.81 ± 0.02 m; body mass index: 22.5 ± 0.9 kg/m^2; %body fat: 16.0 ± 3%), with no history of participating in any regular exercise program, were recruited for the present study. All subjects were informed of the nature and possible risks of the experimental procedures before their informed consent was obtained, the latter after approval by the Medical Ethics Committee of the Academic Hospital, Maastricht, The Netherlands.

Pretesting. All subjects participated in an orientation trial to become familiarized with the exercise protocol and the equipment. Proper lifting technique was demonstrated and practiced for each of the two lower-limb exercises (leg press and leg extension). Thereafter, maximum strength was estimated using the multiple-repetitions testing procedure (29). In another session, ≥1 wk before the first experimental trial, subjects' one-repetition maximum (1-RM) was determined (26). After the subjects warmed up, the load was set at 90–95% of the estimated 1-RM and was increased after each successful lift until failure. A 5-min resting period between the subsequent attempts was allowed. A repetition was valid if the subject was able to complete the entire lift in a controlled manner without assistance. The average 1-RM for the leg press was 185 ± 9 kg, and the mean 1-RM for the leg extension was 106 ± 4 kg.

Diet and activity before testing. All subjects consumed a standardized meal [66.1 ± 2.7 kJ/kg, consisting of 72 energy% (En%) carbohydrate, 23 En% protein, and 5 En% fat] the evening before the trials. All volunteers were instructed to refrain from any sort of heavy physical exercise and to keep their diet as constant as possible 3 days before the trials. In addition, subjects were asked to record their food intake for 48 h before the start of the first experimental trial and to consume the same diet 48 h before the start of the second and third tests.

Experimental trials. Each subject participated in three trials separated by ≥7 days, in which recovery drinks containing CHO, CHO+PRO, or CHO+PRO+Leu were tested in a randomized and double-blind fashion. Each trial lasted ~8 h. Repeated boluses of a given test drink were ingested after the exercise protocol to ensure a continuous supply of ample glucose and amino acids to the muscle. Plasma and muscle samples were collected during a 6-h postexercise period. These trials 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 vastus lateralis muscle.

Protocol. At 8:00 AM, after an overnight fast, subjects reported to the laboratory, and a Teflon catheter was inserted into an antecubital vein for stable isotope infusion. A second Teflon catheter was inserted in a heated dorsal hand vein of the contralateral arm placed in a hot box (60°C), for arterialized blood sampling. After collection of a resting blood sample, a single intravenous dose of L-[*ring*-$^{13}C_6$]phenylalanine (2 μmol/kg) was administered to prime the phenylalanine pool. Thereafter, tracer infusion was started with subjects resting in a supine position. After 75 min of infusion, a second blood sample was collected, and subjects started the resistance exercise protocol. The exercise session consisted of eight sets of eight repetitions on the horizontal leg press machine (Technogym, Rotterdam, The Netherlands) and eight sets of eight repetitions on the leg extension machine (Technogym). Both exercises were performed at 80% of the subjects' individual 1-RM with 2-min rest intervals between the sets and in total required ~45 min to complete. All subjects were verbally encouraged during the test to complete the whole protocol. At the end of the resistance exercise protocol ($t = 0$), subjects rested supine, and an arterialized blood sample from the heated hand vein as well as a muscle biopsy from the vastus lateralis muscle were collected. Subjects then received an initial bolus (3 ml/kg) of a given test drink. Repeated boluses (3 ml/kg) were taken every 30 min until $t = 330$ min after exercise. Blood samples were subsequently taken from the heated hand vein at $t = 15$, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min, with an additional muscle biopsy taken at 360 min after exercise.

Muscle biopsies. Muscle biopsies were obtained from the middle region of the vastus lateralis (15 cm above the patella) and ~2 cm below entry through the fascia by means of the percutaneous needle biopsy technique (5). Muscle samples were immediately freed from blood, visible fat, and connective tissue, rapidly frozen in liquid nitrogen, and stored at −80°C for measurement of the amino acid enrichment in the muscle free amino acid pool and in mixed muscle protein.

Beverages. Subjects received a beverage volume of 3 ml/kg every 30 min to ensure a given dose of 0.3 g carbohydrate/kg (50% as glucose and 50% as maltodextrin) and 0.2 g/kg of a protein hydrolysate every hour, with or without the addition of 0.1 $g \cdot kg^{-1} \cdot h^{-1}$ leucine. The total amount of protein (0.2 $g \cdot kg^{-1} \cdot h^{-1}$) provided in both the CHO+PRO and CHO+PRO+Leu trials by far exceeded the calculated amount of protein that was estimated to provide sufficient precursor substrate to sustain maximal protein synthesis rates for ≥6 h (50). Repeated boluses were administered to enable a continuous ample supply of amino acids in the circulation, preventing perturbations in L-[*ring*-$^{13}C_6$]phenylalanine enrichments. The whey protein hydrolysate used in the present study contained 9.95% leucine; consequently, the total amount of leucine administered in the CHO+PRO and CHO+PRO+Leu trial was 0.02 vs. 0.12 $g \cdot kg^{-1} \cdot h^{-1}$, respectively.

The compositions of all test drinks are listed in Table 1. Glucose and maltodextrin were obtained from AVEBE (Veendam, The Netherlands). Whey protein hydrolysate was prepared by Numico Research (Wageningen, The Netherlands). Leucine was purchased from BUFA (Uitgeest, The Netherlands). To make the taste comparable in all trials, beverages were uniformly flavored by adding 0.2 g of sodium saccharinate solution (25% wt/wt), 1.8 g of citric acid solution (50% wt/wt), and 5 g of cream vanilla flavor (Numico Research) for each liter of beverage. Trials were performed in a randomized order, with test drinks provided in a double-blind fashion.

Tracer infusion. L-[*ring*-$^{13}C_6$]phenylalanine (99% enriched; Cambridge Isotopes, Andover, MA) was dissolved in 0.9% saline before infusion. The phenylalanine pool was primed with an infusion dose of 2 μmol/kg. Thereafter, continuous intravenous infusion of the iso-

Table 1. *Composition of beverages*

Test Drink	CHO	CHO+PRO	CHO+PRO+Leu
Whey protein		33.3	33.3
Leucine			16.6
Glucose	25	25	25
Maltodextrin	25	25	25
Sodium saccharinate	0.2	0.2	0.2
Citric acid	1.8	1.8	1.8
Cream vanilla	5	5	5
Water	≤1.00 liter		

Values are expressed in g/liter beverage. CHO, carbohydrate; CHO+PRO, CHO + protein; CHO+PRO+Leu, CHO + PRO + free leucine.

topes was performed using a calibrated IVAC 560 pump (San Diego, CA) with an average infusion rate (IR) of 0.046 ± 0.001 $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

Analysis. Blood samples were collected in EDTA-containing tubes and centrifuged at 1,000 *g* and 4°C for 5 min. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C. Insulin was analyzed by radioimmunoassay (Insulin RIA kit; Linco Research, St. Charles, MO). Plasma (500 μl) for amino acid analysis was deproteonized on ice with 100 μl of 24% (wt/vol) 5-sulfosaliculic acid and mixed (vortexing), and the clear supernatant was collected after centrifugation. Plasma amino acid 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 (15). The exact phenylalanine concentration in the infusates (8.06 ± 0.21 mmol/l) was measured using the same method. Plasma phenylalanine and tyrosine were derivatized to their *tert*-butyldimethylsilyl (TBDMS) derivatives and their ^{13}C enrichments determined by electron ionization gas chromatography-mass spectrometry (GC-MS, Finnigan Incos XL) using selected ion monitoring of masses 336 and 342 for unlabeled and labeled phenylalanine, respectively, and masses 466 and 472 for unlabeled and labeled tyrosine (51).

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 2 ml of ice-cold 2% perchloric acid (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 and L-[*ring*-$^{13}C_6$]-tyrosine enrichments could be measured using their TBDMS derivatives on a GC-MS. The protein pellet was washed with three additional 2-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 heated to 120°C and 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) with 2 M NH_4OH. Thereafter, the eluate was dried, and the purified amino acid fraction was derivatized into the *N*-acetyl-methyl (NAM)-esters to determine the ^{13}C enrichment of protein bound phenylalanine enrichment using GC-IRMS (Finnigan, MAT 252).

Calculations. Infusion of L-[*ring*-$^{13}C_6$]phenylalanine with muscle and arterialized blood sampling was used to simultaneously assess whole body amino acid kinetics and FSR of mixed muscle protein. Whole body rates of appearance (R_a) and disappearance (R_d) of phenylalanine were calculated using the non-steady-state Steele equations, adapted for stable isotope methodology (41).

$$R_a = \frac{F - V[(C_2 + C_1)/2]\,[(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2} \quad (1)$$

$$R_d = R_a - V \cdot \left(\frac{C_2 - C_1}{t_2 - t_1}\right) \quad (2)$$

where F is the infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$); V is the distribution volume for phenylalanine (125 ml/kg) (14); C_1 and C_2 are the phenylalanine concentrations (mmol/l) in arterialized plasma at *times 1* (t_1) and *2* (t_2), respectively; and E_1 and E_2 are the plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichments [expressed in tracer-to-tracee ratio (TTR)]. As whole body R_a comprises the phenylalanine R_a arising from protein breakdown (B) and protein intake (I), whole body protein breakdown can be calculated as follows:

$$B = R_a - I \quad (3)$$

As whole body R_d comprises the phenylalanine R_d from the free amino acid pool in the blood due to protein synthesis (S) and oxidation, whole body protein synthesis can be calculated as R_d minus oxidation. The rate of phenylalanine oxidation was calculated using the phenylalanine balance model from Thompson et al. (42). Whole body phenylalanine oxidation (Q_{pt}) can be determined from the conversion (hydroxylation) of L-[*ring*-$^{13}C_6$]phenylalanine to L-[*ring*-$^{13}C_6$]tyrosine without the necessity to measure $^{13}CO_2$ enrichment in breath gases (42). The rate of hydroxylation can be calculated using the formula

$$Q_{pt} = \frac{P_t}{P_p} \cdot \frac{Q_{p^2}}{\left(\frac{E_p}{E_t} - 1\right) \cdot (F - Q_p)} \quad (4)$$

where P_t/P_p (=0.73) is the molar ratio of fluxes of tyrosine and phenylalanine arising from protein catabolism (31); Q_p equals R_d under the steady-state conditions that we observed. E_p and E_t are the L-[*ring*-$^{13}C_6$]phenylalanine and L-[*ring*-$^{13}C_6$]tyrosine enrichments [expressed in atom percent excess (APE)], respectively. Whole body protein synthesis was calculated using

$$S = R_d - Q_{pt} \quad (5)$$

Mixed muscle protein FSR was calculated by dividing the increment in enrichment in the product, i.e., protein bound L-[*ring*-$^{13}C_6$]phenylalanine (TTR), by the enrichment of the precursor. Due to the large muscle sample size that is required to make an estimate of the enrichment in the aminoacyl-tRNA pool (generally regarded to be the true protein synthesis precursor pool) and the inherent analytic complexity of such measurements, we used plasma L-[*ring*-$^{13}C_6$]phenylalanine (TTR) and free muscle L-[*ring*-$^{13}C_6$]phenylalanine (TTR) enrichments as surrogates to provide an estimate of the lower boundary (based on plasma precursor enrichments) and the higher boundary (based on intracellular muscle precursor enrichments) for the true FSR of mixed muscle proteins. Muscle FSRs were calculated as follows:

$$FSR = \frac{\Delta E_p \cdot 2}{E_{precursor} \cdot t} \cdot 100 \quad (6)$$

where ΔE_p is the Δincrement of protein bound L-[*ring*-$^{13}C_6$]phenylalanine during incorporation periods. $E_{precursor}$ is *1*) the average plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment during the time period for determination of amino acid incorporation; *2*) the free muscle L-[*ring*-$^{13}C_6$]phenylalanine enrichment in muscle biopsy taken at 6 h after exercise; and *3*) the free muscle L-[*ring*-$^{13}C_6$]phenylalanine enrichment in muscle biopsy taken at 6 h after exercise corrected for the contribution of extracellular water, as previously described (50). *t* Indicates the time interval (h) between biopsies, and the factor 100 is needed to express the FSR in percent per hour (%/h). The factor 2 arises because, in the NAM-ester of L-[*ring*-$^{13}C_6$]phenylalanine molecule, 6 of a total of 12 carbon atoms are labeled.

Statistics. All data are expressed as means ± SE. The plasma essential amino acid, insulin, and glucose responses were calculated as area under the curve above predrink values. Statistical analysis of the data was performed using a one-factor repeated-measures analysis of variance (ANOVA) for non-time-dependent variables. 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 difference between trials, Tukey's post hoc test was applied to locate these differences. Statistical significance was set at $P < 0.05$.

RESULTS

Plasma insulin levels increased in all trials during the first 15–30 min after initial beverage ingestion. After this strong initial increase, insulin concentrations plateaued after 3–4 h of

recovery. Plasma insulin concentrations in the CHO+PRO and CHO+PRO+Leu trials increased more compared with the CHO trial (Fig. 1*A*). The insulin response, expressed as area under the curve (above baseline values) during the entire 6-h postexercise period (Fig. 1*B*), was substantially greater in the CHO+PRO+Leu trial compared with the CHO and CHO+PRO trials (12.6 ± 1.2 vs. 3.7 ± 0.4 and 7.4 ± 0.9 U·6 h·liter^{-1}, respectively, $P < 0.01$), which represents a +240 ± 19 and +77 ± 11% increase, respectively. The observed insulin response during the recovery period in the CHO+PRO trial was increased, with +98 ± 17% compared with the CHO trial ($P < 0.05$). Plasma glucose levels significantly increased following administration of the first beverage, after which they tended to decrease. The glucose response, expressed as area under the curve above baseline values, was significantly lower in the CHO+PRO+Leu trial compared with the CHO trial (96 ± 62 vs. 320 ± 65 mmol·6 h·liter^{-1}, $P < 0.05$), with an intermediate value for the CHO+PRO trial (216 ± 48 mmol·6 h·liter^{-1}).

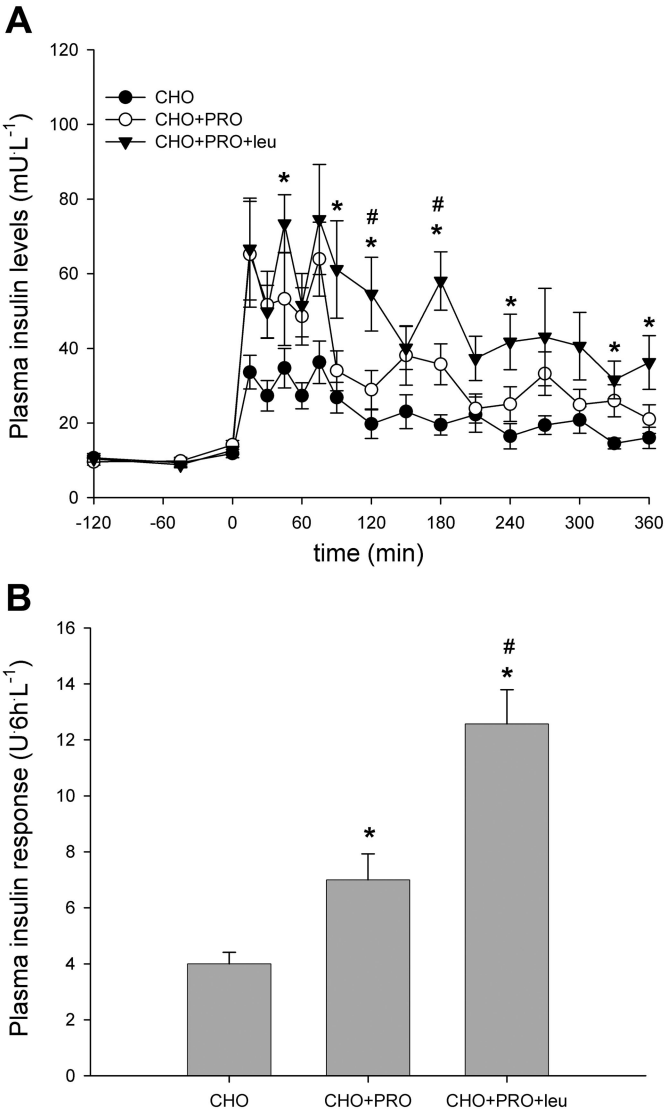

Fig. 1. A: plasma insulin concentration (expressed in mU/l). *B*: plasma insulin response (expressed as area under the curve minus baseline values) during the different trials while carbohydrate (0.3 g·kg^{-1}·h^{-1}; CHO), carbohydrate and protein (0.3 and 0.2 g·kg^{-1}·h^{-1}, respectively; CHO+PRO), or a carbohydrate and protein plus free leucine mixture (0.3, 0.2, and 0.1 g·kg^{-1}·h^{-1}, respectively; CHO+PRO+Leu) is ingested. Values are means ± SE. *Significantly different from CHO; #significantly different from CHO+PRO: $P < 0.05$.

Plasma leucine, phenylalanine, and tyrosine concentrations are reported in Fig. 2. Plasma essential amino acid (EAA) responses (area under the curve above baseline) are reported in Fig. 3. Resting plasma phenylalanine concentration averaged 53.6 ± 1.4 μmol/l. After ingestion of the first bolus, plasma phenylalanine concentrations increased in the CHO+PRO and CHO+PRO+Leu trials ($P < 0.05$), whereas a slight, nonsignificant decrease was observed in the CHO trial during the first 2 h of postexercise recovery (Fig. 2*B*). The plasma phenylalanine response (Fig. 3) was negative in the CHO trial, whereas it was positive in the CHO+PRO and CHO+PRO+Leu trials (−2.92 ± 1.00 vs. 3.25 ± 0.72 and 1.47 ± 0.81 mmol·6 h·liter^{-1}, respectively, $P < 0.01$). Plasma leucine concentrations (Fig. 2*A*) strongly increased after ingestion of the first bolus ($P < 0.01$) in both the CHO+PRO+Leu and CHO+PRO trials, whereas a slight decrease ($P < 0.05$) was observed in the CHO trial during the first 2 h of recovery. The plasma leucine response (Fig. 3) was negative in the CHO trial, positive in the CHO+PRO trial, and significantly greater in the CHO+PRO+Leu trial compared with both other trials (−9 ± 5, 46 ± 12, and 468 ± 62 mmol·6 h·liter^{-1}, respectively, $P < 0.05$). The observed plasma insulin response was strongly correlated with the observed plasma leucine concentration ($r = 0.773$, $P < 0.001$). Basal plasma tyrosine concentrations averaged 65.3 ± 0.8 μmol/l in all trials. Plasma tyrosine concentrations increased after ingestion of the first bolus in the CHO+PRO and CHO+PRO+Leu trials ($P < 0.05$). In the CHO trial, the concentration decreased ($P < 0.05$) during the first 2 h after exercise (Fig. 2*C*). Whereas plasma tyrosine responses (Fig. 3) were negative in the CHO trial, they were positive in both the CHO+PRO and CHO+PRO+Leu trials (−5.0 ± 1.5 vs. 11.2 ± 2.3 and 4.3 ± 0.8 mmol·6 h·liter^{-1}, respectively, $P < 0.01$).

The time courses of the plasma L-[*ring*-$^{13}C_6$]phenylalanine and L-[*ring*-$^{13}C_6$]tyrosine enrichments are shown in Fig. 4. Plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment increased after exercise in the CHO trial. In the CHO+PRO and CHO+PRO+Leu trials, plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment slightly decreased to reach plateau values at $t = 60$ min. Plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment was significantly greater in the CHO trial compared with the CHO+PRO and CHO+PRO+Leu trials ($P < 0.05$). Plasma L-[*ring*-$^{13}C_6$]tyrosine enrichment increased after exercise in the CHO trial but remained unchanged in the CHO+PRO and CHO+PRO+Leu trials. Plasma L-[*ring*-$^{13}C_6$]tyrosine enrichment was significantly higher in the CHO compared with the CHO+PRO and CHO+PRO+Leu trials ($P < 0.05$). Mean plasma amino acid enrichment, plasma phenylalanine R_a and R_d, muscle free amino acid enrichment, and the increment in muscle protein enrichment are presented in Table 2.

Whole body protein breakdown (Fig. 5), calculated over the 6 h of postexercise recovery, was lower in the CHO+PRO and CHO+PRO+Leu trials compared with the CHO trial ($P < 0.05$). Whole body protein synthesis (Fig. 5), as calculated by $R_d - R_{ox}$ (rate of oxidation), was increased in the CHO+PRO and CHO+PRO+Leu trials compared with the CHO trial ($P < 0.05$). The rate of postexercise whole body phenylalanine

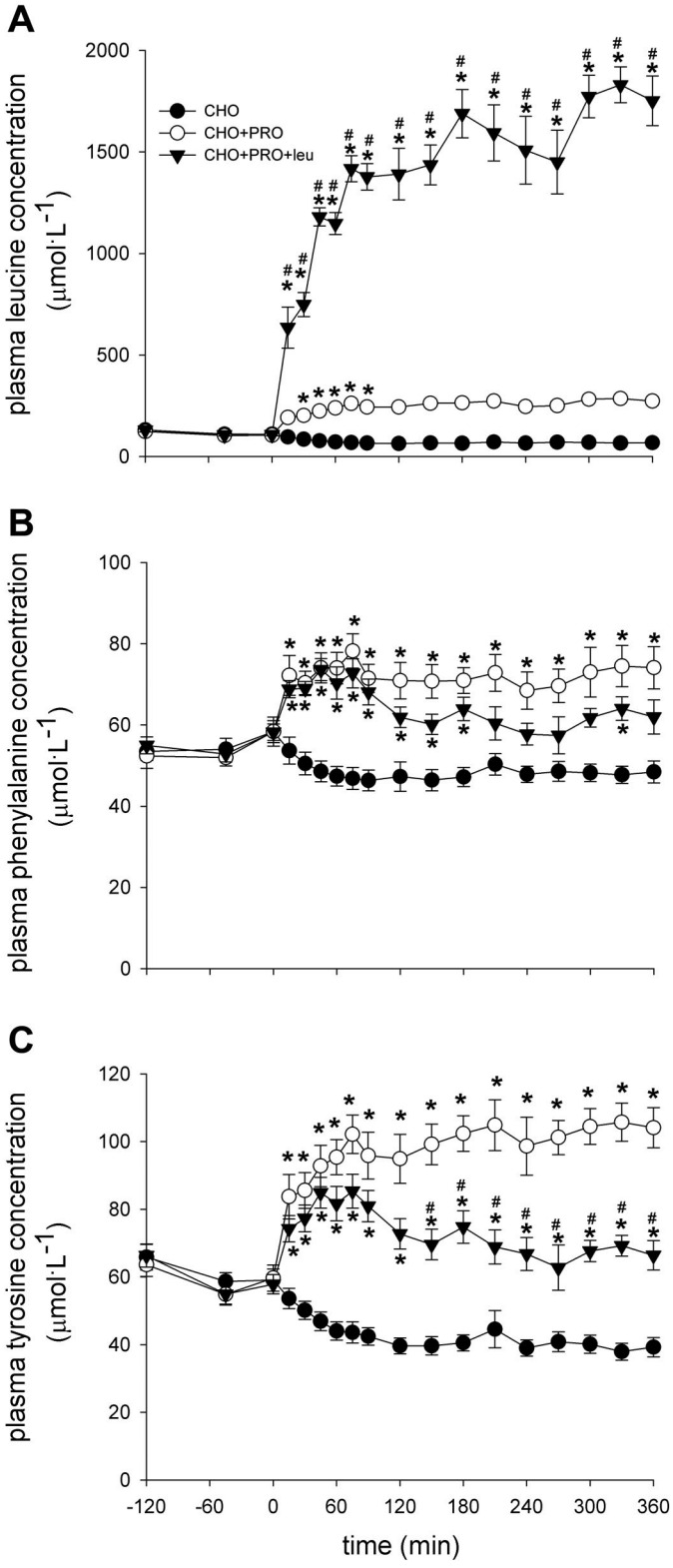

Fig. 2. Plasma leucine (*A*), phenylalanine (*B*), and plasma tyrosine (*C*) concentrations (μmol/l) during CHO, CHO+PRO, and CHO+PRO+Leu trials. Values are means ± SE. *Significantly different from CHO; #significantly different from CHO+PRO: $P < 0.05$.

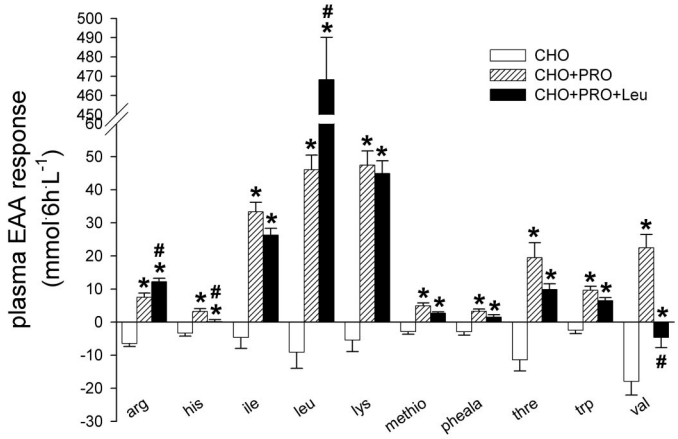

Fig. 3. Plasma essential amino acid (EAA) response (expressed as area under the curve minus baseline values) during CHO, CHO+PRO, and CHO+PRO+Leu trials. Values are means+SE. *Significantly different from CHO; #significantly different from CHO+PRO: $P < 0.05$.

oxidation (Fig. 5), as calculated from the conversion of phenylalanine to tyrosine, was decreased in the CHO+PRO+Leu trial compared with the CHO+PRO and CHO trials ($P < 0.05$). Whole body protein balance (Fig. 5) was negative in the CHO trial, whereas protein balance was positive in the CHO+PRO and CHO+PRO+Leu trials. In addition, whole body protein balance was higher in the CHO+PRO+Leu trial than in the CHO+PRO trial ($P < 0.01$). Whole body protein breakdown correlated with the plasma insulin and leucine responses ($r = -0.670$ and -0.647, respectively, $P < 0.01$). Whole body net protein balance was positively correlated with the plasma insulin and leucine response ($r = 0.678$ and 0.626, respectively, $P < 0.01$).

Mixed muscle protein FSRs were calculated using the precursor-product equation outlined in METHODS. FSR, with the mean plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment as precursor (Fig. 6), was significantly higher in the CHO+PRO+Leu trial compared with the CHO trial (0.095 ± 0.005 vs. 0.061 ± 0.009%/h, respectively, $P < 0.05$), with intermediate values observed in the CHO+PRO trial (0.0820 ± 0.0104%/h). When the free intracellular L-[*ring*-$^{13}C_6$]phenylalanine enrichment was used as the precursor, FSR values were higher than when calculated with plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment as precursor but revealed the same intervention effect ($P = 0.052$). FSR values averaged 0.089 ± 0.012, 0.110 ± 0.013, and 0.128 ± 0.007%/h for the CHO, CHO+PRO, and CHO+PRO+Leu trial, respectively. When these values were corrected for the contribution of extracellular water to the measured free muscle L-[*ring*-$^{13}C_6$]phenylalanine enrichment (50), FSR values averaged 0.097 ± 0.013, 0.117 ± 0.014, and 0.136 ± 0.007%/h for the CHO, CHO+PRO, and CHO+PRO+Leu trial, respectively, with the same intervention effect ($P = 0.073$). Significant correlations were observed between FSR and the amount of leucine ingested ($r = 0.471$, $P < 0.05$), phenylalanine R_d ($r = 0.531$, $P < 0.01$), whole body protein synthesis ($r = 0.548$, $P < 0.01$), and whole body protein balance ($r = 0.507$, $P < 0.02$).

DISCUSSION

In the present study, we simultaneously assessed whole body protein turnover as well as the FSR of mixed muscle protein by

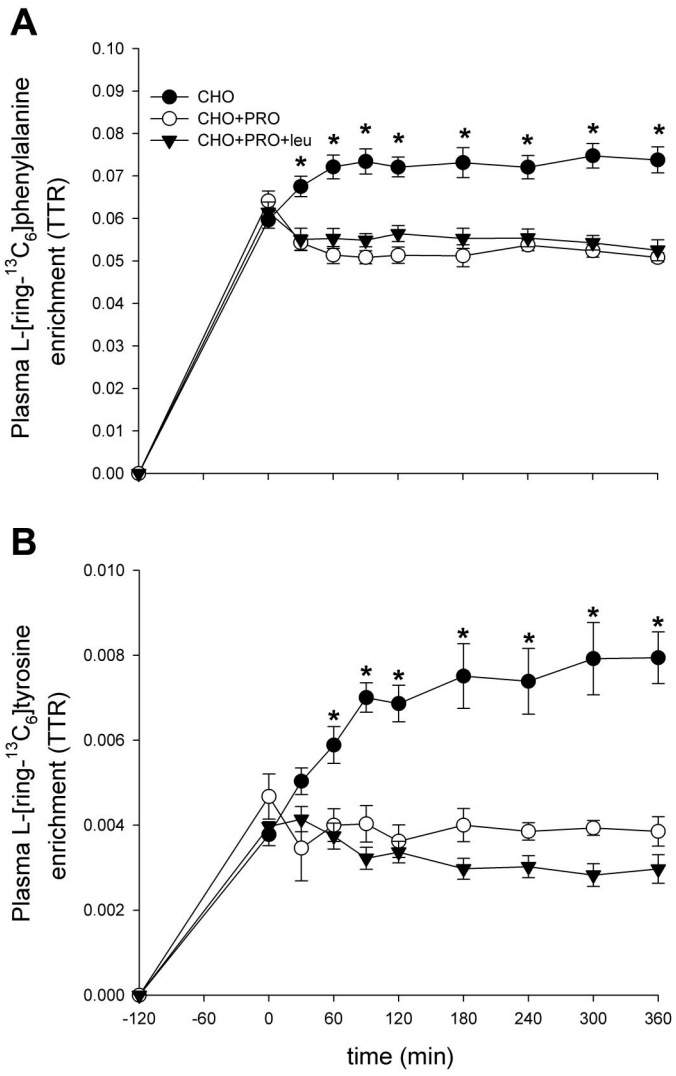

Fig. 4. Plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichment (*A*) and plasma L-[*ring*-$^{13}C_6$]tyrosine enrichment (*B*) during CHO, CHO+PRO, and CHO+PRO+Leu trials. TTR, tracer-to-tracee ratio. Values are means ± SE. *Significantly different from CHO+PRO and CHO+PRO+Leu: $P < 0.05$.

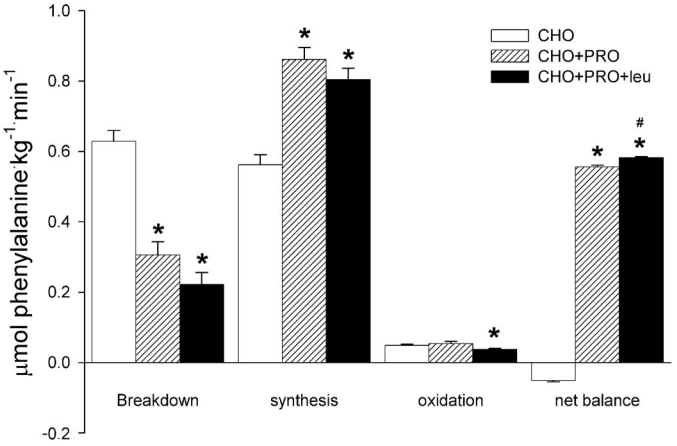

Fig. 5. Rates of whole body protein breakdown, synthesis, oxidation, and net balance (expressed as μmol phenylalanine·kg·min^{-1}) during CHO, CHO+PRO, and CHO+PRO+Leu trials. Values are means+SE. *Significantly different from CHO; #significantly different from CHO+PRO: $P < 0.05$.

the incorporation of labeled phenylalanine in the vastus lateralis muscle after resistance exercise. The coingestion of leucine and protein with carbohydrate significantly increased whole body net protein balance compared with carbohydrate intake only and the combined ingestion of carbohydrate and protein. In addition, muscle protein synthesis rates were higher when protein and free leucine were coingested compared with the ingestion of carbohydrate only. Ingestion of carbohydrate and protein resulted in intermediate muscle protein synthesis rates, which were not significantly different from rates observed after coingestion of protein and leucine and carbohydrate ingestion only. Thus the present study shows that the combined ingestion of protein and leucine with carbohydrate stimulates protein anabolism, measured using both tracer incorporation and whole body tracer methodology.

It has been shown that both muscle protein synthesis (7, 35, 44) and protein breakdown rates (7, 35) are accelerated following resistance exercise. Resistance exercise exerts its effect either via the increased local production of IGF-I in the muscle (21) and/or via other contraction-induced signaling events. Although muscle protein synthesis is stimulated during the postexercise recovery phase (35), net muscle protein balance remains negative in the absence of food intake (7, 35, 36). It has been shown that the administration of amino acids with or without carbohydrate results in a rapid increase in muscle protein synthesis rate, whereas the protein degradation rate is decreased (8, 11, 39, 44, 45). This results in an improved (positive) net muscle protein balance at rest and/or during postexercise recovery.

The combined intake of free leucine and protein with carbohydrate has been shown to increase the plasma insulin

Table 2. *Plasma and muscle tracer data after resistance exercise*

	Plasma AA Enrichment, APE	Plasma R_a, μmol·kg^{-1}·min^{-1}	Plasma R_d, μmol·kg^{-1}·min^{-1}	Muscle AA Pool Enrichment, APE	ΔEnrichment Muscle Protein, APE
L-[*ring*-$^{13}C_6$]phenylalanine					
CHO	6.84±0.28*	0.613±0.030*	0.611±0.031*	4.83±0.15	0.0141±0.0020
CHO+PRO	4.85±0.09	0.918±0.037	0.916±0.038	3.77±0.05	0.0130±0.0016
CHO+PRO+Leu	4.99±0.22	0.834±0.035	0.841±0.034	3.85±0.12	0.0144±0.0006
L-[*ring*-$^{13}C_6$]tyrosine					
CHO	0.75±0.06*	NA	NA	1.06±0.29	NA
CHO+PRO	0.38±0.02	NA	NA	0.66±0.28	NA
CHO+PRO+Leu	0.30±0.01	NA	NA	0.76±0.20	NA

Values are means ± SE. AA, amino acids; R_a and R_d, rates of appearance and disappearance, respectively; APE, atom percent excess; NA, not applicable. *Significantly different from CHO+PRO and CHO+PRO+Leu trials, $P < 0.05$.

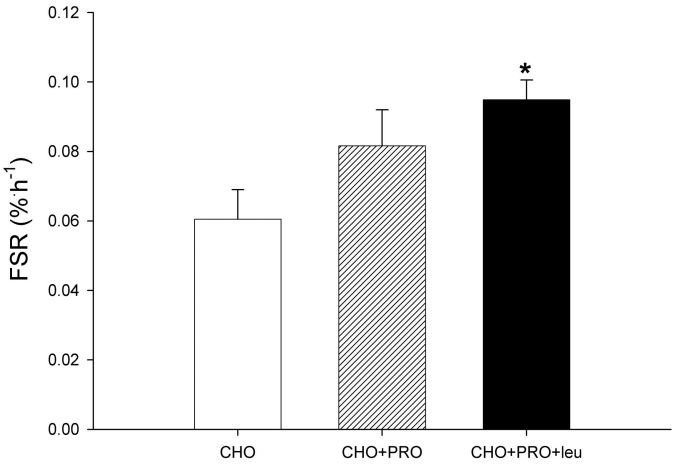

Fig. 6. Fractional synthetic rate (FSR) of mixed muscle protein during the recovery phase from resistance exercise during CHO, CHO+PRO, and CHO+PRO+Leu trials. Values are means + SE. *Significantly different from CHO: $P < 0.05$.

response (47–49). Increased amounts of circulating insulin have been reported to increase net muscle protein balance during conditions of hyperaminoacidemia (18, 19, 22), which occurs primarily by inhibition of muscle protein breakdown rather than by stimulating protein synthesis. Furthermore, leucine ingestion has been reported to stimulate muscle protein synthesis, independently of circulating plasma insulin levels, by increasing the phosphorylation (activation) of key proteins involved in the regulation of protein synthesis in rat (1, 4) and human skeletal muscle (23, 28). An insulinotropic mixture containing carbohydrate, protein, and leucine could therefore likely represent an efficient nutritional intervention to optimize net muscle accretion during postexercise recovery.

Using whole body tracer balance methodology, we observed that the protein breakdown rates exceeded the protein synthesis rates in the CHO trial, leading to a negative net protein balance (Fig. 5). The latter is in line with recently published data by Borsheim et al. (10), who showed that protein balance over the (leg) muscle remains negative after resistance exercise when only carbohydrate is ingested. However, measurements over the muscle do not necessarily account for all of the changes observed on a whole body level. Whole body protein breakdown rates were significantly suppressed in the CHO+PRO and CHO+PRO+Leu trials compared with the CHO trial, whereas protein synthesis rates were increased (Fig. 5). Coingestion of protein or protein and leucine with carbohydrate resulted in 50 ± 2 and 62 ± 2% reductions in protein degradation, respectively, compared with carbohydrate ingestion only. In addition, protein synthesis was 54 ± 5 and 45 ± 5% increased in the CHO+PRO and CHO+PRO+Leu trials, respectively, compared with the CHO trial. When whole body protein synthesis rates were expressed as a percentage of the phenylalanine R_d, we found the highest values in the CHO+PRO+Leu trial (95.6 ± 0.1 vs. 92.0 ± 0.4 and 94.2 ± 0.4% in the CHO and CHO+PRO trials respectively, $P < 0.05$), which could be attributed to a decreased rate of phenylalanine oxidation in the CHO+PRO+Leu compared with the CHO+PRO trial, with intermediate values found in the CHO trial. Such a reduction in amino acid oxidation after administration of leucine has previously been established (32). Our findings are in accord with earlier reports (27, 30, 39) showing that the combined ingestion of a single bolus of protein/amino acids and carbohydrate improves protein balance in the post-resistance exercise period. In the present study, we extend on those findings by investigating whether additional leucine could further promote protein anabolism. Therefore, in both the CHO+PRO and CHO+PRO+Leu trials we provided an ample supply of protein (0.2 $g \cdot kg^{-1} \cdot h^{-1}$). This amount by far exceeds the amount of protein that was estimated to allow sustained maximal protein synthesis rates (50) and was selected to ensure that precursor substrate availability would not be limiting. Repeated boluses were administered, as opposed to a single-bolus approach, to ensure a continuous supply of amino acids. Consequently, we show that the coingestion of leucine even further increases the net protein balance compared with carbohydrate and protein ingestion (Fig. 5).

Most studies have applied the (whole body) tracer balance methodology to investigate the effect of postexercise nutrition on protein metabolism. Unfortunately, whole body tracer methods have some important limitations, as they do not allow the direct measurement of muscle protein synthesis rates. As such, they do not provide information on the contribution of individual tissues to protein metabolism and their response to nutritional and/or exercise intervention and do not enable the discrimination between active and inactive muscle. Therefore, we also measured the incorporation rate of labeled phenylalanine in skeletal muscle tissue after resistance exercise to determine the FSR of mixed muscle protein in the active vastus lateralis muscle. We used plasma L-[*ring*-$^{13}C_6$]phenylalanine and free muscle L-[*ring*-$^{13}C_6$]phenylalanine enrichments to provide an estimate of the lower boundary (based on plasma precursor enrichments) and the higher boundary (based on intracellular muscle precursor enrichments) for the true FSR of mixed muscle proteins. Both methods provided similar results. Because we observed a higher variation in intracellular muscle precursor enrichment, we based the FSR values on plasma precursor enrichments. Mixed muscle FSR averaged 0.0605 ± 0.0085%/h during 6 h of postexercise recovery while carbohydrate was ingested. Combined ingestion of leucine and protein with carbohydrate resulted in a significantly increased FSR compared with carbohydrate ingestion alone (Fig. 6). The combined ingestion of protein and carbohydrate without free leucine resulted in an intermediate value and was not significantly different from the lower values in the CHO trial or the higher values reported in the CHO+PRO+Leu trials (Fig. 6). Therefore, our data on the muscle level also show that the combined ingestion of leucine and protein with carbohydrate can effectively stimulate muscle protein synthesis.

The presented FSR data are in line with our observations using whole body tracer methods, showing that coingestion of leucine and protein with carbohydrate accelerates protein synthesis and improves net protein balance compared with carbohydrate ingestion only. With the whole body tracer methodology, our data indicate that the combined ingestion of leucine and protein with carbohydrate can reduce protein breakdown. The latter seems to be in line with earlier reports by Nair et al. (32), who showed that leucine infusion significantly decreases protein breakdown as well as amino acid oxidation rate. In addition, data from the same study showed a decrease in the plasma concentrations of the other EAAs during leucine infusion. In the present study, we also observed a reduced plasma

EAA response in the CHO+PRO+Leu trial compared with the CHO+PRO trial, although the same amount of EAA (with the exclusion of the supplemented leucine) was consumed. However, no differences were observed in phenylalanine R_d between the CHO+PRO+Leu and CHO+PRO trials. This diminished plasma EAA response following leucine supplementation could be attributed to a reduced release from the muscle and indicate a reduced protein breakdown and/or reduced protein oxidation rate (Fig. 5).

We recently reported that coingestion of leucine, phenylalanine, and protein with carbohydrate results in an increased plasma insulin response in the postabsorptive resting state (46, 49) and during recovery from endurance exercise (47, 48). In the present study, we demonstrated that combined ingestion of leucine and protein with carbohydrate (without phenylalanine) can substantially increase the insulin response by ~250% compared with the ingestion of only carbohydrate. Circulating insulin concentrations play an important role in regulating protein metabolism. The mechanism by which insulin mediates muscle hypertrophy is still a subject of debate, as some reports (9, 18, 19) indicate that insulin infusion reduces protein breakdown whereas others (6, 20) have reported an enhanced muscle protein synthesis rate when sufficient amino acids were made available. However, in the absence of an ample supply of amino acids, insulin does not seem to stimulate muscle protein synthesis (9). In the present study, we found plasma insulin responses to be negatively correlated with whole body protein degradation ($r = -0.641$, $P < 0.01$), whereas whole body protein synthesis was positively correlated with plasma insulin response ($r = 0.451$, $P < 0.05$). Muscle protein synthesis rates (FSR) did not correlate with the plasma insulin response, whereas mixed muscle protein FSR did correlate with the amount of leucine that was ingested ($r = 0.471$, $P < 0.05$). Whether the observed differences can be attributed to the insulin- or non-insulin-dependent stimulatory effects of leucine remains to be established.

The combined ingestion of leucine and protein with carbohydrate in the recovery period from resistance exercise could stimulate muscle protein synthesis in several ways. It provides amino acids as precursors for muscle protein synthesis, and the added leucine further increases plasma insulin concentrations and could also directly stimulate protein synthesis. Leucine has been shown to have the potential to affect muscle protein metabolism by decreasing the rate of protein degradation (32), most likely via increases in circulating insulin, and the phosphorylation of key proteins involved in the regulation of protein synthesis (23, 28, 37). The latter has been shown to occur even in the absence of an increase in circulating insulin concentrations. These studies indicate that leucine has the ability to function as a nutritional signaling molecule that modulates muscle protein synthesis and/or breakdown following food intake. The stimulatory effect of leucine on protein synthesis occurs at the level of translation initiation and involves signaling through mammalian target of rapamycin (mTOR) (24, 37). The protein kinase referred to as mTOR is thought to serve as a convergence point for leucine- and insulin-mediated effects on translation initiation (24, 37). Maximal rates of protein synthesis rates probably require both leucine and insulin signaling and the anabolic signal of resistance exercise. However, under normal in vivo conditions, it is impossible to discriminate between the effects of leucine and insulin and resistance exercise (IGF-I and mechanogrowth factor) on muscle protein synthesis, as all these signals are substantially elevated in the CHO+PRO+Leu trial.

In conclusion, the combined ingestion of protein and leucine with carbohydrate improves whole body protein balance during recovery from resistance exercise compared with the ingestion of carbohydrate or carbohydrate with protein. The combined ingestion of both leucine and protein with carbohydrate augments postexercise mixed muscle protein synthesis compared with the ingestion of only carbohydrate. The present data indicate that the additional ingestion of free leucine in combination with protein and carbohydrate likely represents an effective strategy to increase muscle anabolism following resistance exercise.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Annemie Gijsen, Annita Rousseau, and Jos Stegen, and the enthusiastic support of the subjects who volunteered to participate in these trials.

GRANTS

This work was partly supported by a grant from Numico Research.

REFERENCES

1. **Anthony JC, Anthony TG, Kimball SR, Vary TC, and Jefferson LS.** Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *J Nutr* 130: 139–145, 2000.
2. **Anthony JC, Anthony TG, and Layman DK.** Leucine supplementation enhances skeletal muscle recovery in rats following exercise. *J Nutr* 129: 1102–1106, 1999.
3. **Anthony JC, Lang CH, Crozier SJ, Anthony TG, MacLean DA, Kimball SR, and Jefferson LS.** Contribution of insulin to the translational control of protein synthesis in skeletal muscle by leucine. *Am J Physiol Endocrinol Metab* 282: E1092–E1101, 2002.
4. **Anthony JC, Reiter AK, Anthony TG, Crozier SJ, Lang CH, MacLean DA, Kimball SR, and Jefferson LS.** Orally administered leucine enhances protein synthesis in skeletal muscle of diabetic rats in the absence of increases in 4E-BP1 or S6K1 phosphorylation. *Diabetes* 51: 928–936, 2002.
5. **Bergstrom J.** Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* 35: 609–616, 1975.
6. **Biolo G, Declan Fleming RY, and Wolfe RR.** Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J Clin Invest* 95: 811–819, 1995.
7. **Biolo G, Maggi SP, Williams BD, Tipton KD, and Wolfe RR.** Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol Endocrinol Metab* 268: E514–E520, 1995.
8. **Biolo G, Tipton KD, Klein S, and Wolfe RR.** An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol Endocrinol Metab* 273: E122–E129, 1997.
9. **Biolo G, Williams BD, Fleming RY, and Wolfe RR.** Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes* 48: 949–957, 1999.
10. **Borsheim E, Cree MG, Tipton KD, Elliott TA, Aarsland A, and Wolfe RR.** Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. *J Appl Physiol* 96: 674–678, 2004.
11. **Borsheim E, Tipton KD, Wolf SE, and Wolfe RR.** Essential amino acids and muscle protein recovery from resistance exercise. *Am J Physiol Endocrinol Metab* 283: E648–E657, 2002.
12. **Chesley A, MacDougall JD, Tarnopolsky MA, Atkinson SA, and Smith K.** Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol* 73: 1383–1388, 1992.
13. **Collin-Vidal C, Cayol M, Obled C, Ziegler F, Bommelaer G, and Beaufrere B.** Leucine kinetics are different during feeding with whole protein or oligopeptides. *Am J Physiol Endocrinol Metab* 267: E907–E914, 1994.

14. **Engelen MP, Deutz NE, Mostert R, Wouters EF, and Schols AM.** Response of whole body protein and urea turnover to exercise differs between patients with chronic obstructive pulmonary disease with and without emphysema. *Am J Clin Nutr* 77: 868–874, 2003.
15. **Fekkes D, van Dalen A, Edelman M, and Voskuilen A.** Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated pre-column derivatization with o-phthaldialdehyde. *J Chromatogr B Biomed Appl* 669: 177–186, 1995.
16. **Floyd JC Jr, Fajans SS, Conn JW, Knopf RF, and Rull J.** Stimulation of insulin secretion by amino acids. *J Clin Invest* 45: 1487–1502, 1966.
17. **Floyd JC Jr, Fajans SS, Knopf RF, and Conn JW.** Evidence that insulin release is the mechanism for experimentally induced leucine hypoglycemia in man. *J Clin Endocrinol Metab* 28: 266–276, 1963.
18. **Fryburg DA, Jahn LA, Hill SA, Oliveras DM, and Barrett EJ.** Insulin and insulin-like growth factor-I enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms. *J Clin Invest* 96: 1722–1729, 1995.
19. **Gelfand RA and Barrett EJ.** Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J Clin Invest* 80: 1–6, 1987.
20. **Gore DC, Wolf SE, Sanford AP, Herndon DN, and Wolfe RR.** Extremity hyperinsulinemia stimulates muscle protein synthesis in severely injured patients. *Am J Physiol Endocrinol Metab* 286: E529–E534, 2004.
21. **Hameed M, Orrell RW, Cobbold M, Goldspink G, and Harridge SD.** Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* 547: 247–254, 2003.
22. **Hillier TA, Fryburg DA, Jahn LA, and Barrett EJ.** Extreme hyperinsulinemia unmasks insulin's effect to stimulate protein synthesis in the human forearm. *Am J Physiol Endocrinol Metab* 274: E1067–E1074, 1998.
23. **Karlsson HK, Nilsson PA, Nilsson J, Chibalin AV, Zierath JR, and Blomstrand E.** Branched-chain amino acids increase $p70^{S6K}$ phosphorylation in human skeletal muscle after resistance exercise. *Am J Physiol Endocrinol Metab* 287: E1–E7, 2004.
24. **Kimball SR and Jefferson LS.** Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids. *Biochem Biophys Res Commun* 313: 423–427, 2004.
25. **Koopman R, Pannemans DL, Jeukendrup AE, Gijsen A, Senden JMG, Halliday D, Saris WHM, van Loon LJC, and Wagenmakers AJM.** Combined ingestion of protein and carbohydrate improves protein balance during ultra-endurance exercise. *Am J Physiol Endocrinol Metab* 287: E712–E720, 2004.
26. **Kraemer WJ and Fry AC.** Strength testing; development and evaluation of methodology. In: *Physiological Assesment of Human Fitness*, edited by Maud PJ and Foster C. Leeds, UK: Human Kinetics, 1995, p. 115–133.
27. **Levenhagen DK, Carr C, Carlson MG, Maron DJ, Borel MJ, and Flakoll PJ.** Postexercise protein intake enhances whole body and leg protein accretion in humans. *Med Sci Sports Exerc* 34: 828–837, 2002.
28. **Liu Z, Jahn LA, Long W, Fryburg DA, Wei L, and Barrett EJ.** Branched chain amino acids activate messenger ribonucleic acid translation regulatory proteins in human skeletal muscle, and glucocorticoids blunt this action. *J Clin Endocrinol Metab* 86: 2136–2143, 2001.
29. **Mayhew JL, Piper FC, and Ware JS.** Anthropometric correlates with strength performance among resistance trained athletes. *J Sports Med Phys Fitness* 33: 159–165, 1993.
30. **Miller SL, Tipton KD, Chinkes DL, Wolf SE, and Wolfe RR.** Independent and combined effects of amino acids and glucose after resistance exercise. *Med Sci Sports Exerc* 35: 449–455, 2003.
31. **Munro HN and Fleck A.** Analysis of tissues and body fluids for nitrogenous constituents. In: *Mammalian Protein Metabolism*, edited by Munro HN. New York: Academic, 1969, p. 423–525.
32. **Nair KS, Schwartz RG, and Welle S.** Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol Endocrinol Metab* 263: E928–E934, 1992.
33. **Pacy PJ, Garrow JS, Ford GC, Merritt H, and Halliday D.** Influence of amino acid administration on whole body leucine kinetics and resting metabolic rate in postabsorptive normal subjects. *Clin Sci (Lond)* 75: 225–231, 1988.
34. **Pallotta JA and Kennedy PJ.** Response of plasma insulin and growth hormone to carbohydrate and protein feeding. *Metabolism* 17: 901–908, 1968.
35. **Phillips SM, Tipton KD, Aarsland A, Wolf SE, and Wolfe RR.** Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol Endocrinol Metab* 273: E99–E107, 1997.
36. **Pitkanen HT, Nykanen T, Knuutinen J, Lahti K, Keinanen O, Alen M, Komi PV, and Mero AA.** Free amino acid pool and muscle protein balance after resistance exercise. *Med Sci Sports Exerc* 35: 784–792, 2003.
37. **Proud CG.** mTOR-mediated regulation of translation factors by amino acids. *Biochem Biophys Res Commun* 313: 429–436, 2004.
38. **Rabinowitz D, Merimee TJ, Maffezzoli R, and Burgess JA.** Patterns of hormonal release after glucose, protein, and glucose plus priotein. *Lancet* 2: 454–456, 1966.
39. **Rasmussen BB, Tipton KD, Miller SL, Wolf SE, and Wolfe RR.** An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J Appl Physiol* 88: 386–392, 2000.
40. **Roy BD, Tarnopolsky MA, MacDougall JD, Fowles J, and Yarasheski KE.** Effect of glucose supplement timing on protein metabolism after resistance training. *J Appl Physiol* 82: 1882–1888, 1997.
41. **Steele R.** Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann NY Acad Sci* 82: 420–430, 1959.
42. **Thompson GN, Pacy PJ, Merritt H, Ford GC, Read MA, Cheng KN, and Halliday D.** Rapid measurement of whole body and forearm protein turnover using a [$^2H_5$]phenylalanine model. *Am J Physiol Endocrinol Metab* 256: E631–E639, 1989.
43. **Tipton KD, Borsheim E, Wolf SE, Sanford AP, and Wolfe RR.** Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion. *Am J Physiol Endocrinol Metab* 284: E76–E89, 2003.
44. **Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, and Wolfe RR.** Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol Endocrinol Metab* 276: E628–E634, 1999.
45. **Tipton KD, Rasmussen BB, Miller SL, Wolf SE, Owens-Stovall SK, Petrini BE, and Wolfe RR.** Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab* 281: E197–E206, 2001.
46. **Van Loon LJ, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH, and Keizer HA.** Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. *Diabetes Care* 26: 625–630, 2003.
47. **Van Loon LJ, Kruijshoop M, Verhagen H, Saris WH, and Wagenmakers AJ.** Ingestion of protein hydrolysate and amino acid-carbohydrate mixtures increases postexercise plasma insulin responses in men. *J Nutr* 130: 2508–2513, 2000.
48. **Van Loon LJ, Saris WH, Kruijshoop M, and Wagenmakers AJ.** Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *Am J Clin Nutr* 72: 106–111, 2000.
49. **Van Loon LJ, Saris WH, Verhagen H, and Wagenmakers AJ.** Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am J Clin Nutr* 72: 96–105, 2000.
50. **Wagenmakers AJ.** Tracers to investigate protein and amino acid metabolism in human subjects. *Proc Nutr Soc* 58: 987–1000, 1999.
51. **Wolfe RR.** *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York: Wiley-Liss, 1992.