Coingestion of carbohydrate with protein does not further augment postexercise muscle protein synthesis

René Koopman,[1] Milou Beelen,[1] Trent Stellingwerff,[1] Bart Pennings,[1] Wim H. M. Saris,[2] Arie K. Kies,[3] Harm Kuipers,[1] and Luc J. C. van Loon[1,2]

Departments of [1]Movement Sciences and [2]Human Biology, Nutrition and Toxicology Research Institute Maastricht, Maastricht University, Maastricht; and [3]DSM Food Specialties, R&D, Biochemistry and Nutrition Department, Delft, The Netherlands

Koopman R, Beelen M, Stellingwerff T, Pennings B, Saris WH, Kies AK, Kuipers H, Loon LJ. Coingestion of carbohydrate with protein does not further augment postexercise muscle protein synthesis. *Am J Physiol Endocrinol Metab* 293: E833–E842, 2007. First published July 3, 2007; doi:10.1152/ajpendo.00135.2007.— The present study was designed to assess the impact of coingestion of various amounts of carbohydrate combined with an ample amount of protein intake on postexercise muscle protein synthesis rates. Ten healthy, fit men (20 ± 0.3 yr) were randomly assigned to three crossover experiments. After 60 min of resistance exercise, subjects consumed 0.3 $g \cdot kg^{-1} \cdot h^{-1}$ protein hydrolysate with 0, 0.15, or 0.6 $g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate during a 6-h recovery period (PRO, PRO + LCHO, and PRO + HCHO, respectively). Primed, continuous infusions with L-[*ring*-$^{13}C_6$]phenylalanine, L-[*ring*-$^2H_2$]tyrosine, and [6,6-$^2H_2$]glucose were applied, and blood and muscle samples were collected to assess whole body protein turnover and glucose kinetics as well as protein fractional synthesis rate (FSR) in the vastus lateralis muscle over 6 h of postexercise recovery. Plasma insulin responses were significantly greater in PRO + HCHO compared with PRO + LCHO and PRO (18.4 ± 2.9 vs. 3.7 ± 0.5 and 1.5 ± 0.2 $U \cdot 6 \ h^{-1} \cdot l^{-1}$, respectively, $P < 0.001$). Plasma glucose rate of appearance (R_a) and disappearance (R_d) increased over time in PRO + HCHO and PRO + LCHO, but not in PRO. Plasma glucose R_a and R_d were substantially greater in PRO + HCHO vs. both PRO and PRO + LCHO ($P < 0.01$). Whole body protein breakdown, synthesis, and oxidation rates, as well as whole body protein balance, did not differ between experiments. Mixed muscle protein FSR did not differ between treatments and averaged 0.10 ± 0.01, 0.10 ± 0.01, and 0.11 ± 0.01%/h in the PRO, PRO + LCHO, and PRO + HCHO experiments, respectively. In conclusion, coingestion of carbohydrate during recovery does not further stimulate postexercise muscle protein synthesis when ample protein is ingested.

resistance exercise; protein metabolism; nutrition; recovery

POSTEXERCISE NUTRITION IS INSTRUMENTAL to enhance recovery and to facilitate the adaptive response to regular exercise training (28). In the endurance-trained athlete, rapid restoration of depleted muscle glycogen stores is essential to enhance postexercise recovery and, as such, to maintain performance capacity (15). Therefore, endurance athletes generally aim to maximize postexercise muscle glycogen synthesis rates by ingesting large amounts of carbohydrate during recovery (30, 40). Coingestion of relative small amounts of protein and/or amino acids has been suggested to further accelerate muscle glycogen repletion and/or to reduce muscle damage (40, 44).

It has been firmly established (4, 18, 19, 23, 25) that postexercise protein and/or amino acid intake is essential to allow net muscle protein accretion. Therefore, athletes involved in resistance-type exercise training like fitness and bodybuilding generally ingest large quantities of protein during postexercise recovery to augment net muscle protein accretion (21, 38). It is generally assumed that carbohydrate should be coingested to maximize the postexercise muscle protein synthetic response. Although ingestion of only carbohydrate does not seem to stimulate postexercise muscle protein synthesis (5, 29), it has been reported to improve net protein balance by inhibiting muscle protein breakdown (5). However, the necessity of carbohydrate coingestion to augment postexercise muscle protein synthesis under conditions where ample protein is ingested remains a matter of debate in both science and sports practice. The latter is attributed to the fact that many recreational athletes perform resistance-type exercise to gain lean muscle mass and reduce fat mass (for health and/or aesthetic purposes) and, therefore, prefer to restrict carbohydrate intake.

In the present study, we assessed the impact of the coingestion of different amounts of carbohydrate on postexercise muscle protein synthesis when ample protein is being ingested. Therefore, a primed, continuous infusion of L-[*ring*-$^{13}C_6$]phenylalanine was combined with plasma and muscle tissue sampling to simultaneously measure mixed-muscle fractional synthesis rate (FSR) and whole body protein turnover during recovery from resistance-type exercise following the ingestion of protein (0.3 $g \cdot kg^{-1} \cdot h^{-1}$) with 0, 0.15, and 0.6 $g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate in healthy, young men.

METHODS

Subjects

Ten healthy, fit male volunteers [age: 20.1 ± 0.3 yr; weight: 71.3 ± 2.7 kg; height: 1.81 ± 0.02 m; body mass index: 21.9 ± 1.0 $kg \cdot m^{-2}$; %body fat: 13.6 ± 1.6%; leg volume: 8.6 ± 0.4 l] with no history of participating in any regular exercise program were recruited for the present study. All subjects were informed on the nature and possible risks of the experimental procedures before their informed consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht, The Netherlands.

Pretesting

Body composition was assessed using the hydrostatic weighing method in the morning following an overnight fast. Residual lung

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volume was measured by the helium dilution technique, using a spirometer (Volugraph 2000; Mijnhart, Bunnik, The Netherlands). Body mass was measured with a digital balance with an accuracy of 0.001 kg (E1200; August Sauter, Albstadt, Germany). Body fat percentage was calculated using Siri's equation (32). Fat-free mass was calculated by subtracting fat mass from total body mass. In addition, single leg volume was determined as described previously (16).

To familiarize subjects with the resistance exercise protocol and the equipment, a familiarization test was performed. Proper lifting technique was demonstrated and practiced for each of the two lower-limb exercises (leg press and leg extension) and for the three upper-body exercises (chest press, shoulder press, and lat pulldown). Thereafter, maximum strength was estimated using the multiple repetitions testing procedure (22). In an additional exercise session, ~1 wk before the first experimental treatment, the subjects' one-repetition maximum (1-RM) was determined (20). After the subjects warmed up, the load was set at 90–95% of the estimated 1-RM and increased after each successful lift until failure. A 5-min resting period between subsequent attempts was allowed. A repetition was valid when subjects were able to complete the entire lift in a controlled manner without assistance. The mean 1-RMs for the leg press and extension were 200 ± 14 and 111 ± 7 kg, respectively.

Diet and Activity Prior to Testing

All subjects consumed a standardized meal [66.1 ± 2.0 kJ/kg body wt consisting of 65 energy percent (En%) carbohydrate, 15 En% protein, and 20 En% fat] on the evening prior to the experiments. All volunteers were instructed to refrain from any sort of heavy physical activity and to keep their diet as constant as possible 3 days before the experiments. In addition, subjects were asked to record their food intake for 48 h before the start of the first experiment and to consume the same diet 48 h prior to the second and third tests.

Experiments

All subjects participated in three experimental treatments, each separated by 7 days, in which subjects first completed ~1 h of resistance exercise (upper body and legs) followed by 6 h of recovery. During recovery, drinks containing either protein only (PRO), protein with a low carbohydrate dose (PRO + LCHO), or protein with a high carbohydrate dose (PRO + HCHO) were given in double-blind, randomized order. A total of 12 boluses were provided every 30 min during each experiment to ensure a continuous and ample supply of both glucose and amino acids during recovery. Plasma and muscle samples were collected during the 6-h recovery period. Tests were designed to simultaneously assess whole body amino acid kinetics and FSR of mixed muscle protein by the incorporation of L-[$ring$-$^{13}C_6$]phenylalanine in the mixed protein pool of tissue samples collected from the vastus lateralis muscle.

Protocol

At 8 AM, after an overnight fast, subjects arrived at the laboratory by car or by public transportation. 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 and placed in a hot box (60°C) for arterialized blood sampling. After basal blood sample collection, a single intravenous dose of [6,6-$^2H_2$]glucose (13.5 μmol/kg), L-[$ring$-$^{13}C_6$]phenylalanine (2 μmol/kg), and L-[$ring$-$^2H_2$]tyrosine (0.775 μmol/kg) was administered to prime the glucose, phenylalanine, and tyrosine pools, respectively. Thereafter, continuous tracer infusion was started (infusion rate of 0.286 ± 0.001 μmol·kg^{-1}·min^{-1} for [6,6-$^2H_2$]glucose, 0.049 ± 0.001 μmol·kg^{-1}·min^{-1} for L-[$ring$-$^{13}C_6$]phenylalanine, and 0.019 ± 0.001 μmol·kg^{-1}·min^{-1} for L-[$ring$-$^2H_2$]tyrosine). Subjects rested in a supine position for 1 h before engaging in the resistance exercise

protocol. After 5 min of warming up on a cycle ergometer (~75 W), subjects completed a session of three upper-body resistance-type exercises that featured three sets of 10 repetitions for each of the exercises. This was performed with resistance set at 40% of their body weight for the chest press and shoulder press and at 50% body weight for the front pulldown (all equipment by Jimsa Benelux, Rotterdam, The Netherlands), with 1-min rest intervals between sets. This was followed by a session of lower-limb exercises consisting of eight sets of 10 repetitions on the leg press and leg extension machines (Technogym, Rotterdam, The Netherlands), both performed at 75% of their individual 1-RM, with 2-min rest intervals between sets. All subjects were verbally encouraged during exercise, and the entire exercise protocol required ~1 h to complete. At the end of the exercise protocol ($t = 0$ min), subjects rested supine, and an arterialized blood sample and muscle biopsy were collected. Subjects then received an initial bolus (2.5 ml/kg) of a given test drink. Repeated boluses (2.5 ml/kg) were ingested every 30 min until $t = 330$ min. Arterialized blood samples were collected at $t = 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 270, 300, 330,$ and 360 min, with a second muscle biopsy taken at $t = 360$ min from the contralateral limb. Muscle biopsies were obtained from the middle region of the vastus lateralis (15 cm above the patella) and ~3 cm below entry through the fascia using the percutaneous needle biopsy technique (2). Muscle samples were dissected carefully and freed from any visible nonmuscle material. The muscle sample was immediately frozen in liquid nitrogen.

Beverages

Subjects received a beverage volume of 2.5 ml/kg every 30 min to ensure a given dose of 0.3 g/kg of a casein protein hydrolysate per hour combined with either 0 g·kg^{-1}·h^{-1} carbohydrate (PRO treatment), 0.15 g·kg^{-1}·h^{-1} carbohydrate (PRO + LCHO treatment), or 0.6 g·kg^{-1}·h^{-1} carbohydrate (PRO + HCHO treatment). The amount of protein (0.3 g/kg) provided in all experimental treatments was selected to exceed the estimated amount of protein needed to provide sufficient precursor substrate to sustain maximal protein synthesis rates (0.15%·h) for a period of $\geq$6 h (41). Repeated boluses were administered to enable a continuous supply of amino acids in the circulation, preventing large perturbations in plasma [6,6-$^2H_2$]glucose, L-[$ring$-$^{13}C_6$]phenylalanine, and L-[$ring$-$^2H_2$]tyrosine enrichments.

Glucose and maltodextrin were obtained from AVEBE (Veendam, The Netherlands). The casein protein hydrolysate (85.3% protein; PeptoPro) was prepared by DSM Food Specialties (Delft, The Netherlands) and involved the enzymatic hydrolysis of casein protein by specific endopeptidases and proline-specific endoprotease. To make the taste comparable in all treatments, beverages were uniformly flavored by adding 0.2 g sodium-saccharinate solution (25% wt/wt), 0.9 g of citric acid solution (50% wt/wt), and 5 g of cream vanilla flavor (Quest International, Naarden, The Netherlands) for each liter of beverage. Treatments were performed in a randomized order, with test drinks provided in a double-blind fashion.

Plasma Sample 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. Plasma glucose (Uni Kit III, 07367204; Roche, Basel, Switzerland) concentrations were analyzed with the COBAS-FARA semiautomatic analyzer (Roche). Insulin was analyzed by radioimmunoassay (Insulin RIA kit; Linco Research, St. Charles, MO). Plasma (500 μl) for amino acid analyses was deproteinized on ice with 100 μl of 24% (wt/vol) 5-sulphosalicylic acid and mixed, 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 precol-

umn derivatization procedure and a ternary solvent system (9). The exact phenylalanine and tyrosine concentrations in the infusates were measured using the same method and averaged 4.19 ± 0.02 and 1.59 ± 0.01 mmol/l, respectively. 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 gas chromatography-mass spectrometry (GC-MS; 6890N GC/5973N MSD; Agilent) using selected ion monitoring of masses 336 and 342 for unlabeled and labeled phenylalanine, respectively, and masses 466, 468, and 472 for unlabeled and labeled ($ring$-$^2H_2$ and $ring$-$^{13}C_6$, respectively) tyrosine (43). Plasma [$^2H_2$]glucose enrichment was determined by GC-MS analysis of the butylboronic acid-acetate derivatives on an electron impact GC-MS (6890N GC/5973N MSD; Agilent, and GC column, DB-5MS; J&W Scientific) using selected ion monitoring of masses 297 and 299 for unlabeled and labeled glucose (24). We applied standard regression curves in all isotopic enrichment analysis to assess linearity of the mass spectrometer and to control for the loss of tracer.

Muscle Sample 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 was 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 8 volumes (8 × dry wt of isolated muscle fibers × wet/dry ratio) of ice-cold 2% perchloric acid 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 concentrations in the supernatant were measured by HPLC after precolumn derivatization with o-phthaldialdehyde (39) and are presented in nanomoles per·gram wet muscle weight.

The protein pellet was washed with three additional 1.5-ml washes of 2% perchloric acid 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, 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$_4$OH. Thereafter, the eluate was dried and the purified amino acids were derivatized to their N-(O,S)-ethoxycarbonyl ethyl esters for the determination of $^{13}C/^{12}C$ ratios of muscle protein-bound phenylalanine (14). Thereafter, the derivative was measured by GC-IRMS (Finnigan MAT 252, Bremen, FRG) using HP Ultra I GC column (no. 19091A-112; Hewlett-Packard, Palo Alto, CA), combustion interface II, and monitoring of ion masses 44, 45, and 46. By establishing the relationship between the enrichment of a series of [$ring$-$^{13}C_6$]phenylalanine standards of variable enrichment and the enrichment of the N-(O,S)-ethoxycarbonyl ethyl esters of these standards, the muscle protein-bound enrichment of phenylalanine was determined. We applied standard regression curves to assess linearity of the mass spectrometer and to control for loss of tracer. The coefficient of variance for the measurement of L-[$ring$-$^{13}C_6$]phenylalanine enrichment in mixed muscle protein averaged $4.3 \pm 0.6\%$.
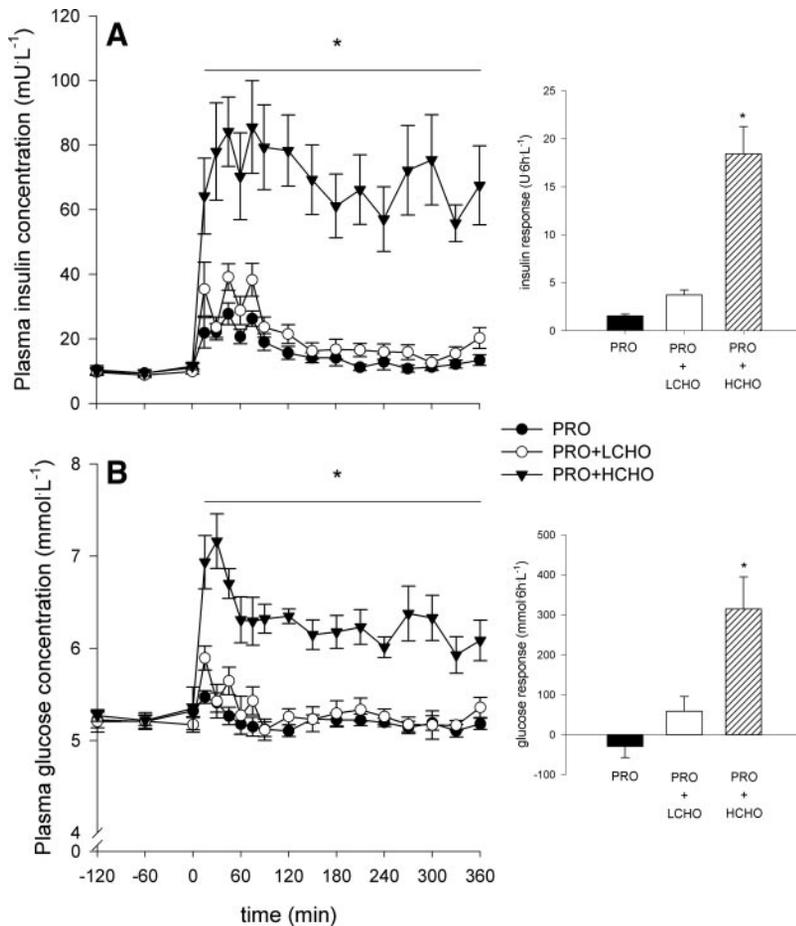

Fig. 1. Plasma insulin (*A*) and glucose (*B*) concentrations and responses (expressed as area under the curve − baseline values) in healthy men ($n = 10$) following ingestion of protein only (PRO; 0.3 g·kg^{-1}·h), protein with a low-carbohydrate dose (PRO + LCHO; 0.3 and 0.15 g·kg^{-1}·h, respectively), and protein with a high carbohydrate dose (PRO + HCHO; 0.3 and 0.6 g·kg^{-1}·h, respectively). Values represent means ± SE. Data were analyzed with repeated-measures ANOVA (treatment × time) or ANOVA. *Significantly different from PRO and PRO + LCHO ($P < 0.001$).

Calculations

Glucose metabolism. Infusion of $[^2H_2]$glucose with arterialized blood sampling was used to assess whole body rate of appearance (R_a) and disappearance (R_d) of glucose as previously described using the single-pool non-steady-state calculations of Steele (33), which were adapted for stable-isotope research as previously described (43) and summarized by the following equations:

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

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

where F is the infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), V is the distribution volume for glucose (160 ml/kg) (8), C_1 and C_2 are the glucose concentrations (mmol/l) in arterialized blood at *times 1* (t_1) and *2* (t_2), respectively, and E_1 and E_2 are the plasma $[6,6-^2H_2]$glucose enrichments (expressed in tracer-to-tracee ratio) at *times 1* and *2*, respectively.

Protein metabolism. 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. (34) and Short et al. (31). Briefly, phenylalanine and tyrosine turnover [flux (Q)] were measured from the isotope dilution at isotopic steady state:

$$Q = i \cdot \left(\frac{E_i}{E_p} - 1\right) \quad (3)$$

where *i* is the isotope infusion rate ($\mu mol \cdot kg$ body $wt^{-1} \cdot h^{-1}$) 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 R_a of meal protein from the gut (I) and protein breakdown (B); whole body protein synthesis rate was calculated as flux minus oxidation.

$$Q = S + O = B + I \quad (4)$$

$$S = Q - O \quad (5)$$

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 (34) using the formula

$$Q_{pt} = Q_t \cdot \frac{E_t}{E_p} \cdot \frac{Q_p}{(i_p + Q_p)} \quad (6)$$

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 of mixed muscle protein synthesis 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. Plasma L-[*ring*-$^{13}C_6$]phenylalanine and free muscle L-[*ring*-$^{13}C_6$]phenylalanine enrichments were used 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 fractional synthesis rate of mixed muscle proteins. Muscle FSRs were calculated as follows (19):

$$FSR = \frac{\Delta E_p}{E_{precursor} \cdot t} \cdot 100 \quad (7)$$

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 in the muscle biopsy taken at 6 h postexercise, and *3*) the free muscle L-[*ring*-$^{13}C_6$]phenylalanine enrichment in the muscle biopsy taken at 6 h postexercise corrected for the contribution of extracellular water, as previously described (41); *t* indicates the time interval (h) between biopsies, and the factor 100 is needed to express the FSR in percent per hour.

Statistics

A complete randomized design was used to assess the impact of coingesting different amounts of carbohydrate (0, 0.15, or 0.6 $g \cdot kg^{-1} \cdot h^{-1}$) with protein on plasma amino acid and glucose kinetics and whole body and muscle protein synthesis rates during postexercise recovery. All data are expressed as means $\pm$ SE. The plasma insulin, glucose, phenylalanine, tyrosine, and branched-chain amino acid (BCAA; leucine, isoleucine, and valine) responses were calculated as area under the curve (AUC) above baseline values. A
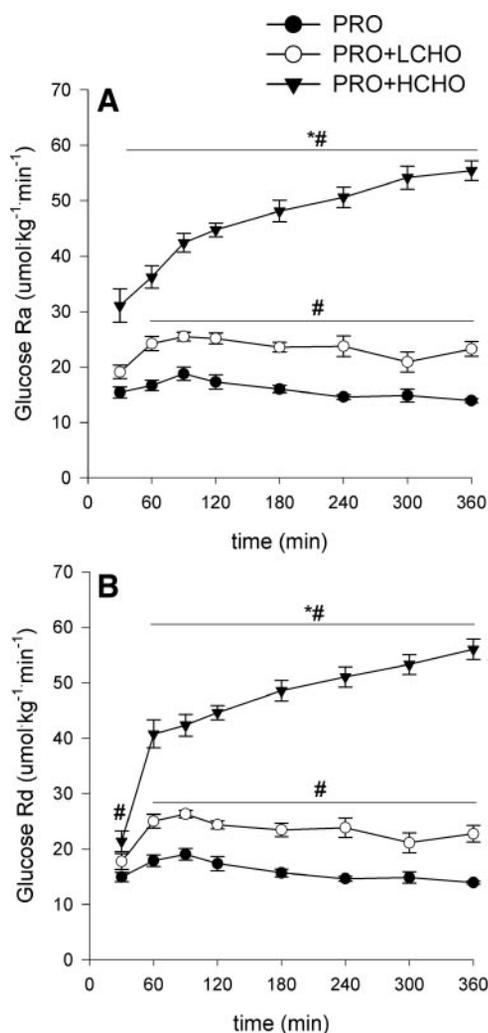

Fig. 2. Plasma rate of appearance (R_a; *A*) and rate of disappearance (R_d; *B*) of glucose in the PRO, PRO + LCHO, and PRO + HCHO treatments in healthy men (*n* = 10). Values represent means $\pm$ SE. Data were analyzed with repeated-measures ANOVA (treatment $\times$ time). Glucose R_a: treatment effect, $P < 0.01$; time effect, $P < 0.001$; interaction of treatment and time, $P < 0.01$. Glucose R_d: treatment effect, $P < 0.01$; time effect, $P < 0.001$; interaction of treatment and time, $P < 0.01$. #Significantly different from PRO (Scheffe's test, $P < 0.05$); *significantly different from PRO + LCHO (Scheffe's test, $P < 0.05$).

two-factor repeated-measures analysis of variance (ANOVA; general linear model) with time [degrees of freedom (DF): 9] and treatment (DF: 2) as factors was used to compare differences between treatments over time. For non-time-dependent variables, a one-factor ANOVA (DF: 2) was used to compare differences between treatments. In case of significant differences between treatments, a Scheffe post hoc test was applied to locate these differences. Statistical significance was set at $P < 0.05$. All calculations were performed using StatView 5.0 (SAS Institute, Cary, NC).

RESULTS

Resistance Exercise

The average workload that was applied in the upper-body exercises was 29 ± 1 kg for both chest and shoulder press and 34 ± 1 kg for front pulldown (lat pulley). For the lower-limb exercises, subjects were able to complete four sets at 75% of 1-RM (average weight: leg press 150 ± 10 kg, leg extension 84 ± 5 kg) and finished the last four sets at 65% of 1-RM (average weight: leg press 136 ± 8 kg, leg extension 71 ± 8 kg). All reductions in weight or the number of sets were recorded during the first experiment and replicated exactly in the subsequent tests. As such, each subject lifted exactly the same amount of weight and performed the same number of repetitions in all three randomized experiments.

Plasma Analyses

The plasma insulin and glucose responses, expressed as AUC above baseline values during the entire 6-h postexercise period, are shown in Fig. 1. The plasma insulin responses were significantly greater in the PRO + HCHO compared with the PRO + LCHO and PRO treatments (18.4 ± 2.9 vs. 3.7 ± 0.5 and 1.5 ± 0.2 U·6 h·l^{-1}, respectively, $P < 0.001$). The plasma glucose responses were significantly greater in the PRO + HCHO compared with the PRO + LCHO and PRO treatments (315 ± 80 vs. 59 ± 37 and -30 ± 28 mmol·6 h·l^{-1}, respectively, $P < 0.001$). Plasma insulin and glucose responses showed a significant correlation ($r = 0.53$, $P < 0.01$).

Plasma R_a and R_d over time are reported in Fig. 2. Plasma glucose R_a increased over time following the ingestion of the drinks in the PRO + HCHO and PRO + LCHO treatments, whereas no significant increase was observed in the PRO
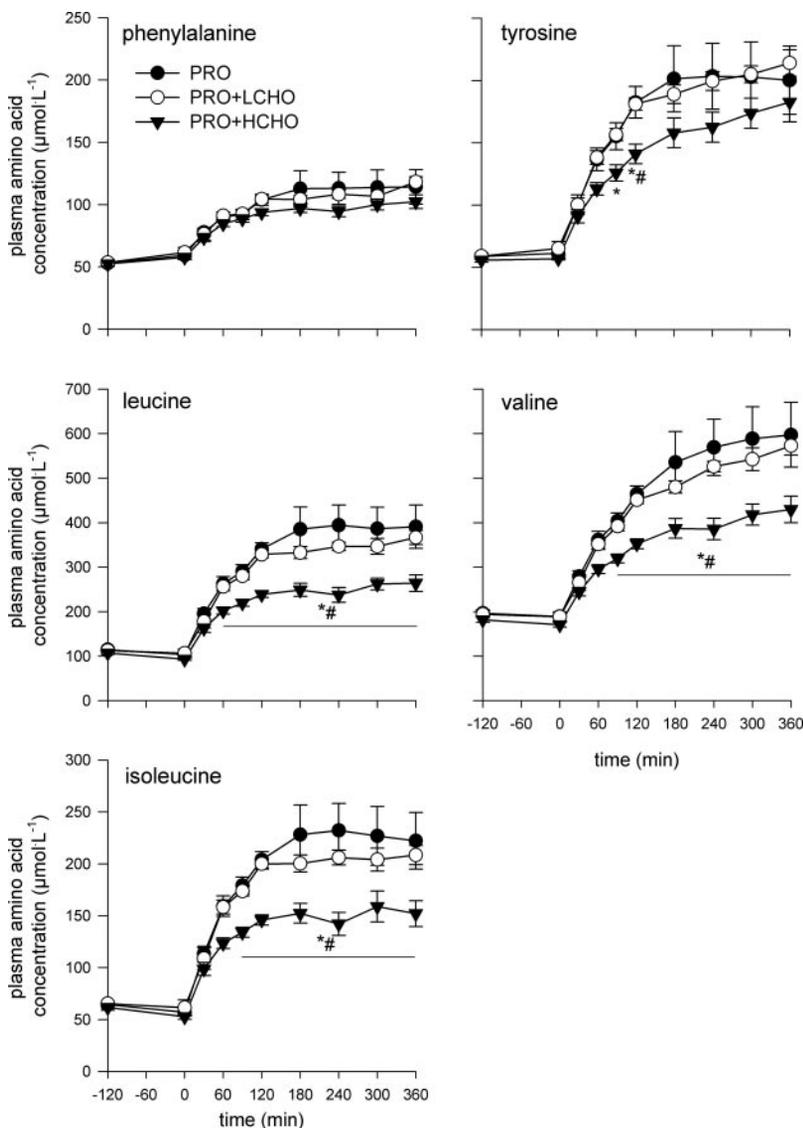

Fig. 3. Plasma phenylalanine, tyrosine, and branched-chain amino acid (leucine, isoleucine, and valine) concentrations (μmol/l) in the PRO, PRO + LCHO, and PRO + HCHO treatments in healthy men ($n = 10$). Values are means ± SE. Data were analyzed with repeated-measures ANOVA (treatment × time). Plasma phenylalanine: treatment effect, $P = 0.074$; time effect, $P < 0.001$; interaction of treatment and time, $P = 0.74$. Plasma tyrosine: treatment effect, $P < 0.05$; time effect, $P < 0.001$; interaction of treatment and time, $P = 0.052$. Plasma leucine: treatment effect, $P < 0.001$; time effect, $P < 0.001$; interaction of treatment and time, $P < 0.001$. Plasma valine: treatment effect, $P < 0.001$; time effect, $P < 0.001$; interaction of treatment and time, $P < 0.001$. Plasma isoleucine: treatment effect, $P < 0.001$; time effect, $P < 0.001$; interaction of treatment and time, $P < 0.001$. #Significantly different from PRO (Scheffe's test, $P < 0.05$); *significantly different from PRO + LCHO (Scheffe's test, $P < 0.05$).

treatment. Plasma glucose R_a increased to a greater extent in the PRO + HCHO compared with the PRO + LCHO treatment ($P < 0.01$). Total plasma glucose R_a (AUC) was lower in the PRO treatment compared with the PRO + LCHO and PRO + HCHO treatments (5.2 ± 0.2 vs. 7.7 ± 0.4 and 15.7 ± 0.5 mmol·6 h·kg^{-1}, respectively, $P < 0.001$). Plasma glucose R_d increased over time following the ingestion of the drinks in the PRO + HCHO and PRO + LCHO treatments, whereas no significant increase was observed in the PRO treatment. Plasma glucose R_d increased to a greater extent in the PRO + HCHO treatment compared with the PRO + LCHO treatment ($P < 0.05$). Total plasma glucose R_d was lower in the PRO treatment compared with PRO + LCHO and PRO + HCHO (5.3 ± 0.2 vs. 7.7 ± 0.4 and 15.7 ± 0.5 mmol·6 h·kg^{-1}, respectively, $P < 0.001$).

Plasma phenylalanine, tyrosine, leucine, valine, and isoleucine concentrations over time are reported in Fig. 3. Overall, their concentrations increased over time following the intake of the protein drinks (PRO, PRO + LCHO, and PRO + HCHO). No differences in plasma phenylalanine concentrations were observed between treatments. Plasma tyrosine concentrations were significantly different between treatments ($P < 0.05$). At $t = 90$ min, plasma tyrosine concentrations were lower in the PRO + HCHO compared with the PRO treatment and at $t = 120$ min lower than values observed in the PRO + LCHO and PRO treatments ($P < 0.05$). The plasma leucine, valine, and isoleucine (BCAA) concentrations were lower from $t = 90$ to $t = 360$ min in the PRO + HCHO compared with the PRO + LCHO and PRO treatments. No differences in plasma phenylalanine and tyrosine responses (expressed as AUC) were observed between treatments. In contrast, plasma BCAA responses were significantly lower in the PRO + HCHO vs. PRO + LCHO and PRO treatments ($P < 0.05$). The observed plasma leucine, valine, and isoleucine responses were negatively correlated with the concomitant insulin response ($r = -0.61$, $P < 0.001$; $r = -0.57$, $P = 0.001$; and $r = -0.53$, $P < 0.01$; respectively).

The time course of the plasma L-[$ring$-$^{13}C_6$]phenylalanine, L-[$ring$-$^2H_2$]tyrosine, and L-[$ring$-$^{13}C_6$]tyrosine enrichments are shown in Fig. 4. Overall, their enrichments declined after the ingestion of the first bolus of test drink, after which they reached plateau values at $t = 90–120$ min postexercise. No differences in plasma L-[$ring$-$^{13}C_6$]phenylalanine, L-[$ring$-$^2H_2$]tyrosine, and L-[$ring$-$^{13}C_6$]tyrosine enrichments were observed between treatments.

Muscle Analyses

Muscle free phenylalanine, tyrosine, leucine, isoleucine, and valine concentrations were significantly higher in the biopsies taken at $t = 360$ min compared with the biopsy samples collected at $t = 0$ min ($P < 0.001$) in the PRO, PRO + LCHO, and PRO + HCHO treatment. No differences were observed in muscle free phenylalanine concentrations in the biopsy sample taken at $t = 360$ min postexercise between treatments (102 ± 4 vs. 114 ± 17 and 113 ± 11 nmol/g wet muscle wt in PRO, PRO + LCHO, and PRO + HCHO, respectively, $P > 0.05$) Muscle free tyrosine concentrations in the biopsy sample taken at $t = 360$ min postexercise averaged 188 ± 16, 202 ± 18, and 188 ± 11 nmol/g wet muscle wt, respectively. Muscle free leucine and isoleucine concentrations were significantly higher
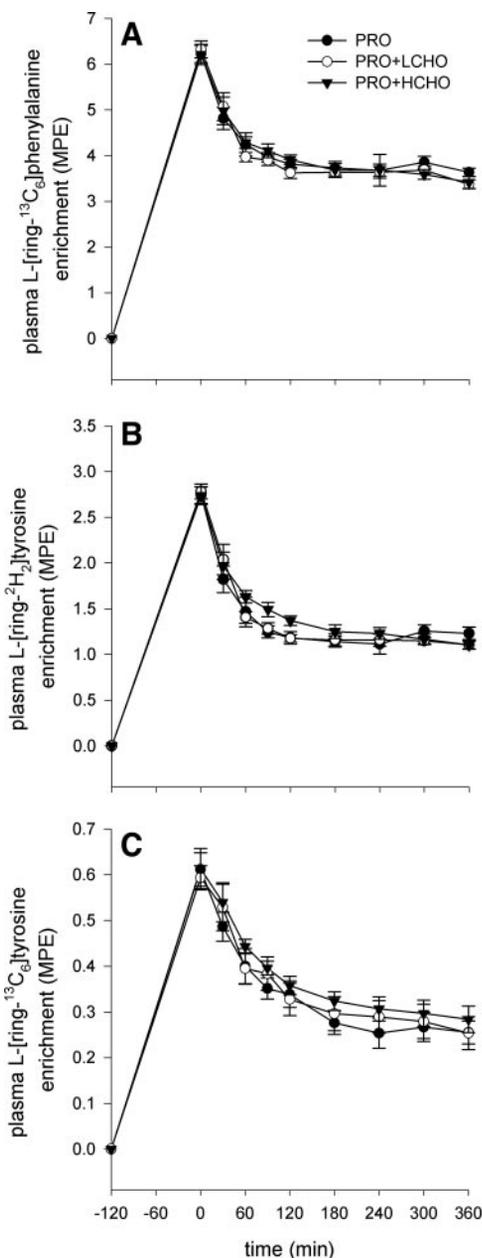

Fig. 4. Plasma L-[$ring$-$^{13}C_6$]phenylalanine (*A*), L-[$ring$-$^2H_2$]tyrosine (*B*), and L-[$ring$-$^{13}C_6$]tyrosine enrichment [mole percent excess (MPE); *C*] during the PRO, PRO + LCHO, and PRO + HCHO treatments in healthy men ($n = 10$). Values represent means $\pm$ SE. Data were analyzed with repeated-measures ANOVA (treatment $\times$ time). Plasma L-[$ring$-$^{13}C_6$]phenylalanine enrichment: treatment effect, $P = 0.93$; time effect, $P < 0.001$; interaction of treatment and time, $P = 0.91$. Plasma L-[$ring$-$^2H_2$]tyrosine enrichment: treatment effect, $P = 0.86$; time effect, $P < 0.001$; interaction of treatment and time, $P = 0.46$. Plasma L-[$ring$-$^{13}C_6$]tyrosine enrichment: treatment effect, $P = 0.96$; time effect, $P < 0.001$; interaction of treatment and time, $P = 1.00$.

in biopsy samples taken at $t = 360$ min postexercise in the PRO vs. the PRO + HCHO treatment (380 ± 49 vs. 302 ± 66 and 317 ± 91 vs. 219 ± 43 nmol/g wet muscle wt, respectively, $P < 0.05$). Intermediate values were observed in the PRO + LCHO treatment, i.e., 335 ± 25 and 225 ± 36 nmol/g wet muscle wt, respectively ($P > 0.05$). Muscle free valine concentrations were significantly higher in muscle biopsies taken at $t = 360$ min in the PRO compared with the PRO +

HCHO treatment (361 ± 55 vs. 293 ± 58 nmol/g wet muscle wt). Intermediate values were observed in the PRO + LCHO treatment (343 ± 28 nmol/g wet muscle wt, $P > 0.05$).

Mean plasma amino acid enrichments over the latter 4 h of postexercise recovery, muscle free amino acid pool enrichments in the 6-h postexercise biopsies, and the increments in muscle protein pool enrichment are presented in Table 1. Muscle free L-[ring-$^{13}C_6$]phenylalanine and L-[ring-$^2H_2$]tyrosine enrichment were significantly higher in the biopsy samples taken at $t = 360$ min vs. those taken at $t = 0$ min ($P < 0.001$). No differences in muscle L-[ring-$^{13}C_6$]phenylalanine, L-[ring-$^2H_2$]tyrosine, and L-[ring-$^{13}C_6$]tyrosine enrichments were observed between treatments. In addition, no differences in tracer incorporation rate into muscle protein were observed between treatments (Table 1).

Whole Body Protein Metabolism

Phenylalanine flux during the latter 4 h of postexercise recovery did not differ between treatments (107 ± 3, 111 ± 3, and 112 ± 6 μmol·kg^{-1}·h^{-1} in the PRO, PRO + LCHO, and PRO + HCHO treatments, respectively). In agreement, tyrosine flux also did not differ between treatments (87 ± 4, 87 ± 4, and 89 ± 4 μmol·kg^{-1}·h^{-1} in the PRO, PRO + LCHO, and PRO + HCHO treatments, respectively). Whole body protein breakdown, oxidation, and synthesis rates did not differ between treatments (Fig. 5A). As such, no differences in whole body net protein balance were observed between PRO, PRO + LCHO, and PRO + HCHO ($P > 0.05$).

Mixed Muscle Protein Synthesis Rates

Mixed muscle protein FSR, using mean plasma L-[ring-$^{13}C_6$]phenylalanine enrichment as the precursor (Fig. 5B), averaged 0.10 ± 0.01, 0.10 ± 0.01, and 0.11 ± 0.01%/h in the PRO, PRO + LCHO, and PRO + HCHO treatments, respectively. No differences were observed between treatments. When using the free intracellular L-[ring-$^{13}C_6$]phenylalanine enrichment as precursor, FSR values tended to be higher and showed no treatment effect (0.11 ± 0.01, 0.12 ± 0.01, and 0.13 ± 0.01%/h in the PRO, PRO + LCHO, and PRO + HCHO treatments, respectively). After correction for extracellular water (41), FSR values averaged 0.13 ± 0.02, 0.13 ±

0.01, and 0.13 ± 0.01%/h in the PRO, PRO + LCHO, and PRO + HCHO treatments, respectively.

Correlations

Plasma glucose responses and glucose R_a and R_d were positively correlated with the ingested amount of carbohydrate ($r = 0.84, 0.66, 0.97$, and 0.97, respectively, $P < 0.01$) and the concomitant plasma insulin response ($r = 0.55, 0.79$, and 0.79, respectively, $P < 0.05$). Plasma phenylalanine disposal rate did not correlate significantly with either plasma glucose or insulin responses or glucose R_a or R_a.

Significant correlations were observed between plasma insulin concentrations and circulating leucine, isoleucine, and valine levels ($r = -0.46, -0.38$, and -0.39, respectively, $P < 0.05$). Muscle free leucine and valine concentrations (determined in the 6-h postexercise biopsy) were negatively correlated with the plasma insulin response ($r = -0.46$ and -0.39, respectively, $P < 0.05$). Muscle free leucine, valine, and tyrosine concentrations correlated with their plasma concentrations, whereas no such correlation was observed for isoleucine and phenylalanine.

Plasma glucose, but not insulin, responses were significantly correlated with both plasma and muscle free L-[ring-$^{13}C_6$]tyrosine enrichment and whole body protein oxidation rate ($r = 0.44, 0.55$, and 0.55, respectively, $P < 0.05$). In agreement, whole body protein synthesis efficiency and net protein balance were negatively correlated with the plasma glucose response ($r = -0.56$ and -0.51, respectively, $P < 0.05$).

DISCUSSION

In the present study, we assessed the impact of carbohydrate coingestion on postexercise muscle protein synthesis when ample protein is being administered. We determined whole body protein turnover and mixed muscle protein synthesis rates by measuring the incorporation rate of labeled phenylalanine in vastus lateralis muscle following postexercise ingestion of protein (0.3 g·kg^{-1}·h^{-1}) with 0, 0.15, or 0.6 g·kg^{-1}·h^{-1} carbohydrate in healthy, young males. Our data show that carbohydrate coingestion does not affect mixed muscle protein synthesis rate during recovery from resistance-type exercise under conditions where ample protein is being ingested.

Table 1. *Plasma and muscle AA tracer enrichments*

	Treatment			P Value
	PRO	PRO + LCHO	PRO + HCHO	
Plasma AA enrichments				
L-[ring-$^{13}C_6$]phenylalanine	0.0385±0.0009	0.0372±0.0007	0.0379±0.0011	0.650
L-[ring-$^2H_2$]tyrosine	0.0115±0.0005	0.0116±0.0005	0.0124±0.0006	0.361
L-[ring-$^{13}C_6$]tyrosine	0.0029±0.0003	0.0027±0.0003	0.0030±0.0002	0.747
Muscle AA enrichments				
L-[ring-$^{13}C_6$]phenylalanine	0.0357±0.0013	0.0323±0.0007	0.0328±0.0013	0.103
L-[ring-$^2H_2$]tyrosine	0.0124±0.0029	0.0111±0.0028	0.0108±0.0021	0.909
L-[ring-$^{13}C_6$]tyrosine	0.0093±0.0009	0.0096±0.0005	0.0084±0.0008	0.494
δEnrichment muscle protein				
L-[ring-$^{13}C_6$]phenylalanine	0.00025±0.00003	0.00024±0.00002	0.00027±0.00003	0.826

Values expressed means ± SE; $n = 10$ young, healthy males. PRO, protein only; PRO + LCHO, protein plus low amount of carbohydrate; PRO + HCHO, protein plus high amount of carbohydrate. AA, amino acid. Data were analyzed with ANOVA. Plasma AA enrichments represent the average plasma AA enrichment during the last 4 h of recovery. Muscle AA enrichments represent the AA enrichment in muscle biopsy taken after 6 h of postexercise recovery. δEnrichment muscle protein represents the increment in muscle protein enrichment from 0 to 6 h following exercise. No differences between treatments.
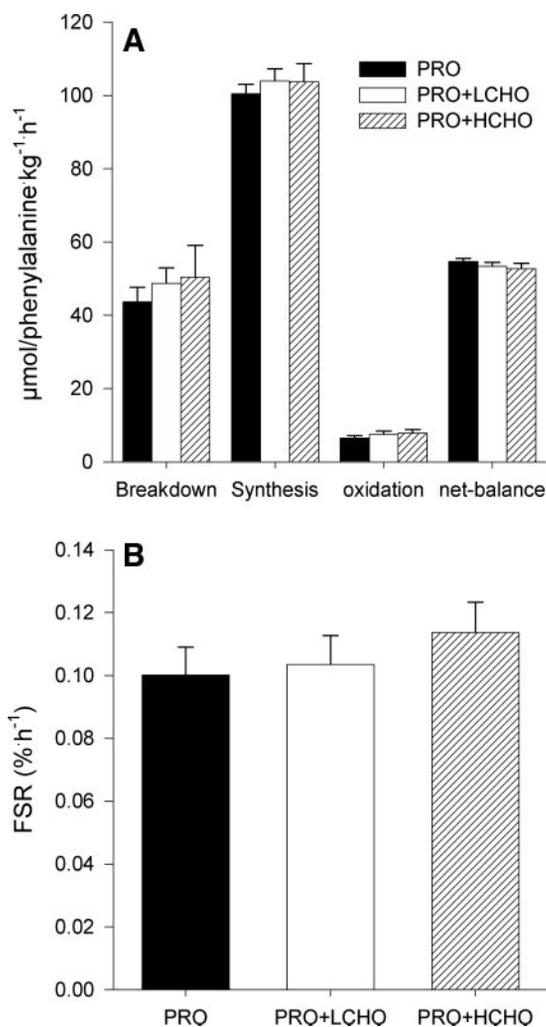
Fig. 5. Whole body protein breakdown, synthesis, and oxidation rates and net protein balance (expressed as μmol phenylalanine·kg^{-1}·h^{-1}; *A*) and fractional synthesis rate (FSR) of mixed muscle protein (*B*) in the PRO, PRO + LCHO, and PRO + HCHO treatments in healthy men (*n* = 10). Values represent means ± SE. Data were analyzed with ANOVA. No differences were observed between treatments in whole body protein breakdown (*P* = 0.68), synthesis (*P* = 0.74), oxidation (*P* = 0.69), whole body protein net balance (*P* = 0.53), and FSR in skeletal muscle tissue (*P* = 0.51).

It has been firmly established that postexercise protein and/or amino acid administration stimulates muscle protein synthesis (3, 6, 18, 19, 26, 35–37), resulting in a positive net muscle protein balance (3, 6, 35, 42). In the present study, we demonstrate that whole body protein synthesis exceeds protein breakdown, resulting in a positive net protein balance when protein is ingested during postexercise recovery (Fig. 5*A*). It has been suggested that carbohydrate coingestion is needed to further augment the anabolic response to exercise. The latter has been attributed to the proposed efficacy of carbohydrate ingestion to elevate plasma insulin concentrations (5, 18, 19, 23, 29) and, as such, to inhibit postexercise muscle protein breakdown (5, 29). Muscle protein synthesis does not seem to be affected by the ingestion of carbohydrate only during postexercise recovery (5, 29). However, hyperinsulinemia has been shown to further stimulate muscle protein synthesis during conditions of hyperaminoacidemia (10, 12, 13). Consequently, it remains to be established whether coingestion

of carbohydrate with an ample amount of protein can further augment the muscle protein synthetic response to exercise.

The coingestion of either a small (0.15 g·kg^{-1}·h^{-1}) or large (0.6 g·kg^{-1}·h^{-1}) amount of carbohydrate substantially raised plasma insulin concentrations (Fig. 1) and stimulated plasma glucose appearance and disappearance (Fig. 2). However, the greater plasma insulin and glucose responses (Fig. 1) were not accompanied by differences in whole body anabolic response to resistance-type exercise under the conditions where ample protein was being ingested (0.3 g·kg^{-1}·h^{-1}). We observed no differences in whole body protein synthesis and degradation rates between treatments (Fig. 5*A*). Mixed muscle protein FSR was not affected by the coingestion of either a small or large amount of carbohydrate (Fig. 5*B*). As such, our data indicate that carbohydrate coingestion is not required to maximize the postexercise muscle protein synthetic response when ample protein is being administered.

Coingestion of carbohydrate with protein resulted in substantially greater plasma glucose responses compared with the ingestion of protein only (Fig. 1*B*). We assessed plasma glucose kinetics to ensure that exogenous glucose was indeed taken up from the gut in the PRO + LCHO and PRO + HCHO treatments (Fig. 2). The greater glucose appearance rate in PRO + HCHO was accompanied by higher plasma insulin levels compared with the other treatments (Fig. 1, *A* and *B*). Hyperinsulinemia has been reported to attenuate muscle protein breakdown following resistance-type exercise (5, 29). Although we did not assess skeletal muscle protein breakdown rates, we observed lower plasma and muscle BCAA levels when carbohydrate was coingested. The latter may imply that either protein breakdown was reduced and/or that amino acid loss via transamination/oxidation was enhanced.

Consequently, the further rise in circulating insulin levels following carbohydrate coingestion did not seem to modulate postexercise protein metabolism. Even in the absence of carbohydrate coingestion, plasma insulin levels averaged 16.5 ± 1.6 μU/ml during the 6-h recovery period in the PRO treatment. Interestingly, it was recently suggested by Rennie et al. (27) that an increase in insulin levels above 10–15 μU/ml does not further enhance muscle protein synthesis and/or reduce protein degradation. Therefore, the present data as well as data from previous studies (1, 7, 11) seem to confirm that insulin plays a merely permissive role in stimulating muscle protein anabolism. Therefore, the present findings imply that when ample protein is ingested during recovery from resistance-type exercise, carbohydrate coingestion is not warranted to maximize the protein synthetic response to resistance exercise. However, as muscle glycogen content can be reduced by 30–40% following a single session of resistance-type exercise (17), carbohydrate coingestion would be preferred when trying to accelerate muscle glycogen repletion.

In conclusion, coingestion of carbohydrate does not further augment muscle protein synthesis rates during recovery from resistance-type exercise under conditions where ample protein is ingested.

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