Co-ingestion of carbohydrate with protein does not further augment post-exercise muscle protein synthesis

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Abstract

The present study was designed to assess the impact of co-ingestion of various amounts of carbohydrate combined to an ample amount of protein intake on post-exercise muscle protein synthesis rates. Ten healthy, fit men (20±0.3 y) were randomly assigned to 3 cross-over experiments. After 60 min of resistance exercise, subjects consumed 0.3 g·kg⁻¹·h⁻¹ protein hydrolysate with 0, 0.15, or 0.6 g·kg⁻¹·h⁻¹ carbohydrate during a 6 h recovery period (PRO, PRO+LCHO, and PRO+HCHO, respectively). Primed, continuous infusions with L-[ring-¹³C₆]phenylalanine, L-[ring-²H₂]tyrosine, and [6,6-²H₂]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 post-exercise recovery. Plasma insulin responses were significantly greater in PRO+HCHO compared to PRO+LCHO and PRO (18.4±2.9 vs 3.7±0.5 and 1.5±0.2 U·6h·L⁻¹, respectively: P<0.001). Plasma glucose rate of appearance (Ra) and disappearance (Rd) increased over time in PRO+HCHO and PRO+LCHO but not in PRO. Plasma glucose Ra and Rd 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⁻¹ in the PRO, PRO+LCHO and PRO+HCHO experiments, respectively. In conclusion, co-ingestion of carbohydrate during recovery does not further stimulate post-exercise muscle protein synthesis when ample protein is ingested.

Keywords: resistance exercise, muscle, protein metabolism, nutrition, recovery
Introduction

Post-exercise 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 post-exercise recovery and, as such, to maintain performance capacity (15). Therefore, endurance athletes generally aim to maximize post-exercise muscle glycogen synthesis rates by ingesting large amounts of carbohydrate during recovery (30, 40). Co-ingestion 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 that post-exercise protein and/or amino acid intake is essential to allow net muscle protein accretion (4, 18, 19, 23, 25). Therefore, athletes involved in resistance type exercise training, like fitness and bodybuilding, generally ingest large quantities of protein during post-exercise recovery to augment net muscle protein accretion (21, 38). It is generally assumed that carbohydrate should be co-ingested to maximize the post-exercise muscle protein synthetic response. Even though ingestion of only carbohydrate does not seem to stimulate post-exercise 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 co-ingestion to augment post-exercise 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 esthetic purposes) and, therefore, prefer to restrict carbohydrate intake.

In the present study, we assess the impact of the co-ingestion of different amounts of carbohydrate on post-exercise 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·kg$^{-1}$·h$^{-1}$) with 0, 0.15, and 0.6 g·kg$^{-1}$·h$^{-1}$ carbohydrate in healthy, young men.
Methods

Subjects
10 healthy, fit male volunteers (age: 20.1±0.3 y; weight: 71.3±2.7 kg; height: 1.81±0.02 m; BMI: 21.9±1.0 kg·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 Ethical 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 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 GmbH, Albstadt, Germany). Body fat percentage was calculated using Siri’s equation (32). Fat Free mass (FFM) 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 2 lower-limb exercises (leg press and leg extension) and for the 3 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, at least 1 wk before the first experimental treatment, the subjects’ 1 repetition maximum (1RM) was determined (20). After warming up, the load was set at 90-95% of the
estimated 1RM, 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-RM 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⁻¹ body weight, consisting of 65 energy% (En%) carbohydrate, 15 En% protein and 20 En% fat) 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 d 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 test.

**Experiments**

All subjects participated in 3 experimental treatments, each separated by 7 d, in which subjects first completed ~1 h of resistance exercise (upper body and legs) followed by 6 h recovery. During recovery, 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 fractional synthesis rate (FSR) of mixed muscle protein by the incorporation of L-[ring-¹³C₆]phenylalanine in the mixed protein pool of tissue samples collected from the vastus lateralis muscle.
**Protocol**

At 8.00 a.m., after an overnight fast, subjects arrived at the laboratory by car or 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 [6,6-²H₂]glucose (13.5 µmol·kg⁻¹), L-[ring-¹³C₆]phenylalanine (2 µmol·kg⁻¹), and L-[ring-²H₂]tyrosine (0.775 µmol·kg⁻¹) was administered to prime the glucose, phenylalanine, and tyrosine pools, respectively. Thereafter, continuous tracer infusion was started (infusion rate (IR) of 0.286±0.001 µmol·kg⁻¹·min⁻¹ for [6,6-²H₂]glucose, 0.049±0.001 µmol·kg⁻¹·min⁻¹ for L-[ring-¹³C₆]phenylalanine, and 0.019±0.001 µmol·kg⁻¹·min⁻¹ for L-[ring-²H₂]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 Watt), subjects completed a session of 3 upper-body resistance type exercises, which featured 3 sets of 10 repetitions for each of the exercises. This was performed with resistance set at 40% of their bodyweight (BW) for the chest press and shoulder press, and at 50% BW for the front pull-down (all equipment by Jimsa Benelux BV, Rotterdam, The Netherlands), with 1 min rest intervals between sets. This was followed by a session of lower-limb exercises, consisting of 8 sets of 10 repetitions on the leg press and leg extension machines (Technogym BV, 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⁻¹) of a given test drink. Repeated boluses (2.5 ml·kg⁻¹) 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 approximately 3 cm below entry through the fascia using the percutaneous needle biopsy technique (2). Muscle samples were dissected carefully, and freed from any visible non-muscle material. The muscle sample was immediately frozen in liquid nitrogen.

**Beverages**

Subjects received a beverage volume of 2.5 ml·kg⁻¹ every 30 min to ensure a given dose of 0.3 g·kg⁻¹ of a casein protein hydrolysate per h 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 CHO (PRO+HCHO treatment). The amount of protein (0.3 g·kg⁻¹) 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⁻¹) for a period of at least 6 h (41). Repeated boluses were administered to enable a continuous supply of amino acids in the circulation, preventing large perturbations in plasma [6,6-²H₂]glucose, L-[ring-¹³C₆]phenylalanine, and L-[ring-²H₂]tyrosine enrichment.

Glucose and maltodextrin were obtained from AVEBE (Veendam, The Netherlands). The casein protein hydrolysate (PeptoPro®, 85.3% protein) 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% w/w), 0.9 g citric acid solution (50% w/w) 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 samples analysis**

Blood samples were collected in EDTA containing tubes and centrifuged at 1000 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 semi-automatic analyzer (Roche). Insulin was analyzed by radioimmunoassay (Insulin RIA kit, LINCO Research Inc., St. Charles, MO, USA). Plasma (500 μL) for amino acid analyses was deproteinized on ice with 100 μL of 24% (w/v) 5-sulphosalicylic acid, 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 precolumn 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⁻¹, respectively. For plasma phenylalanine and tyrosine enrichment measurements, plasma phenylalanine and tyrosine were derivatized to their t-butyldimethylsilyl (TBDMS) derivatives and their $^{13}$C and/or $^2$H enrichments were determined by electron ionization gas chromatography-mass spectrometry (GC-MS, Agilent 6890N GC/5973N MSD Little Falls, USA) 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-$^2$H$_2$ and ring-$^{13}$C$_6$) tyrosine, respectively (43). Plasma [2H$_2$]-glucose enrichment was determined by gas chromatography-mass spectrometry (GC-MS) analysis of the butylboronic acid-acetate derivatives on an electron impact gas chromatography-mass spectrometry (GC-MS, Agilent 6890N GC/5973N MSD Little Falls,
USA, GC column: DB-5MS, J & W Scientific, Folsom, CA, USA) 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 non-muscle fiber material were removed from the muscle fibers under a light microscope. The isolated muscle fiber mass (2-3 mg) was weighed and 8 volumes (8x dry weight of isolated muscle fibers x wet/dry ratio) 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, L-[ring-$^2$H$_2$]tyrosine, and L-[ring-$^{13}$C$_6$]tyrosine enrichments could be measured using their TBDMS derivatives on a GC-MS. The free amino acid concentrations in the supernatant was measured by HPLC, after precolumn derivatization with o-phthaldialdehyde (39) and are presented in nmol·g$^{-1}$, wet muscle weight.

The protein pellet was washed with 3 additional 1.5 ml washes of 2% PCA, dried and the proteins were hydrolyzed in 6M HCl at 120°C for 15-18h. 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, Biorad, Hercules, CA, USA) using 2M 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 (#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 (CV) for the measurement of L-[ring-$^{13}$C$_6$]phenylalanine enrichment in mixed muscle protein averaged 4.3±0.6%.

**Calculations**

**Glucose metabolism**

Infusion of [$^2$H$_2$]glucose with arterialized blood sampling was used to assess whole-body rate of appearance (Ra) and disappearance (Rd) 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:

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

\[
R_d = R_a - V \left( \frac{C_2 - C_1}{t_2 - t_1} \right) \tag{2}
\]

where F is the infusion rate ($\mu$M·kg$^{-1}$·min$^{-1}$); V is the distribution volume for glucose (160 ml·kg$^{-1}$) (8); C$_1$ and C$_2$ are the glucose concentrations (mmol·l$^{-1}$) in arterialized blood at times 1 ($t_1$) and 2 ($t_2$), respectively; and E$_1$ and E$_2$ are the plasma [6,6-$^2$H$_2$]glucose enrichments [expressed in tracer-to-tracee ratio (TTR)] at times 1 and 2, respectively.
Protein metabolism

Infusion of L-[ring-\textsuperscript{13}C\textsubscript{6}]phenylalanine and L-[ring-\textsuperscript{2}H\textsubscript{2}]tyrosine with muscle and arterialized blood sampling was used to simultaneously assess whole-body amino acid kinetics and fractional synthesis rate (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, $Q$) was measured from the isotope dilution at isotopic steady state:

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

where $i$ is the isotope infusion rate ($\mu$mol·kg body weight\textsuperscript{-1}·h\textsuperscript{-1}) and $E_i$ and $E_p$ correspond to the enrichments of infusate and plasma amino acids, respectively. At isotopic steady state, protein flux ($Q$) equals the sum of protein synthesis ($S$) and oxidation ($O$) as well as the sum of the rate of appearance of meal protein from the gut ($I$) and protein breakdown ($B$); whole-body protein synthesis rate was calculated as flux minus oxidation.

$$Q = S + O = B + I$$

$$S = Q - O$$

At isotopic steady state, whole-body phenylalanine oxidation can be determined from the conversion (hydroxylation) of L-[\textsuperscript{ring-13}C\textsubscript{6}]phenylalanine to L-[\textsuperscript{ring-13}C\textsubscript{6}]tyrosine. The rate of hydroxylation ($Q_{pt}$) was calculated (34) using the formula

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

where $Q_t$ and $Q_p$ are the flux rates for L-[\textsuperscript{ring-2}H\textsubscript{2}]tyrosine and labeled phenylalanine, respectively. $E_t$ and $E_p$ are the L-[\textsuperscript{ring-13}C\textsubscript{6}]tyrosine and L-[\textsuperscript{ring-13}C\textsubscript{6}]phenylalanine enrichments in plasma, respectively, and $i_p$ is the infusion rate of the phenylalanine tracer.
Fractional rate of mixed muscle protein synthesis (FSR) was calculated by dividing the increment in enrichment in the product, i.e. protein-bound L-[ring-\(^{13}\)C\(_6\)]phenylalanine, by the enrichment of the precursor. 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 FSR’s were calculated as follows (19):

\[
FSR = \frac{\Delta E_p}{E_{\text{precursor}} \cdot t} \cdot 100
\]

where \(\Delta E_p\) is the delta increment of protein bound L-[ring-\(^{13}\)C\(_6\)]phenylalanine during incorporation periods. \(E_{\text{precursor}}\) is (I) the average plasma L-[ring-\(^{13}\)C\(_6\)]phenylalanine enrichment during the time period for determination of amino acid incorporation; (II) the free muscle L-[ring-\(^{13}\)C\(_6\)]phenylalanine in the muscle biopsy taken at 6h post-exercise; (III) the free muscle L-[ring-\(^{13}\)C\(_6\)]phenylalanine enrichment in the muscle biopsy taken at 6h post-exercise corrected for the contribution of extra-cellular 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 h (\%·h\(^{-1}\)).

**Statistics**

A complete randomized design was used to assess the impact of co-ingesting different amounts of carbohydrate (0, 0.15 or 0.6 g·kg\(^{-1}\)·h\(^{-1}\)) with protein on plasma amino acid and glucose kinetics and whole-body and muscle protein synthesis rates during post-exercise recovery. All data are expressed as means±SEM. The plasma insulin, glucose, phenylalanine, tyrosine and BCAA (branched chain amino acids; leucine, isoleucine and valine) responses were calculated as area under the curve above baseline values. A 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 inc., Cary, NC, USA).
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 pull-down (lat-pulley). For the lower limb exercises subjects were able to complete 4 sets at 75% of 1-RM (average weight: leg press 150±10; leg extension 84±5 kg) and finished the last 4 sets at 65% of 1-RM (average weight: leg press 136±8; leg extension 71±8 kg). All reductions in weight or the number of sets were recorded during the first experiment and exactly replicated in the subsequent tests. As such, each subject lifted exactly the same amount of weight and performed the same number of repetitions in all 3 randomized experiments.

**Plasma analyses**

The plasma insulin and glucose responses, expressed as area under the curve above baseline values (AUC) during the entire 6 h post-exercise period, are shown in Figure 1. The plasma insulin responses were significantly greater in the PRO+HCHO compared to the PRO+LCHO and PRO treatments (18.4±2.9 vs 3.7±0.5 and 1.5±0.2 U·6h·L⁻¹, respectively; P<0.001). The plasma glucose responses were significantly greater in the PRO+HCHO compared to the PRO+LCHO and PRO treatments (315±80 vs 59±37 and -30±28 mmol·6h·L⁻¹, respectively; P<0.001). Plasma insulin and glucose responses showed a significant correlation (r=0.53, P<0.01).

Plasma glucose appearance (Ra) and disappearance (Rd) rates over time are reported in Figure 2. Plasma glucose Ra 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 Ra increased to a greater extent in the PRO+HCHO
compared to the PRO+LCHO treatment (P<0.01). Total plasma glucose Ra (AUC) was lower in the PRO treatment compared to the PRO+LCHO and PRO+HCHO treatments (5.2±0.2 vs 7.7±0.4 and 15.7±0.5 mmol·6h·kg$^{-1}$, respectively, P<0.001). Plasma glucose Rd 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 Rd increased to a greater extent in the PRO+HCHO treatment compared to the PRO+LCHO treatment (P<0.05). Total plasma glucose Rd was lower in the PRO treatment compared to PRO+LCHO and PRO+HCHO (5.3±0.2 vs 7.7±0.4 and 15.7±0.5 mmol·6h·kg$^{-1}$, respectively; P<0.001).

Plasma phenylalanine, tyrosine, leucine, valine, and isoleucine concentrations over time are reported in Figure 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 to 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 to 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-$^2$H$_2$]tyrosine and L-[ring-$^{13}$C$_6$]tyrosine enrichments are shown in Figure 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 post-exercise. No differences in plasma L-[ring-$^{13}$C$_6$]phenylalanine, L-[ring-$^{2}$H$_2$]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 when compared to 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 post-exercise between treatments (102±4 vs 114±17 and 113±11 nmol·g wet muscle weight$^{-1}$ in PRO, PRO+LCHO and PRO+HCHO, respectively, P>0.05) Muscle free tyrosine concentrations in the biopsy sample taken at t=360 min post-exercise averaged 188±16, 202±18 and 188±11 nmol·g wet muscle weight$^{-1}$, respectively. Muscle free leucine and isoleucine concentrations were significantly higher in biopsy samples taken at t=360 min post-exercise in the PRO vs the PRO+HCHO treatment (380±49 vs 302±66, and 317±91 vs 219±43 nmol·g wet muscle weight$^{-1}$, respectively, P<0.05). Intermediate values were observed in the PRO+LCHO treatment, ie 335±25 and 225±36 nmol·g wet muscle weight$^{-1}$, respectively (P>0.05). Muscle free valine concentrations were significantly higher in muscle biopsies taken at t=360 min in the PRO compared to the PRO+HCHO treatment (361±55 nmol·L$^{-1}$ vs. 293±58 nmol·g wet muscle weight$^{-1}$). Intermediate values were observed in the PRO+LCHO treatment (343±28 nmol·g wet muscle weight$^{-1}$; P>0.05).

Mean plasma amino acid enrichments over the latter 4 h of post-exercise recovery, muscle free amino acid pool enrichments in the 6 h post-exercise 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-$^{2}$H$_2$]tyrosine enrichment were significantly higher in the
biopsy samples taken at t=360 min versus those taken at t=0 min (P<0.001). No differences in muscle L-[ring-\textsuperscript{13}C\textsubscript{6}]phenylalanine, L-[ring-\textsuperscript{2}H\textsubscript{2}]tyrosine and L-[ring-\textsuperscript{13}C\textsubscript{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 post-exercise recovery did not differ between treatments (107±3, 111±3, and 112±6 µmol·kg\textsuperscript{-1}·h\textsuperscript{-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\textsuperscript{-1}·h\textsuperscript{-1} in the PRO, PRO+LCHO and PRO+HCHO treatments, respectively). Whole-body protein breakdown, oxidation and synthesis rates did not differ between treatments (Figure 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 fractional synthesis rates (FSR), using mean plasma L-[ring-\textsuperscript{13}C\textsubscript{6}]phenylalanine enrichment as the precursor (Figure 5B), averaged 0.10±0.01, 0.10±0.01 and 0.11±0.01 %·h\textsuperscript{-1} in the PRO, PRO+LCHO, and PRO+HCHO treatments, respectively. No differences were observed between treatments. When using the free intracellular L-[ring-\textsuperscript{13}C\textsubscript{6}]phenylalanine enrichment as precursor, FSR values tend to be higher, and showed no treatment effect (0.11±0.01, 0.12±0.01, and 0.13±0.01 %·h\textsuperscript{-1} in the PRO, PRO+LCHO, and PRO+HCHO treatment, respectively). After correction for extra-cellular water (41), FSR values averaged 0.13±0.02, 0.13±0.01, and 0.13±0.01 %·h\textsuperscript{-1} in the PRO, PRO+LCHO, and PRO+HCHO treatments, respectively.
Correlations

Plasma glucose responses and glucose Ra and Rd 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 Ra or Rd.

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 post-exercise 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, while 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-13C₆]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 co-ingestion on post-exercise 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 post-exercise 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 co-ingestion does not affect mixed muscle protein synthesis rate during recovery from resistance type exercise under conditions where ample protein is being ingested.

It has been firmly established that post-exercise 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 post-exercise recovery (Figure 5A). It has been suggested that carbohydrate co-ingestion 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 post-exercise muscle protein breakdown (5, 29). Muscle protein synthesis does not seem to be affected by the ingestion of carbohydrate only during post-exercise 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 co-ingestion of carbohydrate with an ample amount of protein can further augment the muscle protein synthetic response to exercise.

The co-ingestion 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 (Figure 1) and stimulated
plasma glucose appearance and disappearance (Figure 2). However, the greater plasma insulin and glucose responses (Figure 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 (Figure 5A). Mixed muscle protein fractional synthesis rate (FSR) was not affected by the co-ingestion of either a small or large amount of carbohydrate (Figure 5B). As such, our data indicate that carbohydrate co-ingestion is not required to maximize the post-exercise muscle protein synthetic response when ample protein is being administered.

Co-ingestion of carbohydrate with protein resulted in substantially greater plasma glucose responses when compared to the ingestion of only protein (Figure 1B). 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 (Figure 2). The greater glucose appearance rate in PRO+HCHO was accompanied by higher plasma insulin levels compared to the other treatments (Figure 1A/B). Hyperinsulinemia has been reported to attenuate muscle protein breakdown following resistance type exercise (5, 29). Though we did not assess skeletal muscle protein breakdown rates, we observed lower plasma and muscle BCAA levels when carbohydrate was co-ingested. 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 co-ingestion did not seem to modulate post-exercise protein metabolism. Even in the absence of carbohydrate co-ingestion, plasma insulin levels averaged 16.5±1.6 µU·ml⁻¹ 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⁻¹ does not further enhance muscle protein synthesis and/or reduce protein degradation. Therefore, the present data as well data
from previous studies (1, 7, 11) seem to confirm that insulin merely plays a 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 co-ingestion 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 co-ingestion would be preferred when trying to accelerate muscle glycogen repletion.

In conclusion, co-ingestion 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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RK, AKK and LJCvL designed the study. RK organized and carried out the clinical experiments with the assistance of MB, TS, LJCvL and BP. RK performed the statistical analysis and wrote the manuscript together with LJCvL. WHMS and HK provided medical assistance. AKK is a researcher with DSM Food Specialties, Delft, The Netherlands, no other author had any financial or personal conflicts of interest.
References


FIGURE LEGENDS

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

Figure 2. Plasma rate of appearance (A, Ra) and rate of disappearance (B, Rd) of glucose in the PRO, and PRO+LCHO, and PRO+HCHO treatments in healthy men (n=10). Values represent means±SEM. Data were analyzed with ANOVA repeated measures (treatment x time). Glucose Ra: treatment effect, P<0.01; time effect, P<0.001; interaction of treatment and time, P<0.01. Glucose Rd: 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).

Figure 3. Plasma phenylalanine, tyrosine, and BCAA (leucine, isoleucine, and valine) concentrations (µmol·L⁻¹), in the PRO and PRO+LCHO and PRO+HCHO treatments in healthy men (n=10). Values are means±SEM. Data were analyzed with ANOVA repeated measures (treatment x 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).

Figure 4. Plasma L-[ring-\textsuperscript{13}C\textsubscript{6}]phenylalanine (A), L-[ring-\textsuperscript{2}H\textsubscript{2}]tyrosine (B), and L-[ring-\textsuperscript{13}C\textsubscript{6}]tyrosine enrichment (Mole Percent Excess, MPE) (C) during the PRO, PRO+LCHO and PRO+HCHO treatments healthy men (n=10). Values represent means±SEM. Data were analyzed with ANOVA repeated measures (treatment x time). Plasma L-[ring-\textsuperscript{13}C\textsubscript{6}]phenylalanine enrichment: treatment effect, P=0.93; time effect, P<0.001; interaction of treatment and time, P=0.91. Plasma L-[ring-\textsuperscript{2}H\textsubscript{2}]tyrosine enrichment: treatment effect, P=0.86; time effect, P<0.001; interaction of treatment and time, P=0.46. Plasma L-[ring-\textsuperscript{13}C\textsubscript{6}]tyrosine enrichment: treatment effect, P=0.96; time effect, P<0.001; interaction of treatment and time, P=1.00.

Figure 5. Whole-body protein breakdown, synthesis, and oxidation rates and net protein balance (expressed as µmol phenylalanine kg\textsuperscript{-1} h\textsuperscript{-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±SEM. 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).
Table 1  Plasma and muscle amino acid tracer enrichments ¹

<table>
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<tr>
<th>Treatments</th>
<th>PRO</th>
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<td><strong>Plasma AA enrichments</strong></td>
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<tr>
<td>L-[ring-$^{13}$C$_6$]phenylalanine</td>
<td>0.0385 ± 0.0009</td>
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<tr>
<td>L-[ring-$^2$H$_2$]tyrosine</td>
<td>0.0115 ± 0.0005</td>
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<td>0.361</td>
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<tr>
<td>L-[ring-$^{13}$C$_6$]tyrosine</td>
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<td>0.0027 ± 0.0003</td>
<td>0.0030 ± 0.0002</td>
<td>0.747</td>
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<td><strong>Muscle AA enrichments</strong></td>
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<tr>
<td>L-[ring-$^{13}$C$_6$]phenylalanine</td>
<td>0.0357 ± 0.0013</td>
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<td>0.0328 ± 0.0013</td>
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<tr>
<td>L-[ring-$^2$H$_2$]tyrosine</td>
<td>0.0124 ± 0.0029</td>
<td>0.0111 ± 0.0028</td>
<td>0.0108 ± 0.0021</td>
<td>0.909</td>
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<tr>
<td>L-[ring-$^{13}$C$_6$]tyrosine</td>
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<td>0.0096 ± 0.0005</td>
<td>0.0084 ± 0.0008</td>
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<td>L-[ring-$^{13}$C$_6$]phenylalanine</td>
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<td>0.00024 ± 0.00002</td>
<td>0.00027 ± 0.00003</td>
<td>0.826</td>
</tr>
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</table>

¹ Values expressed means ± SEM, n=10 young healthy males. PRO, protein only; PRO+LCHO, protein + low amount of carbohydrate; PRO+HCHO, protein + high amount of carbohydrate. Data were analyzed with ANOVA. Plasma amino acid (AA) enrichments represent the average plasma amino acid enrichment during the last 4h of recovery. Muscle AA enrichments represent the amino acid enrichment in muscle biopsy taken after 6 h of post-exercise recovery. δ Enrichment muscle protein represents the increment in muscle protein enrichment from 0 to 6h following exercise. No differences between treatments.