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During endurance exercise

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HIGHLIGHTED TOPIC | Regulation of Protein Metabolism in Exercise and Recovery

Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans

Krista R. Howarth, Natalie A. Moreau, Stuart M. Phillips, and Martin J. Gibala

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Howarth KR, Moreau NA, Phillips SM, Gibala MJ. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. J Appl Physiol 106: 1394–1402, 2009. First published November 26, 2008; doi:10.1152/japplphysiol.90333.2008. — Coingestion of protein with carbohydrate (CHO) during recovery from exercise can affect muscle glycogen synthesis, particularly if CHO intake is suboptimal. Another potential benefit of protein feeding is an increased synthesis rate of muscle proteins, as is well documented after resistance exercise. In contrast, the effect of nutrient manipulation on muscle protein kinetics after aerobic exercise remains largely unexplored. We tested the hypothesis that ingesting protein with CHO after a standardized 2-h bout of cycle exercise would increase mixed muscle fractional synthetic rate (FSR) and whole body net protein balance (WBNB) vs. trials matched for total CHO or total energy intake. We also examined whether postexercise glycogen synthesis could be enhanced by adding protein or additional CHO to a feeding protocol that provided 1.2 g CHO·kg−1·h−1, which is the rate generally recommended to maximize this process. Six active men ingested drinks during the first 3 h of recovery that provided either 1.2 g CHO·kg−1·h−1 (L-CHO), 1.2 g CHO + 0.4 g protein·kg−1·h−1 (PRO-CHO), or 1.6 g CHO·kg−1·h−1 (H-CHO) in random order. Based on a primed constant infusion of [1-13C]leucine, we assessed FSR using [1-13C]leucine was positive only during PRO-CHO, and this was mainly attributable to a reduced rate of protein breakdown. Glycogen synthesis rate was not different between trials. We conclude that ingesting protein with CHO during recovery from aerobic exercise increased muscle FSR and improved WBNB, compared with feeding strategies that provided CHO only and were matched for total CHO or total energy intake. However, adding protein or additional CHO to a feeding strategy that provided 1.2 g CHO·kg−1·h−1 did not further enhance glycogen resynthesis during recovery, protein turnover; stable isotopes; amino acids; glycogen.

The primary purpose of the present study was to determine whether ingesting protein with CHO during recovery from prolonged exercise would increase mixed skeletal muscle protein fractional synthetic rate (FSR) and improve whole body protein balance compared with CHO alone. A secondary purpose was to determine whether protein or additional CHO to a feeding strategy that provided 1.2 g CHO·kg−1·h−1, which is the rate generally recommended to maximize postexercise glycogen synthesis (6, 11), would further augment this process during recovery. On three occasions during recovery from a 2-h bout of standardized cycle exercise, subjects ingested drinks that provided either 1.2 g CHO·kg−1·h−1, 1.2 g CHO + 0.4 g protein·kg−1·h−1, or 1.6 g CHO·kg−1·h−1. Our primary hypothesis was that coingestion of protein with CHO would increase FSR and whole body protein balance compared with drinks matched for total CHO or total energy intake. We also tested the secondary hypothesis that ingesting protein or additional CHO would result in a higher rate of muscle glycogen synthesis compared with the control trial that provided 1.2 g CHO·kg−1·h−1.

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METHODS

Subjects

Six healthy men (age 22 ± 1 yr; mass 90 ± 5 kg; height 184 ± 2 cm; body mass index 26.4 ± 0.8 kg/m²) volunteered for the study. The subjects were recreationally active and habitually engaged in a variety of activities that included running, cycling, weightlifting, and intramural sports several times per week, but none were specifically training for a particular sport or event. Their peak oxygen uptake (V\textsubscript{O\textsubscript{2 peak}}), determined using an incremental test on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0) and an on-line gas collection system (Moxus Modular V\textsubscript{O\textsubscript{2}}, AEI Technologies, Pittsburgh, PA), was 4.4 ± 0.3 l/min. A preliminary screening process was employed to confirm that subjects were free of risk factors associated with cardiovascular, pulmonary, or metabolic disease. The experimental procedures and potential risks were fully explained to the subjects before the study, and all subjects provided written, informed consent. The experimental protocol was approved by the Hamilton Health Sciences/Faculty of Health Sciences, McMaster University Research Ethics Board.

Overview of Experimental Design

Each subject served as his own control and performed three experimental trials in random order, separated by at least 7 days. Each trial consisted of a 2-h standardized bout of exercise (see below) followed by a 4-h recovery period during which subjects ingested one of three experimental beverages (Fig. 1). All trials were performed in an identical manner, the only difference being the composition of the beverage ingested during recovery. Beverages were ingested at a rate of 750 ml/h, in 15-min intervals for the first 3 h of recovery, and formulated to deliver either 1.2 g CHO·kg body mass\textsuperscript{−1}·h\textsuperscript{−1} (L-CHO), 1.2 g CHO + 0.4 g protein·kg body mass\textsuperscript{−1}·h\textsuperscript{−1} (PRO-CHO; matched to L-CHO in terms of total CHO ingested), or 1.6 g CHO·kg body mass\textsuperscript{−1}·h\textsuperscript{−1} (H-CHO; matched to PRO-CHO in terms of total energy ingested). The CHO source was maltodextrin (Glucidex IT 19, Roquette Frères), and the protein source was a hydrolyzed whey protein concentrate (American Casein, HLA-198). To make the drinks comparable in taste, 5 g of Sucralose (Splenda), 2.5 g of sodium chloride, and orange powder flavoring were added to 750 ml of each beverage. To maintain constant infusion of \textsuperscript{[\textit{ring}-\textsuperscript{2}H\textsubscript{2}]}Phe, an amount of isotope equivalent to 9% of the Phe present in the whey protein was added to the PRO-CHO drinks. Beverages were ingested during the first 3 h of recovery since pilot testing revealed some individuals experienced considerable gastrointestinal distress (particularly bloating and an urge to vomit) if drinks were ingested after this time (i.e., over the entire 4-h recovery period).

Physical Activity and Nutritional Controls

Subjects were asked to keep their habitual exercise pattern and dietary intake as constant as possible over the course of the experiment, but given the within-subject design (i.e., each subject served as his own control), these parameters were not standardized between subjects. Subjects were specifically instructed to perform no physical activity, aside from activities of daily living, for 48 h before each trial. Subjects were also asked to maintain food records during the 48 h before each trial. Following the first experimental trial, subjects were instructed to replicate their individual nutritional pattern over the course of the second and third trials and again record food intake, noting any deviations from the first trial. Food records were subsequently analyzed (Nutritionist Five dietary analysis software, First Data Bank, San Bruno, CA), and results confirmed no difference in total energy intake or macronutrient composition between trials (data not shown). In addition to these general nutrition controls, all subjects were provided with a standardized, prepackaged meal on the day before each experimental trial. Subjects were instructed to ingest the meal as breakfast at ~0700 on the day of the trial, after having fasted overnight. The meal provided 700 kcal and was derived from 82% carbohydrate, 10% fat, 8% protein. Subjects were instructed not to consume any other food or drink, except for water, before reporting to the laboratory.

Experimental Trial Details

Subjects arrived at the laboratory at ~0900 on the day of each experimental trial. On arrival, subjects were weighed and a catheter was inserted into an antecubital vein. After a resting blood sample was obtained, a baseline breath sample was collected into a 100-liter Douglas bag that was connected to an on-line gas collection system (Moxus) for the determination of carbon dioxide output (V\textsubscript{CO\textsubscript{2}}). A 10-ml sample of expired air was transferred into a Vacutainer tube and used for subsequent analysis of background breath \textsuperscript{13}CO\textsubscript{2} enrichment using isotope ratio mass spectrometry (IRMS) as previously described (29). Subjects then received a bolus infusion of Na\textsuperscript{13}CO\textsubscript{3} (0.295 mg/kg) to prime the bicarbonate pool and a primed constant infusion of two stable isotope labeled amino acids, \textsuperscript{1}-\textsuperscript{[\textit{ring}-\textsuperscript{2}H\textsubscript{2}]}phenylalanine (prime, 2 \textmu mol/kg; infusion, 0.05 \textmu mol·kg\textsuperscript{−1}·min\textsuperscript{−1}) and \textsuperscript{1}-\textsuperscript{[\textit{ring}-\textsuperscript{13}C\textsubscript{5}]}leucine (prime, 1 mg/kg; infusion, 1 mg·kg\textsuperscript{−1}·h\textsuperscript{−1}) for the determination of whole body and skeletal muscle protein kinetics as described below.

At ~1000, or ~3 h following ingestion of the standardized meal, subjects mounted an ergometer (Lode) and initiated a 2-h bout of standardized variable-intensity cycling to reduce muscle glycogen content. The protocol was modeled after Kuipers et al. (13) and consisted of 12 × 10-min stages that alternated between 50% V\textsubscript{O\textsubscript{2 peak}} (174 ± 7 W) and a higher workload that began at 80% V\textsubscript{O\textsubscript{2 peak}} (278 ± 11 W) and decreased by 5% V\textsubscript{O\textsubscript{2 peak}} every other stage to a final workload of 55% V\textsubscript{O\textsubscript{2 peak}} (191 ± 8 W). Immediately following exercise, the lateral portion of one thigh was prepared for the extraction of a needle biopsy sample from the vastus lateralis muscle (2) and a second catheter was inserted into the contralateral arm for blood sampling. A biopsy sample and blood sample were obtained within 10 min of the cessation of exercise (which corresponded to ~5 h

\textbf{Fig. 1.} Overview of experimental protocol.
following the standardized breakfast), and these samples were considered “0 h” samples that corresponded to the start of the recovery period. Blood samples were collected at 15 min intervals for the initial hour of recovery and every 30 min thereafter, and a second biopsy sample was obtained after 4 h of recovery. All biopsies for a given trial were obtained from separate incision sites on the same leg, and legs were alternated between trials. Expired breath samples were collected in triplicate during the final 15 min of the first and fourth hours of recovery.

**Gastrointestinal Distress Measurements**

Subjects were asked to complete a gastrointestinal (GI) distress questionnaire immediately after exercise and each hour of recovery. The questionnaire was adapted from Jensjø et al. (11) and assessed 11 parameters of GI distress including nausea, bloating, GI cramps, need to belch, need to vomit, diarrhea, urge to urinate, urge to defecate, headache, dizziness, and body chills. Each parameter was ranked on a scale from 1 to 10, with 1 being absence of any distress and 10 being distress strongly present (minimum score 11, maximum score 110).

**Muscle Analyses**

Upon removal from the leg, each muscle sample was immediately frozen in liquid nitrogen and subsequently stored at −86°C before analyses. Muscle samples were subsequently divided into two pieces while still frozen. One portion was freeze-dried, powdered, and subsequently dissected free of obvious blood and connective tissue. After this process was completed, the freeze-dried samples were stored at −86°C before analysis of glycogen using fluorometry and amino acids using HPLC. The remaining piece of frozen muscle was used for subsequent determination of mixed muscle fractional synthetic rates (FSR) using gas chromatography mass spectrometry (GCMS).

**Glycogen.** A ~2-mg aliquot of freeze-dried muscle was incubated in 2.0 N HCl and heated for 2 h at 100°C to hydrolyze the glycogen to glucosyl units. The solution was subsequently neutralized with an equal volume of 2.0 N NaOH and analyzed for glucose using an enzymatic assay adapted for fluorometry (20).

**Amino acids.** Freeze-dried muscle was extracted on ice using 0.5 M perchloric acid (PCA) (containing 1 mM EDTA), neutralized with 2.2 M KHCO₃, and the resulting supernatant was used for the determination of free amino acids using HPLC. The concentrations of amino acids were determined using a protocol described by Moore et al. (18). Briefly, extracts were derivatized before injection using Waters AccQ-Fluor reagent kit (Millford, MA) by heating for 30 min at 55°C to form the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivative of all physiological amino acids. Samples and standards (Sigma, St. Louis, MO) were run on a Waters 2695 HPLC separations module (Millford, MA) through a Nova-Pak C₁₈, 4-µm column to separate the amino acids. The amino acids were detected using a Waters 474 scanning fluorescence detector (Millford, MA) with an excitation and emission wavelengths of 250 nm and 395 nm, respectively. Amino acid peak areas were integrated, compared with known standards, and analyzed using the Waters Millenium 32 software package. The method achieved reliable separation of 17 of the 20 physiological amino acids.

**Mixed muscle FSR.** Muscle samples collected at 0 h and 4 h were used for the measurement of mixed muscle FSR based on [1-¹³C]leucine infused as described by Wilkinson et al. (29). Briefly, frozen muscle samples were weighed and ice-cold acetoneitrile (100 µl/mg) was added to extract the intracellular free amino acids. Samples were then manually homogenized, vortexed for 10 min, and centrifuged at 15,000 rpm for 2 min at 4°C. The supernatant was collected and the procedure repeated. The pooled supernatant was then dried under N₂ gas for later analysis of the muscle intracellular free Phe enrichments. The remaining muscle pellets were washed twice with distilled H₂O, once with absolute ethanol, and then freeze dried overnight. The dried pellets were subsequently weighed and hydrolyzed with 6 N HCl (400 µl/mg) for 24 h at 110°C. A 300-µl aliquot of each bound protein hydrolysate was pass over a C₁₈ reverse-phase chromatography spin column (Harvard Apparatus, Holliston, MA). The eluted samples were then dried under N₂ gas. Samples were derivatized to the tert-butyldimethyl silyl (tBDMS) derivative of phenylalanine using 50 µl N-methyl-N-(tert-butyldimethyl) trifluoroacetamide (MTBSTFA) + 1% tert-butyldimethylchloro-rosilane (TBDMCS) (Pierce Chemical, Rockford, IL) + 50 µl anhydrous acetonitrile and heated for 15 min at 100°C. The Phe isotopic enrichments were determined on an electron-impact ionization GC-MS (GC: Agilent 6890N; MS: Hewlett-Packard 5973). Ions were selectively monitored at mass-to-charge (m/z) ratios of 234 and 239 for Phe enrichment in the intracellular muscle extracts, and 237 and 239 in the bound muscle hydrolysate. Bound muscle protein enrichments were determined using the standard curve method (21). FSR was calculated using the equation published by Wolfe (32).

**Blood Analyses**

Blood samples were collected into heparinized and nonheparinized tubes. One 200-µl aliquot of heparinized whole blood was combined with 1,000 µl of 0.6 N perchloric acid (PCA), vortexed, and centrifuged, and the supernatant was collected and stored at −30°C. The PCA extract was subsequently analyzed for glucose using an enzymatic assay adapted for fluorometry (20). A second 200-µl aliquot of heparinized whole blood was combined with 1,000 µl of 0.6 N PCA, vortexed, centrifuged, and neutralized with 500 µl of 1.25 N KHCO₃. The supernatant was collected and stored at −30°C for later analysis of amino acids using HPLC as described above. The remaining heparinized whole blood was centrifuged and the plasma was collected and stored at −30°C for subsequent analysis using GCMS. The isotope enrichment of plasma α-ketoisocaproic acid (α-KIC) was measured on an electron-impact ionization GCMS (GC: Agilent 6890N; MS: Hewlett-Packard 5973, Palo Alto, CA) using a method described previously (24, 29). Nonheparinized tubes were centrifuged and serum was collected and stored at −30°C for subsequent analysis of insulin using a radioimmunossay kit (RIA) (Coat-A-Count, Diagnostic Products, Los Angeles, CA). Area under the curve (AUC) for insulin was measured as total area over 4 h minus baseline.

**Breath Analyses**

The ratio of ¹³CO₂ to ¹²CO₂ was measured in breath samples using an automated breath analysis system (BreathMat Plus, Thermo Finnigan, San Jose, CA) with a method we have described previously (29). Values for breath samples measured in triplicate were corrected for baseline breath ¹³CO₂ enrichment and averaged.

**Calculation of Whole Body Protein Kinetics**

Calculations of whole body Leu flux (Q), oxidation (O), breakdown (B), nonoxidative Leu disposal (NOLD), and net balance (NBL) for the 1-h and 4-h time points of recovery were made using the equations described previously (16), with correction for ingested protein and an estimated splanchnic extraction of 33% based on the results of Arnal et al. (1). A bicarbonate retention factor (c) of 0.83 was used for the retention of carbons in the blood bicarbonate pool during feeding (25).

**Choice of Amino Acid Tracers**

[1-¹³C]leucine was used for the determination of whole body protein kinetics because this tracer allows measurement of kinetics that primarily assess the impact of exercise on skeletal muscle rather than other tissues. The measurement of whole body leucine kinetics facilitates derivation of a number of parameters that are much harder to obtain using other tracers due, for the most part, to the ease of obtaining a representative precursor (i.e., α-KIC). Thus, in a scenario
in which muscle is active and a good tracer is available, leucine is an excellent choice as opposed to another tracer that may not show sensitivity to changes. L-[ring-2H₅]phenylalanine was used for the determination of muscle FSR since it is a highly deuterated and “heavy” isotope (i.e., 5 atomic mass units greater than the endogenous compound).

Statistical Analyses

All muscle and blood data were analyzed using a two-factor (treatment × time) repeated-measures ANOVA, except for muscle FSR and glycogen synthesis rates, which were analyzed using a one-factor (treatment) repeated-measures ANOVA. When a significant main effect or interaction was identified, data were subsequently analyzed using a Tukey honestly significant difference post hoc test. Integrated AUC calculations were performed using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA) and analyzed using a one-factor (treatment) ANOVA. Significance for all analysis was set at \( P \leq 0.05 \). All data are presented as means ± standard error of the mean (SE).

RESULTS

Isotope Analysis

Average blood plasma, muscle intracellular and breath isotope enrichments are summarized in Fig. 2 and show subjects achieved isotopic steady state.

Whole Body Leucine Kinetics

Leucine flux (Table 1) and oxidation (Fig. 3A) were higher during PRO-CHO compared with H-CHO and L-CHO (main effect treatment, \( P < 0.05 \)), with both variables being higher at 4 h vs. 1 h (\( P < 0.05 \)). NOLD was lower after 4 h vs. 1 h (main effect time, \( P < 0.05 \)), with both variables being higher at 4-h period of recovery (main effect for time, \( P < 0.05 \)) during the PRO-CHO trial (\( P < 0.05 \)) (Fig. 3B). While leucine net balance decreased from 1 to 4 h in all trials (main effect time, \( P < 0.05 \)), it remained positive in the PRO-CHO trial whereas it was negative in the other two trials (main effect, \( P < 0.05 \)) (Fig. 3B).

Muscle FSR

Muscle FSR was higher (\( P < 0.05 \)) during recovery in PRO-CHO compared with both H-CHO and L-CHO (Fig. 4).

Muscle Glycogen

Muscle glycogen content was similar between trials after exercise and increased to a similar extent in all trials over the 4-h period of recovery (main effect for time, \( P < 0.05 \)) (Fig. 5). There was no difference between trials in muscle glycogen synthesis rate during recovery (L-CHO, 23 ± 3; H-CHO, 25 ± 7; PRO-CHO, 25 ± 4 mmol·kg dry wt⁻¹·h⁻¹).

Muscle Amino Acids

Muscle amino acids are summarized in Table 2. Total branched-chain amino acid (BCAA) content was higher (\( P < 0.05 \)) in the PRO-CHO trial (\( P < 0.05 \)) compared to both H-CHO and L-CHO (main effect treatment; \( P < 0.05 \)).
Values are means from prolonged exercise while ingesting either 1.2 g CHO-
kg⁻¹·h⁻¹ (L-CHO), 1.6 g CHO-kg⁻¹·h⁻¹ (H-CHO), or 1.2 g CHO + 0.4 g protein-
kg⁻¹·h⁻¹ (PRO-CHO). Values are means ± SE; n = 6. *P < 0.05 vs. other treatments.

Blood Amino Acids

Blood amino acids are summarized in Table 3. After 1 h of recovery, the concentrations of alanine, leucine, isoleucine, valine, arginine, lysine, phenylalanine, proline, threonine, and tyrosine were higher in PRO-CHO vs. the other trials and remained higher for the remainder of the recovery (P < 0.05). Total amino acid were higher during the PRO-CHO trial compared with the L-CHO trial by 1 h of recovery and were higher than both CHO trials by 2.5 h of recovery (P < 0.05). Total BCAA concentrations increased during the first hour of recovery during the PRO-CHO trial compared with the CHO alone trials and were significantly greater by 1 h of recovery and remained higher throughout the remainder of the recovery (P < 0.05) (Fig. 6B).

Blood Glucose and Serum Insulin

Blood glucose was higher after 60–180 min of recovery vs. immediately postexercise, but there was no difference between treatments (main effect for time, P < 0.05; data not shown). Serum insulin increased after 90 min and remained elevated for the remainder of the recovery compared with immediately postexercise, but there was no difference between trials (main effect for time, P < 0.05; data no shown). The AUC for insulin was not different between trials (L-CHO, 79 ± 17; H-CHO, 90 ± 22; PRO-CHO, 104 ± 18 μIU·ml⁻¹·min⁻¹).

Gastrointestinal Distress

Total GI distress scores were higher at 3 and 4 h of recovery (reaching peak values of ~35) compared with immediately postexercise (main effect for time, P < 0.05), but there was no difference between trials.

DISCUSSION

The major finding from the present study was that co-ingestion of protein with CHO during recovery from endurance exercise increased mixed skeletal muscle FSR and induced a more positive whole body net protein balance compared with drinks matched for total CHO or total energy intake. This study was unique in that it was the first to directly examine the effect of manipulating CHO and protein ingestion during recovery from endurance exercise on muscle FSR using the needle biopsy technique. However, our data are consistent with the work of Levenhagen et al. (14, 15), who used arterial-venous difference measurements to show that adding protein to a carbohydrate-fat supplement increased leg protein net balance after exercise (1 h of cycling or recumbent cycling at 60% VO₂peak) compared with the supplement without protein. However, those studies (14, 15) did not employ a trial matched for total energy intake, and thus the authors could not conclusively discern whether the increased muscle FSR was attributable to

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the addition of protein per se or simply higher energy intake. The present data confirm that protein ingestion was necessary to stimulate protein synthesis, since muscle FSR was lower during the H-CHO trial that was matched to PRO-CHO in terms of total energy intake. Our design could not resolve the specific type of proteins that were responsible for the increase in mixed muscle FSR. However, we recently found that 45 min of cycling at 75% of \( V_{\text{O2peak}} \) elicited an increase in mitochondrial protein synthesis (30), and thus it is tempting to speculate that the rise in mixed muscle protein synthesis we observed with protein ingestion in our study was due at least in part to increased mitochondrial protein synthesis.

An increased availability of blood and muscle amino acids, especially the essential amino acids (EAA), has been associated with an increased muscle FSR at rest and following resistance-based exercise (22, 26). In the present study, there was an increase in the concentration of the EAA (leucine, valine, isoleucine, arginine, histidine, lysine, phenylalanine, threonine, and tyrosine) in the blood in the PRO-CHO trial by 1 h of recovery that was greater than that of the CHO-only trials, and the values remained higher for the rest of the recovery period. While the present study did not address potential mechanisms, the higher concentration of EAAs would presumably ensure that their availability was not limiting for the increased protein synthetic rate. Insulin has also been shown to increase resting muscle protein synthesis when the availability of amino acids is not limited (4), although the effect following exercise is equivocal (5). Miller et al. (17) showed that when glucose was added to an amino acid drink ingested during recovery from resistance exercise, there seemed to be a synergistic effect on muscle protein synthesis. In the present study, there was no difference in insulin concentration between the three trials, and thus the increased FSR during the PRO-CHO trial compared with the two CHO-only trials would seem to be related more to the increased availability of amino acids.

With regard to whole body protein balance, several previous studies utilized leucine tracer methodology (12, 14, 15), and all showed that net protein balance was positive only when protein was added to a CHO beverage compared with CHO alone (12, 14, 15). Levenhagen and colleagues (14, 15) attributed the
positive net balance to increased whole body synthesis and no change in breakdown, whereas our data are similar to Koopman et al. (12) in that there was no change in synthesis (represented by NOLD in the present study) and a decrease in breakdown. The discrepancies may be explained in part by methodological differences. First, Levenshagen et al. (14, 15) had subjects ingest a single bolus dose and measured changes after 3 or 6 h of recovery, while in the present study and that of Koopman et al. (12), subjects received smaller doses at frequent intervals over 3–4 h of recovery. The total amount of protein ingested also differed between studies; Levenshagen et al. (14, 15) had subjects ingest 10 g of protein with 8 g CHO and 2 g fat, whereas Koopman et al. (12) had subjects ingest beverages at a rate of 0.7 g CHO + 0.25 g protein·kg⁻¹·h⁻¹, or −18 g protein/h based on the average mass of their subjects, for 6 h of exercise and 4 h of recovery. Subjects in the present study ingested substantially more protein than these other studies (12, 14, 15), and further examination of the dose response of protein ingestion on protein balance after endurance exercise is warranted.

While the changes in whole body protein balance during exercise in the present study are comparable to data from Koopman et al. (12), those authors provided nutrition throughout the exercise session and recovery, whereas subjects in the present study were only fed during recovery. A novel finding from the present study was that whole body protein synthesis decreased from 1 to 4 h of recovery regardless of type of nutrients ingested. This suggests a possible temporal relationship between the effects of exercise on whole body protein synthesis during recovery from aerobic exercise. However, lacking a measurement of resting whole body protein synthesis, we could not resolve whether this represented a return to baseline levels or a potential decrease below resting values. One might question the apparent disconnect between the whole body and muscle protein synthetic responses. However, mixed muscle FSR was calculated based on the postexercise response over 4 h, whereas the leucine balance data was divided into “early” and “late” time bins. Thus, while whole body rates declined so too might have the mixed muscle FSR, but we did not obtain biopsies throughout the recovery period to evaluate this directly. It is possible that the data are congruent, but one measure has the temporal resolution to show an early and a late response, whereas the other does not. In addition, mixed muscle FSR accounts for only −25% of whole body protein synthesis (19), and it could be that the magnitude or direction of change in other proteins is not consistent with that seen for skeletal muscle. We also report that the attenuation in breakdown during PRO-CHO declined during recovery, resulting in lower positive net balance at 4 h compared with 1 h postexercise. While the net balance was still positive compared with that to the CHO-only trials, it occurred while the blood amino acid concentrations remained high, and thus amino acid availability cannot be the only factor involved in the attenuation in breakdown.

Whole body leucine oxidation was increased in the PRO-CHO trial compared with both CHO-alone trials, which is comparable to the results of both Koopman et al. (12) and Levenshagen et al. (14). In addition, the present study showed that the oxidation of leucine was higher at 4 h compared with 1 h of recovery. The increased oxidation at 4 h of recovery occurred when the leucine concentration was high in both the blood and muscle. It is plausible that the increase in leucine

### Table 3. Blood amino acids

<table>
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<th>Time</th>
<th>Arg</th>
<th>Asn</th>
<th>Glu</th>
<th>His</th>
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<th>Val</th>
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<td>28.0</td>
<td>26.3</td>
<td>653.15</td>
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<tr>
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<td>30.6</td>
<td>29.3</td>
<td>33.0</td>
<td>34.3</td>
<td>33.4</td>
<td>34.2</td>
<td>34.3</td>
<td>26.8</td>
<td>26.6</td>
<td>650.84</td>
</tr>
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<td>33.4</td>
<td>30.6</td>
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<td>34.3</td>
<td>26.8</td>
<td>26.6</td>
<td>650.84</td>
</tr>
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Values are means ± SD in μmol·kg⁻¹·h⁻¹.

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concentration increased activation of muscle branched-chain oxoacid dehydrogenase complex (BCOAD), which regulates BCAA oxidation, and is known to increase in activity with an increase in leucine (7). Our results are also comparable to Wilkinson et al. (29), who showed an increase in whole body leucine oxidation during late recovery (3 h) compared with early recovery (45 min) with the ingestion of a CHO-EAA beverage.

With regard to our second hypothesis, we found that adding protein or additional CHO to a drinking strategy that provided 1.2 g CHO·kg⁻¹·h⁻¹ did not enhance muscle glycogen synthesis during recovery. Several investigators have suggested this rate of CHO ingestion is the threshold for maximizing glycogen synthesis during recovery (6, 10) but no previous study has tested this proposal by directly comparing 1.2 g CHO·kg⁻¹·h⁻¹ vs. a higher rate of CHO ingestion. The drinks in the present study were formulated from a 17–22% glucose polymer solution that subjects ingested at a rate of 750 ml/h, which is similar to that provided in other studies (11, 27, 28). One discrepancy from some previous studies is that we did not detect a significant difference in plasma insulin between trials, although insulin tended to be higher during PRO-CHO compared with the two CHO-only conditions. However, higher blood insulin levels do not necessarily translate into a higher muscle glycogen resynthesis rate, especially when large amounts of CHO are ingested (10, 11). For example, Jentjens et al. (11) reported no difference in glycogen resynthesis rate when they compared ingestion of 1.2 g CHO vs. 1.2 g CHO + 0.4 g protein, despite higher insulin in the latter trial. This suggests that other factors such as the rate of gastric emptying and intestinal absorption of ingested glucose, glucose entry into the muscle, or glycogen synthase activity may have been limiting (10). Finally, it is worth noting that there were modest but significant increases in GI distress scores during recovery in the present study, which were mainly due to a sensation of severe bloating in half of our subjects after 3–4 h. This finding was consistent with the opinion of some authors (6, 10), who have proposed that very high rates of CHO ingestion may not be well tolerated by all athletes (10). From a practical standpoint, consideration should therefore be given to potential GI discomfort and the potential ramifications in an applied setting (e.g., on volitional exercise performance after short-term recovery).

In summary, we have shown that ingesting protein with CHO during recovery from prolonged endurance exercise increased muscle FSR and improved whole body net protein balance compared with CHO alone. However, adding protein or additional CHO to a drinking strategy that provided 1.2 g CHO·kg⁻¹·h⁻¹ did not further augment glycogen synthesis. The higher FSR during recovery could promote muscle adaptation during recovery from acute exercise by stimulating the synthesis or repair of proteins that facilitate energy provision and force production. Additional studies are warranted to resolve the specific population(s) of muscle proteins that are stimulated by protein and CHO ingestion after aerobic-based exercise, e.g., myofibrillar vs. mitochondrial.

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