Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery

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Howarth KR, Phillips SM, MacDonald MJ, Richards D, Moreau NA, Gibala MJ. Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. J Appl Physiol 109: 431–438, 2010. First published May 20, 2010; doi:10.1152/japplphysiol.00108.2009.—We examined the effect of carbohydrate (CHO) availability on whole body and skeletal muscle protein utilization at rest, during exercise, and during recovery in humans. Six men cycled at ~75% peak O2 uptake (V˙O2peak) to exhaustion to reduce body CHO stores and then consumed either a high-CHO (H-CHO; 71 ± 3% CHO) or low-CHO (L-CHO; 11 ± 1% CHO) diet for 2 days before the trial in random order. After each dietary intervention, subjects received a primed constant infusion of [1-13C]leucine and 1-[ring-2H3]phenylalanine for measurements of the whole body net protein balance and skeletal muscle protein turnover. Muscle, breath, and arterial and venous blood samples were obtained at rest, during 2 h of two-legged kicking exercise at ~45% of kicking V˙O2peak, and during 1 h of recovery. Biopsy samples confirmed that the muscle glycogen concentration was lower in the L-CHO group versus the H-CHO group at rest, after exercise, and after recovery. The net leg protein balance was decreased in the L-CHO group compared with at rest and compared with the H-CHO condition, which was primarily due to an increase in protein degradation (area under the curve of the phenylalanine rate of appearance: 1,331 ± 162 µmol in the L-CHO group vs. 786 ± 51 µmol in the H-CHO group, P < 0.05) but also due to a decrease in protein synthesis late in exercise. There were no changes during exercise in the rate of appearance compared with rest in the H-CHO group. Whole body leucine oxidation increased above rest in the L-CHO group only and was higher than in the H-CHO group. The whole body net protein balance was reduced in the L-CHO group, largely due to a decrease in whole body protein synthesis. These data extend previous findings by others and demonstrate, using contemporary stable isotope methodology, that CHO availability influences the rates of skeletal muscle and whole body protein synthesis, degradation, and net balance during prolonged exercise in humans.

protein synthesis; protein degradation; energy status

There are limited data regarding the effect of endogenous carbohydrate (CHO) availability on protein metabolism during prolonged exercise. An early study (11) of changes in blood urea nitrogen suggested that when endogenous CHO availability was reduced during exercise, a state of hyperammonemia occurred, possibly indicating increased protein degradation and amino acid oxidation. Van Hall et al. (19) and Blomstrand and Saltin (4) used arterial-venous (a-v) difference measurements to examine the effect of glycogen availability on amino acid flux to derive estimates of net muscle protein turnover. These studies, which relied solely on the chemical net balance, showed a net release of amino acids during exercise, which was augmented under low-glycogen conditions, suggesting an increase in net protein degradation (4, 19). However, the a-v balance method only allows for the measurement of net amino acid balance, and it cannot be determined whether the increased net degradation is a result of decreases in synthesis, increases in degradation, or a combination of both.

The primary purpose of the present study was to examine the effect of glycogen availability on whole body and skeletal muscle protein turnover at rest, during prolonged aerobic exercise, and during recovery using contemporary stable isotope tracer technology. We hypothesized that exercise with low glycogen would increase the net negative protein balance at the whole body and skeletal muscle levels compared with the high-glycogen condition and that this would be primarily due to an increase in protein breakdown with an additional reduction in protein synthesis. The use of stable isotopes to address this topic represents an improvement over previous a-v balance studies (4, 19) because this technology allows for the simultaneous determination of skeletal muscle protein synthesis and degradation instead of simply the net balance. In addition to the primary focus on protein metabolism, the experimental protocol provided an opportunity to simultaneously examine the effect of glycogen availability on skeletal muscle glucose uptake during exercise. Previous studies that have examined this topic have shown conflicting results, including a greater increase in glucose uptake during exercise in the glycogen-depleted condition (4) or no effect of glycogen availability on glucose uptake (9). We sought to address a potential limitation inherent to these previous studies (4, 9) in which one leg performed glycogen-lowering exercise before a two-legged experimental exercise trial. To avoid the potential confounding effects of acute prior exercise in one leg, we had subjects perform the glycogen-lowering exercise in both conditions followed by a 2-day dietary intervention before the main experimental trial.

METHODS

Subjects

Six healthy men with a mean age of 24 ± 1 yr and a body mass of 80 ± 5 kg volunteered for the study. Subjects were 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 O2 uptake (cycle V˙O2peak), as determined using an incremental test on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0) and an online gas collection system (Moxus Modular VO2 System, AEI Technologies, Pittsburgh, PA), was 44 ± 3 ml·kg−1·min−1. A preliminary screening process was used to confirm that subjects were free of risk factors associated with cardiovascular, pulmonary, or metabolic dis-

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eases. The experimental procedures and potential risks were fully explained to the subjects before the study, and all subjects provided written informed consent. This experimental protocol was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board.

Overview of the Experimental Protocol

Each subject served as his own control and performed two experimental trials in random order separated by at least 7 days (Fig. 1). Before each experimental trial, subjects performed a standardized bout of cycle exercise to reduce glycogen content in the vastus lateralis muscle. The glycogen depletion protocol was followed by the ingestion of either a low-CHO (L-CHO) or high-CHO (H-CHO) diet for ~44 h. After the dietary intervention, subjects returned to the laboratory for the experimental trial, which involved 4 h of rest, 2 h of two-leg knee extensor exercise, and 1 h of recovery (Fig. 2). Both experimental trials were performed in an identical manner, with the only difference being the composition of the diet ingested during the period between the glycogen depletion protocol and experimental trial.

Preeexperimental Procedures

Before baseline measurements, each subject was familiarized with the ergometer used for the two-leg knee extensor exercise. Subjects practiced until they were able to perform the exercise without the use of extraneous muscles, so that work was confined to the leg extensor and flexor muscles. On a separate day, subjects performed a graded exercise test on the kicking ergometer to determine their kicking \( \dot{V}O_2 \) peak for this exercise, which corresponded to \( \approx 75\% \) of cycling \( \dot{V}O_2 \) peak. After a rest period, subjects then practiced kicking at \( \approx 45\% \) of the kicking \( \dot{V}O_2 \) peak for 30 min to ensure the workload elicited the desired \( \dot{V}O_2 \) for the main experimental trials.

Background \( ^{13}\text{CO}_2 \) Enrichment and Bicarbonate Correction Trials

The pretest consisted of two background \( ^{13}\text{CO}_2 \) enrichment trials and two bicarbonate retention trials (n = 2 each). The first set of pretrials was used to determine the naturally occurring enrichment of \( ^{13}\text{CO}_2/^{12}\text{CO}_2 \) in the subject’s breath samples. Two of the six subjects from the trials performed the glycogen depletion protocol, followed by the H-CHO or L-CHO diet, and then returned for 2 h of exercise and 1 h of recovery, exactly as in the trials. Breath samples were obtained at rest and at the same time points of breath collection used in the trial. Samples were collected into a 100-liter Douglas bag that was connected to an online gas collection system (Moxus) for the determination of \( \dot{V}O_2 \), rate of CO2 expiration (\( \dot{CO}_2 \)), respiratory exchange ratio, and minute ventilation. A 10-ml sample of the expired air was drawn, placed in a Vacutainer tube, and used for the subsequent analysis of background breath \( ^{13}\text{CO}_2 \) enrichment using isotope ratio mass spectrometry as previously described (21). The same subjects returned \( >1 \) wk later to complete the pretrial again with the opposite diet. A second set of pretrials was used to determine the retention of \( \text{CO}_2 \) in the bicarbonate pool. Two different subjects from the trials performed the glycogen depletion protocol followed by the H-CHO or L-CHO diet. They then returned for a trial exactly like the experimental trial except that no amino acids were infused and only breath samples were collected. Instead, they received a primed constant infusion of \( \text{NaH}^{13}\text{CO}_3 \) (prime: 0.295 mg/kg and infusion: 0.4 mg kg\(^{-1} \) min\(^{-1} \)). Samples were used to measure the recovery of \( \text{CO}_2 \) from the bicarbonate pool. The same subjects returned \( >1 \) wk later to complete the pretrial again with the opposite diet.

Glycogen Depletion Protocol and Dietary Interventions

For the glycogen depletion protocol, subjects reported to the laboratory at 9 AM and commenced riding on a cycle ergometer (Lode) at an intensity equivalent to 75% cycle \( \dot{V}O_2 \) peak until exhaustion. They then had a 10-min break and completed a second ride to exhaustion. In an attempt to create the high- or low-glycogen conditions in the muscle, immediately after the glycogen depletion protocol, subjects assigned to the H-CHO diet consumed high-CHO food and subjects assigned to the L-CHO diet were given a sugar-free beverage and asked to refrain from eating for 2 h. For the next 43–45 h, subjects followed their assigned diet and were instructed to refrain from alcohol and exercise. Subjects were given lists of acceptable food choices for H-CHO or L-CHO foods along with sample diets but were allowed to consume food of their own choosing for the initial trial with no energy restrictions. For the second trial, diets were designed for the subjects in an attempt to match the energy intake of the initial trial but consuming foods from the opposite list of the first
trial. After the 43–45 h of dietary control, subjects returned to the laboratory at 7 AM for the experimental trial. Food records were subsequently analyzed using commercial software (Nutritionist Five, First Data Bank, San Bruno, CA) to evaluate compliance with the prescribed dietary interventions. All subjects consumed a L-CHO beverage (300 kcal, 13% CHO, 21% fat, and 66% protein) 2 h before the start of the infusions in the experimental trials.

**Experimental Trial Details**

Upon arrival at the laboratory, subjects were weighed, a catheter was inserted into an antecubital vein, and a resting blood sample and baseline breath sample were obtained (Fig. 2). Subjects then received a primed constant infusion of L-[ring-2H3]phenylalanine (prime: 2 μmol/kg and infusion: 0.05 μmol·kg⁻¹·min⁻¹) and rested for 2 h. After 2 h of rest, the lateral portion of one thigh was prepared for the extraction of a needle biopsy sample from the vastus lateralis muscle (2), and a biopsy sample was obtained. Immediately after the initial biopsy, subjects received a bolus infusion of NaH13CO3 (0.295 mg/kg) to prime the bicarbonate pool and a primed constant infusion of [1-13C]leucine (prime: 1 mg/kg and infusion: 1 mg·kg⁻¹·h⁻¹) to prime the bicarbonate pool and a primed constant infusion of [1-13C]leucine (prime: 1 mg/kg and infusion: 1 mg·kg⁻¹·h⁻¹). A catheter was then placed in the femoral vein of the inguinal region of one leg and in the radial artery of one arm. These catheters were used to determine a-v difference across the exercising leg. Blood velocity and femoral artery diameter were measured for the calculation of blood flow (BF) using Doppler ultrasound placed below the inguinal ligament on the common femoral artery and ~2–3 cm above its bifurcation. All BF measurements were made while the subjects had their upper body in the upright position, similar to their position during the knee extensor exercise. Blood samples, breath samples, and BF measurements were taken every 0.5 h at rest beginning 3 h from the start of the first infusion. Heart rate (HR) was also monitored for the duration of the trial using a telemetry monitor (Polar Electro, Woodbury, NY).

A second resting biopsy was taken 4 h after the start of phenylalanine infusion. The leg was then prepared for the extraction of the exercise biopsies, and the subject began the knee extensor exercise at 45% of kicking VO2peak. The exercise continued for 2 h, and a muscle biopsy was taken after 10 min and immediately after the exercise. Blood samples and BF measurements were collected at 5, 15, 30, 60, 90, and 120 min of exercise. Breath samples were collected at 60, 90, and 120 min of exercise. Subjects then rested for 1 h, and blood samples and BF measurements were made after 5, 15, 30, 45, and 60 min of recovery. Breath samples were collected after 30, 45, and 60 min of recovery. A final muscle biopsy was obtained after 1 h of recovery. After the final biopsy, the catheters were removed, and subjects were given a light meal before being allowed to leave the laboratory.

**Muscle, Blood, and Breath 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 freeze dried, powdered, and dissected free of blood and connective tissue. For glycogen analysis, ~2 mg 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 (15).

The initial baseline blood sample was taken from the catheter that was inserted into the antecubital vein before the infusion of the isotopes. All other blood samples were collected from the radial artery catheter and femoral vein catheter with pairs of samples being drawn in close temporal proximity at the appropriate sampling times during the experiment. 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 blood glucose using enzymatic assays adapted for fluorometry (15). 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 KHC03. The supernatant was collected and stored at ~30°C for the later analysis of phenylalanine concentrations using HPLC. The remaining heparinized whole blood was centrifuged, and serum was collected and stored at ~30°C for the subsequent analysis of insulin using a radioimmunoassay kit (Coat-A-Count, Diagnostic Products, Los Angeles, CA).

Breath 13CO2 enrichment for each sample was obtained by subtracting the background breath taken at the start of each trial and the mean of the two subjects from the background breath pretrials. Bicarbonate retention factors were calculated using breath 13CO2 enrichment results obtained from the bicarbonate correction pretrials and the corresponding VO2 data according to the following equation: 

\[ c = \frac{[\text{VCO}_2] \times [\text{E}_{\text{CO}_2}] \times t^{-1}}{100} \]

where \( c \) denotes bicarbonate retention factors, E\textsubscript{CO2} is the breath 13CO2 enrichment, and \( i \) is the infusion rate of NaH13CO3 used during the pretrial.

**HPLC**

The concentrations of phenylalanine in the blood was determined using the protocol described by Moore and colleagues (14). Briefly, each extract was derivatized before the injection using a Waters AccQ·Fluor reagent kit (Millford, MA) by heating for 30 min at 55°C to form the 6-aminouquinoliny-1H-hydroxyquinolinimidyl carbamate derivative of all physiological amino acids. Samples and standards (Sigma, St. Louis, MO) were run on a Waters 2695 HPLC separation module through a Nova-Pak C18 4-μm column to separate the amino acids. Amino acids were detected using a Waters 474 scanning fluorescence detector with excitation and emission wavelengths of 250 and 395-nm, respectively. Amino acid peak areas were integrated, compared with known standards, and analyzed using the Waters Millenium software package.

**GC-MS**

\([1-13C]\text{Leucine.} \)

The isotope enrichment of plasma α-ketoisocaproic acid was measured on an electron-impact ionization GC-MS (GC: model 6890N, Agilent, Santa Clara, CA and MS: model 5973, Hewlett-Packard, Palo Alto, CA) using previously described methods (18, 21). The ratio of 13CO2 to 12CO2 was measured in breath samples using an automated breath analysis system (BreathMat plus, Thermo Finnegan, San Jose, CA) using previously described methods (21).

\([1-\text{ring-2H3}]\text{Phenylalanine.} \)

The isotope enrichment of plasma phenylalanine was measured for the calculation of the leg phenylalanine rate of appearance (Ra) and rate of disappearance (Rd). Briefly, 100 μl of plasma were added to 400 μl of ice-cold acetonitrile, and samples were vortexed and centrifuged at 15,000 rpm for 10 min at 4°C. The supernatant was collected, and 200 μl were dried under N2 gas at 70°C. Samples were derivatized to the tert-butyldimethyl silyl deriv-ative of phenylalanine using 50 μl N-methyl-N-(tert-butyldimethylsilyl)trrifluoroacetamide + 1% tert-butydimethylchlororosilane ( Pierce Chemical, Rockford, IL) + 50 μl anhydrous acetonitrile and heated for 15 min at 100°C. Phenylalanine isotopic enrichments were determined on an electron-impact ionization GC-MS (GC: model 6890N, Agilent, and MS: model 5973, Hewlett-Packard), and ions were selectively monitored at mass-to-charge ratios of 234 and 239.

**Calculations**

Calculations of whole body leucine flux, oxidation, nonoxidative leucine disposal (NOLD), and net balance were made using previ-
Analysis was set at Tukey honestly significant difference post hoc test. Significance for all interaction was identified, data were subsequently analyzed using a denoted net release and net muscle protein degradation. Uptake and net muscle protein synthesis and a negative net balance is not metabolized in muscle, so a positive net balance denotes net muscle protein degradation.

A two-pool model was used to calculate the muscle $R_a$ and $R_d$ of phenylalanine across the muscle as an estimate of muscle protein degradation and synthesis, respectively, as previously described (1, 3):

$$NB = (C_a - C_v) \times BF$$

where $NB$ is the net balance, $C_a$ is the arterial phenylalanine or glucose concentration, $C_v$ is the femoral venous phenylalanine or glucose concentration, and $BF$ is the femoral artery BF. Phenylalanine is not metabolized in muscle, so a positive net balance denotes net uptake and net muscle protein synthesis and a negative net balance denotes net release and net muscle protein degradation.

Statistical Analyses

Muscle glycogen utilization during exercise was analyzed using a paired $t$-test. All other muscle and blood data were analyzed using two-factor (diet $\times$ time) repeated-measure ANOVA. Integrated area under the curve calculations were performed using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA) and analyzed using two-factor (diet $\times$ time) ANOVA. When a significant main effect or interaction was identified, data were subsequently analyzed using a Tukey honestly significant difference post hoc test. Significance for all analysis was set at $P \leq 0.05$. All values are presented as means $\pm$ SE.

RESULTS

Dietary Interventions and Muscle Glycogen

Dietary analyses of food records revealed that subjects ingested $6,837 \pm 518$ kcal during the $\sim 44$-h period before the H-CHO trial, with energy derived from $71 \pm 3\%$ CHO, $19 \pm 3\%$ fat, and $10 \pm 1\%$ protein. Total energy intake was similar before the L-CHO trial ($6,172 \pm 739$ kcal, $P = 0.5$); however, the macronutrient distribution was $11 \pm 1\%$ CHO, $64 \pm 2\%$ fat, and $25 \pm 2\%$ protein. Prettrial exercise and nutritional controls achieved their intended goal given that muscle glycogen content was lower at rest and throughout exercise and recovery during the L-CHO trial compared with the H-CHO trial (main effect: diet, $P < 0.05$; Fig. 3A). Net muscle glycogen utilization during the two-leg knee extensor exercise was also lower in the L-CHO trial compared with the H-CHO trial ($P < 0.05$; Fig. 3B).

Cardiorespiratory Measures and Blood Flow

$\dot{V}_O_2$, HR, and ventilation rate (minute ventilation) were higher during exercise compared with rest and recovery (main effect: time, $P < 0.05$), with no differences between the H-CHO and L-CHO trials (Table 1). BF increased from rest and remained higher throughout exercise but returned to resting values during recovery (main effect: time, $P < 0.05$), with no differences between trials (Fig. 4).

Fig. 3. A: muscle glycogen concentrations at rest, during 2 h of two-leg knee extensor exercise (Ex), and after 1 h of recovery. B: utilization during exercise after a glycogen depletion protocol and the subsequent diet of either high CHO (H-CHO) or low CHO (L-CHO) for $\sim 48$ h. Values are means $\pm$ SE; $n = 6$. †Main effect for diet ($P < 0.05$). *$P < 0.05$ vs. the H-CHO trial.

Background Breath $^{13}$CO$_2$ and Bicarbonate Retention Trials

There were no effects of diet, exercise, or recovery on background breath $^{13}$CO$_2$ enrichments; therefore, baseline breath samples obtained at the start of every trial were sufficient for correcting for background breath enrichment. Measurements made in triplicate for each subject during each stage of the trial were averaged to obtain a single retention factor for rest, exercise, and recovery (each). The calculation of bicarbonate retention factors from breath $^{13}$CO$_2$ enrichments and $V_{CO_2}$ values obtained from the bicarbonate retention trials resulted in values of $0.83, 1.07$, and $1.01$ for rest, exercise, and recovery, respectively, in the H-CHO trial and $0.81, 1.06$, and $0.98$ for rest, exercise, and recovery, respectively, in the L-CHO trial.

Whole Body Leucine Balance

Leucine flux was lower during recovery compared with both rest and exercise, but there were no differences between the H-CHO and L-CHO trials (main effect: time, $P < 0.05$; Fig. 5A). Leucine oxidation was increased during exercise in the L-CHO trial only ($P < 0.05$) but was decreased during recovery in both the H-CHO and L-CHO trials, with no differences between trials ($P < 0.05$; Fig. 5B). NOLD decreased during exercise in the L-CHO trial ($P < 0.05$) but returned to resting values during recovery, with no changes in NOLD at any time during the H-CHO trial (Fig. 5C). The net whole body leucine balance was negative at all times in both trials but was reduced further during exercise in the L-CHO trial ($P < 0.05$) compared with...
rest and the H-CHO trial during exercise (Fig. 5D). However, during recovery, the net leucine balance was less negative in both the H-CHO and L-CHO trials compared with both rest and exercise (P < 0.05), with no differences between trials (Fig. 5D).

Muscle Protein Turnover

The phenylalanine net balance was negative at all times during both trials and was lower (P < 0.05) in the L-CHO trial compared with the H-CHO trial throughout exercise, with no differences between trials at rest or during recovery (Fig. 6).

Phenylalanine Ra was higher in the L-CHO trial compared with the H-CHO trial (main effect: diet, P < 0.05) and was increased during exercise compared with rest and recovery (main effect: time, P < 0.05; Fig. 7A). Analysis revealed an increased area under the Ra-time curve in the L-CHO trial during exercise compared with the H-CHO trial, with a decrease in Ra during recovery in both trials (Fig. 7B). Phenylalanine Rq was lower at 90 and 120 min of exercise and 30 and 45 min of recovery in L-CHO trial compared with the H-CHO trial (P < 0.05; Fig. 7C).

Blood Glucose Uptake and Arterial Insulin Concentration

Leg glucose uptake was higher in the L-CHO trial compared with the H-CHO trial (main effect: diet, P < 0.05) and increased compared with rest by 30 min of exercise (main effect: time, P < 0.05; Fig. 8A). Glucose uptake remained higher throughout the remainder of exercise, reaching peak values of 0.43 ± 0.10 and 0.76 ± 0.07 mmol/min at the end of the 2 h of exercise for the H-CHO and L-CHO trials, respectively. Arterial insulin concentrations were lower in the L-CHO trial compared with the H-CHO trial at rest and during exercise and recovery (P < 0.05; Fig. 8B). Insulin concentrations reached a peak during both trials at 5 min of recovery from exercise (main effect: time, P < 0.05; Fig. 8B).

DISCUSSION

The major novel findings from the present study were that 1) the skeletal muscle net protein balance was lower during exercise in the L-CHO trial compared with the H-CHO trial, owing to an increase in protein degradation and a decrease in protein synthesis late in exercise; 2) whole body leucine oxidation was higher during exercise compared with rest only in the L-CHO condition but decreased below rest values during recovery in both conditions; 3) the whole body leucine net balance was more negative during exercise in the L-CHO trial versus the H-CHO trial, primarily due to a lower whole body protein synthesis, but was less negative during recovery regardless of treatment condition; and 4) skeletal muscle glycogenolysis was lower during exercise in the L-CHO trial versus the H-CHO trial and this was associated with a higher rate of blood glucose uptake. These data extend previous findings by others and demonstrate using contemporary stable isotope methodology that CHO availability influences rates of skeletal muscle and whole body protein synthesis, degradation, and net balance during prolonged exercise in humans.

Van Hall et al. (19) and Blomstrand and Saltin (4) have previously reported an increase in net skeletal muscle protein degradation during prolonged aerobic exercise when muscle glycogen content was reduced. In contrast to the present work, those studies used a-v balance measurements of nonmetabolized amino acids across the exercising leg. While quantitative, the a-v balance method only allows for the measurement of the net amino acid balance but does not allow for the determination of specific aspects of protein turnover (i.e., synthesis and degradation). The present study confirms findings from previous studies (4, 19) that have shown increased net amino acid efflux from working human skeletal muscle in the glycogen reduced state. However, by incorporating stable isotope tracer methodology, this study was able to determine that the increased net degradation during prolonged exercise was not only a result of increased degradation of muscle protein but also decreased synthesis late in exercise.

The present study involved two separate trials and was designed such that nutrient intake was the main variable manipulated between treatments to examine the effect of reduced muscle glycogen availability on skeletal muscle protein turnover during exercise. Previous studies (4, 19) used a model in which subjects first performed exercise with one leg only and then ingested a L-CHO diet or fasted overnight to manipulate glycogen content between legs during the main experimental trial. Based on the latter approach, Van Hall et al. (19) reported increased net protein degradation during exercise.
compared with rest in both legs, the magnitude of which was higher in the low-glycogen leg compared with the normal-glycogen leg. In contrast, Blomstrand and Saltin (4) reported no effect of exercise on net protein degradation in the normal-glycogen leg but an increase in the low-glycogen leg. Our findings are similar to those of Van Hall et al. (19) in that exercise reduced the net skeletal muscle protein balance during exercise compared with rest and net balance was more negative in the low- versus high-glycogen condition.

A potential limitation of the present study is that subjects were free to make their own food choices from lists that were provided and we did not prescribe specific diets to precisely regulate total energy and macronutrient intake. As a result, it appeared that subjects were not able to totally replace energy in the CHO-restricted condition with fat and thus maintain constant protein intakes between treatments. Total energy intake was 10% lower in the L-CHO trial versus the H-CHO trial, but this difference was not statistically significant. However, preexercise protein intake was significantly higher in the L-CHO trial (2.4 ± 0.6 vs. 1.1 ± 0.2 g·kg⁻¹·day⁻¹ in the H-CHO trial). It is therefore possible that differences in protein intake in the 2 days before exercise may have influenced our protein kinetic measurements and that the results are not entirely attributable to differences in glycogen availability. Several studies have examined the potential for habitual protein intake to modulate the whole body protein metabolism response to endurance exercise. Overall, no significant differences were found between rates of whole body synthesis and breakdown in the fasted state when protein intakes ranged from 0.9 to 2.5 g·kg⁻¹·day⁻¹ (5–7), although an increase in leucine oxidation has been reported with higher protein intakes. The effect of CHO availability on whole body protein oxidation during aerobic exercise has been investigated using the nitrogen balance method (11). A classic study by Lemon and Mullin (11) showed an increase in urea nitrogen measures in the blood, sweat, and urine during a glycogen-depleted condition compared with a glycogen-loaded condition during 1 h of cycle exercise and recovery. This indicated increased ureagenesis, suggesting greater amino acid catabolism when endogenous CHO availability was limited. The present study used stable isotope tracer methodology to measure whole body leucine oxidation, as leucine is one of the branched-chain amino acids, which are the primary amino acids oxidized in skeletal muscle. We found increased whole body leucine oxidation during exercise in the L-CHO trial versus the H-CHO trial, which is indicative of increased amino acid oxidation under conditions of reduced CHO availability. The increased oxidation of leucine during exercise is supported by studies (10, 20) that have shown an exaggerated increase in the activation of the branched-chain oxo-acid dehydrogenase complex, the limiting step in the oxidation of branched-chain amino acids in skeletal muscle, when glycogen availability is limited.

The decreased leucine net balance during exercise in the L-CHO versus H-CHO trial was primarily due to a reduction in

Fig. 6. Phenylalanine net balance across the leg at rest, during 2 h of two-leg knee extensor exercise, and during 1 h of recovery after a glycogen depletion protocol and subsequent H-CHO or L-CHO diet for ~48 h. Values are means ± SE; n = 6. *P < 0.05 vs. the L-CHO trial at the same time point; †main effect for diet (P < 0.05).
whole body protein synthesis (i.e., NOLD) with no change in degradation (i.e., flux). Although there were no significant changes in whole body synthesis or degradation, a nonsignificant increase in synthesis with a nonsignificant decrease in degradation resulted in a whole body protein balance that was less negative during recovery compared with rest during both trials. Other studies (6, 13, 16) that have examined the whole body net balance during prolonged exercise have shown no change or an increase in whole body leucine net balance during rest, exercise, or recovery. However, a comparison between the present study and these studies is difficult as they used subjects in the fed or fasted states during 4 h of treadmill walking. The type and duration of exercise and differences in dietary controls may help explain the discrepancies between the present and previous studies. Relative work intensity also hampers comparisons between studies, as the oxygen utilization per unit of active muscle mass is higher in leg extensor exercise compared with traditional whole body exercise (8).

The effect of glycogen availability on glucose uptake during exercise was also examined in the present study. While others (4, 9, 17) have examined this topic, data are equivocal due in part to differences in study design and particularly the method used to reduce muscle glycogen stores, as a potential confounding factor is the residual effects of prior exercise. The present study showed a decrease in glycogenolysis during exercise in the glycogen-depleted condition, and this was associated with an increase in glucose uptake compared with the glycogen-loaded condition. Interestingly, studies (4, 17) that used the single-leg depletion model followed by two-leg knee extensor exercise also showed an increase in glucose uptake in the glycogen-depleted leg, but studies (9, 17) that used protocols similar to that used in the present study, where subjects performed glycogen depletion protocols followed by H-CHO or L-CHO diets, showed no changes in glucose uptake in the glycogen-depleted condition. In an attempt to explain the differences between these studies, Steensburg et al. (17) suggested that changes in the delivery of hormones or substrates may negate the effect of glycogen availability on glucose uptake. For example, the model that used separate trial days showed increased insulin concentrations during exercise in the

*Fig. 7.* Leg phenylalanine rate of appearance (Ra; A), area under the curve (AUC) for Ra (B), and rate of disappearance (Rd; C) at rest, during 2 h of two-leg knee extensor exercise, and during 1 h of recovery after a glycogen depletion protocol and subsequent H-CHO or L-CHO diet for ~48 h. Values are means ± SE; n = 6. *P < 0.05 vs. the H-CHO trial at the same time point; †main effect for diet (P < 0.05); ‡main effect for time vs. rest and all recovery time points (P < 0.05).

*Fig. 8.* Leg glucose uptake (A) and arterial insulin concentrations (B) at rest, during 2 h of two-leg knee extensor exercise, and during 1 h of recovery after a glycogen depletion protocol and subsequent H-CHO or L-CHO diet for ~48 h. Values are means ± SE; n = 6. *P < 0.05 vs. the L-CHO trial at the same time point; †main effect for diet (P < 0.05); ‡main effect for time vs. rest and all recovery time points after 15 min postexercise (P < 0.05).
high-glycogen condition compared with the reduced-glycogen condition (9, 17). However, the present study showed greater insulin concentrations in the H-CHO trial and increased glucose uptake in the L-CHO trial. Therefore, insulin did not appear to be the determining factor for exhibiting the effect of glycogen availability on glucose uptake. The present study suggests that during exercise with reduced endogenous carbohydrate availability, muscle glucose uptake is increased despite lower blood insulin levels.

In summary, the present study characterized the effects of CHO availability on skeletal muscle and whole body protein kinetics during prolonged exercise and recovery using contemporary stable isotope methodology. Commencing exercise with reduced muscle glycogen content caused a greater increase in net skeletal muscle protein breakdown, which was primarily due to an increase in protein degradation but also to a decrease in protein synthesis late in exercise. L-CHO availability was also associated with greater oxidation of leucine during exercise and a decreased whole body leucine net balance, primarily due to a decrease in whole body synthesis compared with the H-CHO condition. Finally, muscle glycogenolysis was reduced during exercise in the glycogen-reduced state, and this was associated with increased glucose uptake by working muscle, which appeared to be unrelated to changes in the circulating insulin concentration. These results could have practical implications for athletes and suggest that commencing endurance exercise in a glycogen-replete state may spare body protein by reducing net skeletal muscle and whole body protein degradation. Future studies with more rigorous dietary controls will help to clarify whether the differences observed between treatments in the present study were solely attributable to CHO availability or whether these were influenced by differences in protein intake before exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


