

Carbohydrate Does Not Augment Exercise-Induced Protein Accretion versus Protein Alone

AARON W. STAPLES¹, NICHOLAS A. BURD¹, DANIEL W. D. WEST¹, KATHARINE D. CURRIE¹, PHILIP J. ATHERTON², DANIEL R. MOORE¹, MICHAEL J. RENNIE², MAUREEN J. MACDONALD¹, STEVEN K. BAKER³, and STUART M. PHILLIPS¹

¹Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, Ontario, CANADA; ²School of Graduate Entry Medicine and Health, City Hospital, University of Nottingham, Derby, UNITED KINGDOM; and ³Michael G. DeGroot School of Medicine, Department of Neurology, McMaster University, Hamilton, Ontario, CANADA

ABSTRACT

STAPLES, A. W., N. A. BURD, D. W. WEST, K. D. CURRIE, P. J. ATHERTON, D. R. MOORE, M. J. RENNIE, M. J. MACDONALD, S. K. BAKER, and S. M. PHILLIPS. Carbohydrate Does Not Augment Exercise-Induced Protein Accretion versus Protein Alone. *Med. Sci. Sports Exerc.*, Vol. 43, No. 7, pp. 1154–1161, 2011. **Purpose:** We tested the thesis that CHO and protein coingestion would augment muscle protein synthesis (MPS) and inhibit muscle protein breakdown (MPB) at rest and after resistance exercise. **Methods:** Nine men (age = 23.0 ± 1.9 yr, body mass index = 24.2 ± 2.1 kg·m⁻²) performed two unilateral knee extension trials (four sets × 8–12 repetitions to failure) followed by consumption of 25 g of whey protein (PRO) or 25 g of whey protein plus 50 g of maltodextrin (PRO + CARB). Muscle biopsies and stable isotope methodology were used to measure MPS and MPB. **Results:** The areas under the glucose and insulin curves were 17.5-fold ($P < 0.05$) and 5-fold ($P < 0.05$) greater, respectively, for PRO + CARB than for PRO. Exercise increased MPS and MPB (both $P < 0.05$), but there were no differences between PRO and PRO + CARB in the rested or exercised legs. Phosphorylation of Akt was greater in the PRO + CARB than in the PRO trial ($P < 0.05$); phosphorylations of Akt ($P = 0.05$) and acetyl coA carboxylase- β ($P < 0.05$) were greater after exercise than at rest. The concurrent ingestion of 50 g of CHO with 25 g of protein did not stimulate mixed MPS or inhibit MPB more than 25 g of protein alone either at rest or after resistance exercise. **Conclusions:** Our data suggest that insulin is not additive or synergistic to rates of MPS or MPB when CHO is coingested with a dose of protein that maximally stimulates rates of MPS. **Key Words:** INSULIN, SKELETAL MUSCLE, RESISTANCE EXERCISE, PROTEIN TURNOVER, HYPERTROPHY

An adaptation to resistance exercise is the hypertrophy of skeletal muscle. This process occurs through induction of a chronically positive muscle net protein balance (NPB = muscle protein synthesis (MPS) – muscle protein breakdown (MPB)) (5). In the fasted state, resistance exercise does not result in net protein accretion because MPB exceeds MPS (18,25); however, consumption of protein and/or essential amino acid provision shifts NPB to positive and promotes muscle protein accretion during the acute recovery period after resistance exercise (27). Recent reports suggest that hyperinsulinemia stimulated by the coingestion of CHO with protein does not seem to provide an even greater stimulus for muscle protein accretion (12,17). However, hyperinsulinemia is reported to stimulate

rates of MPS (1–3,10,26) and/or inhibit leg protein breakdown (20), both of which could augment protein accretion. These effects are equivocal, however, in terms of their beneficence for postexercise protein accretion; in particular, they are not seen when protein or amino acid consumption is sufficient to maximally stimulate rates of MPS (12,13,17).

To date, no workers have examined rates of MPS or MPB after the consumption of a bolus of protein with and/or without CHO. Studies have examined the effects of a mixed amino acid infusion (13) and repeated feedings of small doses of protein (17) on MPS, neither of which would yield practical information for athletes or those concerned with gaining muscle mass. In previous works (13,17), the methods used resulted in a sustained hyperaminoacidemia and hyperinsulinemia, which is not typical of what happens when food and/or drinks are consumed in a normal feeding pattern. Indeed, Glynn et al. (12) added 20 g of crystalline essential amino acids (equivalent to about 50 g of intact high-quality protein) to either 90 or 30 g of CHO and reported similar effects on postexercise protein kinetics; however, without a condition in which only protein was consumed, the authors could only comment on how differing doses of CHO affected protein kinetics and not on the effect of CHO *per se* over and above that of protein.

Address for correspondence: Stuart M. Phillips, Ph.D., McMaster University, 1280 Main St. West, Hamilton, Ontario, Canada L8S 4K1; E-mail: phillis@mcmaster.ca.

Submitted for publication November 2010.

Accepted for publication November 2010.

0195-9131/11/4307-1154/0

MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Copyright © 2011 by the American College of Sports Medicine

DOI: 10.1249/MSS.0b013e31820751cb

Thus, the primary purpose of the present study, which is dissimilar to previous works on this question (12,17), was to use direct measures of muscle protein turnover and examine whether a bolus dose of high-quality whey protein, which contains sufficient essential amino acids to maximally stimulate rates of MPS (7,23), coingested with CHO (50 g) would augment rates of MPS and/or inhibit rates of MPB compared with protein alone. Further, we examined intramuscular signaling events shown to be involved in the regulation of mRNA translation and elongation (21). This approach would provide practical insight on the relevance of CHO to a single-dose protein beverage on postexercise muscle protein turnover and intramuscular signaling events. We hypothesized that insulin, if it were to be stimulatory for rates of MPS, might stimulate limb blood flow and enhance amino acid delivery to the limb at rest (2); however, insulin would not further stimulate limb blood flow or rates of MPS after resistance exercise followed by adequate protein consumption (3). We further hypothesized that insulin would augment protein-stimulated inhibition of rates of MPB after exercise but not at rest (9,14,15).

METHODS

Ethics approval. This study conformed to the Helsinki Declaration and was approved by the local research ethics board of McMaster University and Hamilton Health Sciences.

Participants. Nine young recreationally active males (age = 23.0 ± 1.9 yr, weight = 80.3 ± 8.5 kg, body mass index = 24.2 ± 2.1 kg·m⁻²) were recruited to participate in the study. All participants were informed about the purpose of the study, the procedures, the risks, and their rights before providing written consent. Participants were assessed as being healthy on the basis of their responses to a routine medical questionnaire, which was used to screen for conditions precluding participation. Participants were instructed not to change their activity or dietary habits immediately before or between trials.

Study design: pretesting. Unilateral leg extension testing was performed for each leg on two separate occasions, at least 7 d apart, to determine the single-leg one-repetition maximum (1RM) and the 8RM to 12RM. In this way, we familiarized the participants with proper lifting techniques and ensured the reproducibility of their single-leg strength assessments. Emphasis was placed on lifting with control through the full range of motion and lifting until voluntary failure, which was operatively defined as a lift that was not performed with control through the full range of motion. A unilateral exercise model was used so that the nutritional manipulation could be examined under resting (i.e., nonexercised) and exercised conditions in the same individual with the same systemic environment (29,30,32).

Diet and activity before trials. Participants were asked to refrain from heavy leg exercise for 72 h before each of the trials and to refrain from alcohol, caffeine, and other drugs for 24 h before each of the trials. Participants kept a

food record for the day before their first trial and were asked to replicate the diet and approximate eating times before the second trial. Participants were only permitted to consume water during the 10 h before each trial, and they were asked to obtain a full night of sleep on the evenings before each trial.

Trial design. Participants performed two trials, separated by at least 7 d. Each trial consisted of a bout of unilateral knee extension resistance exercise (four sets, 8RM to 12RM to failure) and the consumption of a protein or protein-CHO drink after exercise. The participant's dominant leg (by strength) was randomly assigned, in a counterbalanced fashion, to rest or exercise in each of the trials. After completing the resistance exercise protocol, participants consumed one of two drinks. One drink consisted of 25 g of whey protein isolate (PRO; Protient, Inc., St. Paul, MN), the quantity of which has previously been shown to maximize the rate of MPS at rest and after resistance exercise (7,23). The other drink (PRO + CARB) consisted of 25 g of the same whey protein isolate and 50 g of maltodextrin CHO (Roquette Frères, Lestrem, France). Drinks were administered in a double-blind randomized crossover fashion, with the flavor and appearance of the drinks matched by the addition of cocoa (Nestle, Toronto, ON) and artificial sweetener (Splenda®, McNeil Nutritionals, Ft. Washington, PA). The drinks were also enriched with L-[ring-¹³C₆]-phenylalanine and L-[¹⁵N]-phenylalanine to 4% of the phenylalanine content of the whey protein to ensure a constant isotopic enrichment of the blood and intracellular precursor pools.

Infusion and sampling protocol. On arrival at the laboratory, the participant's height and weight were recorded. The participant then lay supine on the bed for 30 min before preparation for the ultrasound measures to eliminate any residual blood flow effects due to transport to the laboratory. Six electrodes were placed on the participant's chest for continuous HR monitoring. An MLE1054-V Finometer® MIDI with medium finger cuff (Finapres Medical Systems, Amsterdam, The Netherlands) was used to monitor blood pressure during the ultrasound measures. The ultrasound probe was placed on the surface of the skin over the femoral artery, and a baseline image inclusive of a 10-heart cycle sequence of the femoral artery was recorded from each leg. A polyethylene catheter was then inserted in an antecubital vein of each forearm. After taking a baseline blood sample, a primed ($2.0 \mu\text{mol}\cdot\text{kg}^{-1}$) continuous infusion ($0.05 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) of L-[ring-¹³C₆]-phenylalanine and L-[¹⁵N]-phenylalanine was started in one catheter. The isotopes (Cambridge Isotope, Inc., Andover, MA) were dissolved in 0.9% saline, filtered through a 0.2- μm filter, and infused using a calibrated syringe pump (Harvard Apparatus, Holliston, MA). The blood sampling catheter was kept patent throughout the trial using a 0.9% saline drip.

Participants were moved to a leg extension machine and warmed up the leg to be exercised by performing 15 repetitions with a light weight (~40% of 1RM). Four minutes of

recovery was given between the warm-up and the first trial set. The participant completed the first of four 8RM to 12RM sets, with 4 min of recovery between the sets. Each set was performed to failure, with the weight adjusted between sets to ensure that failure occurred between 8 and 12 repetitions. After the fourth set, the participant returned to the bed to commence a 180-min recovery period. At 15 min after exercise, another blood sample was taken, a 10-heart cycle sequence was recorded of the femoral artery of both legs, and one of the drinks was consumed. At 120 min after exercise, the L-[¹⁵N]-phenylalanine tracer infusion was stopped. Additional blood samples were taken at 30, 60, 90, 120, 135, 150, 165, and 180 min after exercise, and additional ultrasound sequences were recorded for both legs at 30, 60, 90, 120, and 180 min after exercise (Fig. 1).

Percutaneous muscle biopsies of the vastus lateralis of both the rested and the exercised legs were taken at 60 and 180 min after exercise using a Bergström needle (modified for manual suction) under 2% xylocaine local anesthesia. Muscle biopsies were taken from separate incisions, each approximately 4–5 cm proximal from the first incision site, which was made ~10 cm above the knee.

Blood analyses. All blood samples were collected into 4-mL evacuated containers containing lithium heparin (Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ). Vacutainers were gently inverted three to five times to ensure thorough mixing before being placed on ice. Within 5 min of blood collection, whole blood was drawn from the Vacutainer, and the blood glucose concentration was measured on a OneTouch® Ultra2 blood glucose meter (LifeScan, Inc., Milpitas, CA). A perchloric acid (PCA) protein-extracted blood sample was subsequently collected by adding 100 μL of the whole blood to 500 μL of cold 0.6 mol·L⁻¹ PCA and a norleucine internal standard. The PCA-whole blood solution was briefly placed on the vortex to ensure mixing and left on ice for subsequent processing. The whole blood and the PCA-whole blood solution were processed as previously described (22), and the recovered

plasma and PCA extracts were stored at -80°C. Plasma insulin concentrations were measured using a commercially available immunoassay kit (ALPCO™ Diagnostics, Salem, NH). To isolate plasma amino acids, 50 μL of plasma was added to 500 μL of ice-cold acetonitrile. The solution was subjected to vortex and centrifugation, and the supernatant was collected and dried under nitrogen. The enrichment of the plasma amino acids was determined by examining the heptafluorobutyryl isobutyl derivative of the phenylalanine fragment on the gas chromatograph-mass spectrometer (models 6890 GC and 5973 MS; Hewlett-Packard, Palo Alto, CA). Whole blood amino acid concentrations were analyzed by high-performance liquid chromatography as previously described (22), with a method that achieves separation of all essential amino acids.

Blood flow analyses. At each blood flow collection time point, the diameter of the femoral artery was determined from digital B-mode ultrasound images, and the mean blood velocity (MBV) in the femoral artery was measured by using pulsed Doppler ultrasonography (10-MHz linear array probe, model System 5; GE Medical Systems, Horten, Norway) while the ultrasound probe was placed on the skin surface 2–3 cm proximal to the bifurcation of the femoral artery into the superficial and profundus segments. Digital images were acquired at a frame rate of 11 frames per second. The diameters were determined using electronic calipers (Echopac V; GE Medical Systems) on the end-systolic and end-diastolic frames. The value at each time point represents the average of five consecutive end-systole and five consecutive end-diastole measurements. For determination of velocity, the ultrasound gate was maintained at full width to ensure that the entire vessel cross section was exposed to complete insonation with constant intensity, and the signal was adjusted for insonation angle (11). The audio signal from the pulse wave Doppler was processed through an external spectral analyzer (Neurovision 500M TCD; Multigon Industries, Yonkers, NY) that generated the continuous mean voltage signal, proportional in magnitude to the MBV. This

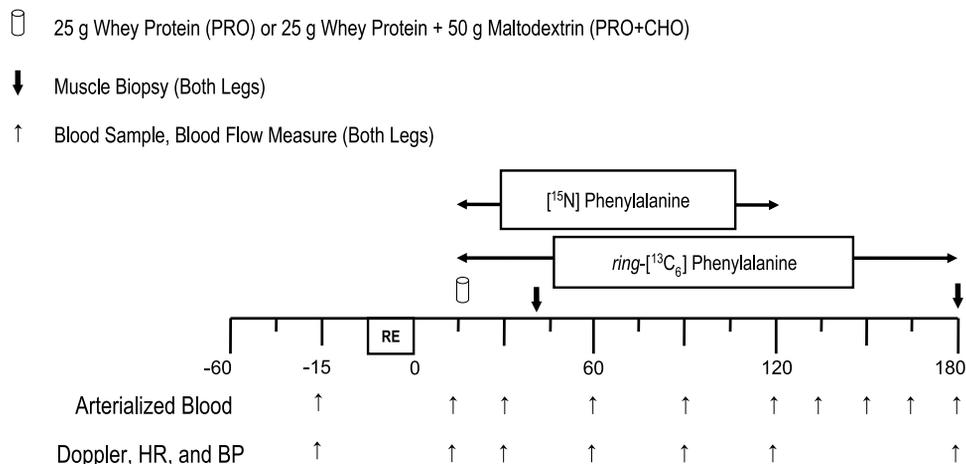


FIGURE 1—Protocol schematic.

signal was analog-to-digital converted and sampled at 200 Hz (Powerlab model ML795; ADInstruments, Colorado Springs, CO) and stored on a computer for subsequent analysis (Chart v5.5.3; ADInstruments). The average MBV was calculated by integrating the total area under the MBV profile for 10 subsequent heart cycles at each time point.

Mixed muscle protein and intramuscular signaling analyses. Mixed muscle proteins and muscle intracellular free amino acids were isolated as previously described (4,6). Approximately, 30 μL of the intracellular free amino acid fraction was aliquoted into two Eppendorf tubes for quantification by high-performance liquid chromatography as described for the blood amino acid concentrations (22), and this method achieved clear separation of phenylalanine and norleucine. The methods for determination of the extent of phosphorylation of protein kinase B (Akt^{Ser473}), eukaryotic elongation factor (eEF2^{Thr56}), 70-kDa S6 protein kinase (p70S6K^{Thr389}), eukaryotic initiation factor 4E binding protein 1 (4EBP1^{Thr37/46}), acetyl coA carboxylase- β (ACC^{Ser729}), and extracellular signal-regulated kinase 1/2 (ERK^{Thr202/Tyr204}) were described in our previous work (6) using antibodies from New England Biolabs (Hitchin, UK) and α -actin from Sigma-Aldrich (Poole, UK) United Kingdom.

Calculations. Mean limb blood flow (Q) was calculated from the mean velocity and area using the formula: Q ($\text{mL}\cdot\text{min}^{-1}$) = $\text{MBV}\pi r^2 60$, where MBV is the mean velocity of the blood ($\text{cm}\cdot\text{s}^{-1}$), r is the mean radius of the artery during the cardiac cycle (cm), and 60 is a constant ($\text{s}\cdot\text{min}^{-1}$) to convert the calculated flow from milliliters per second to milliliters per minute. The mean radius of the artery was calculated from the diameter assuming the artery is circular ($r = d/2$). The mean diameter was calculated from the maximum diameter of the artery during systole (d_{max} , cm) and minimum diameter of the artery during diastole (d_{min} , cm) weighted to the percentage of time spent at each diameter in the cardiac cycle: $d = (1/3)d_{\text{max}} + (2/3)d_{\text{min}}$.

The mixed MPS was calculated at isotopic plateau using the standard precursor-product method. Briefly, MPS ($\%\cdot\text{h}^{-1}$) = $\Delta E_m/E_p \times 1/t \times 100$, where ΔE_m is the change in the isotopic enrichment of the mixed muscle proteins between biopsies, E_p is the mean isotopic enrichment of the intracellular amino acid precursor pool, t is the incorporation time between biopsies (h), and 100 is a constant to convert the calculated proportion of muscle synthesized per hour to a percentage of muscle synthesized per hour. The mixed MPB was calculated using the two-precursor pool adaptation of the precursor-product method as described elsewhere (25,33), and the ratio of the free amino acid pool size to the bound amino acid pool size (QM/T), which was directly measured and not assumed, was used to express MPB in the same units as MPS ($\%\cdot\text{h}^{-1}$).

Statistics. This study was a within-subject repeated-measures design. ANOVA were carried out with relevant preplanned comparisons using STATISTICA[®] v5.1 software (StatSoft, Inc., Tulsa, OK). Strength (1RM, 8RM to

12RM), external work (set, trial), and the area under the curve (glucose, insulin) data were analyzed using paired t -tests. Cardiovascular data and blood metabolites, hormones, and enrichments were analyzed using a two-factor (drink (PRO and PRO + CARB) \times time) repeated-measures ANOVA. Mixed MPS and mixed MPB were analyzed using a two-factor (drink (PRO and PRO + CARB) \times activity (nonexercise and exercise)) repeated-measures ANOVA. Intracellular amino acid enrichments, signaling molecule phosphorylation data, and bulk blood flow data were analyzed using a three-factor (drink \times activity \times time) repeated-measures ANOVA. Where significant differences were observed in the ANOVA, a Tukey *post hoc* test was performed to determine differences between values. For all analyses, statistical significance was set at $P \leq 0.05$. Values are presented as means \pm SEM.

RESULTS

Strength and external work. There were no differences in the pretesting 1RM (PRO = 84 kg vs PRO + CARB = 83 kg, $P = 0.85$) or 8RM to 12RM (PRO = 62 kg vs PRO + CARB = 61 kg, $P = 0.35$) between legs. External work was approximated by multiplying the number of repetitions performed by the mass lifted on each repetition because each repetition was performed over the same range of motion and at the same cadence (and thus the same velocity). The total external work completed by each leg during its respective exercise trial was identical between conditions (PRO = 2235 kg vs PRO + CARB = 2242 kg, $P = 0.91$).

Blood metabolites, hormones, and enrichments. Plasma glucose and plasma insulin responses were greater for PRO + CARB than PRO ($P < 0.05$), with differences at 30–135 min ($P < 0.05$) and 30–120 min ($P < 0.05$) for glucose and insulin, respectively (Figs. 2A and B). Total exposure to plasma glucose and insulin, as determined by area under the curve analysis, were 17.5-fold and 5-fold greater for PRO + CARB than PRO ($P < 0.05$ each). Plasma concentrations of leucine, branched-chain amino acids, and essential amino acids (EAA) (Fig. 2C) were greater for PRO than PRO + CARB at 60 and 90 min ($P < 0.05$). Plasma concentrations of phenylalanine increased after consumption of the drink ($P < 0.05$) and thereafter declined to baseline values but with no differences between drinks ($P = 0.69$). Plasma [*ring*-¹³C₆]-phenylalanine enrichments were not different between drinks (PRO = 0.061% vs PRO + CARB = 0.061%, $P = 0.52$) throughout the trial, and plasma [¹⁵N]-phenylalanine enrichments were not different between drinks (PRO = 0.064% vs PRO + CARB = 0.065%, $P = 0.46$) during the L-[¹⁵N]-phenylalanine infusion. The slope of the plasma phenylalanine enrichment curves was not significantly different from 0, indicating that a prerequisite isotopic steady state was achieved ([¹³C₆]-phenylalanine, $P = 0.51$; [¹⁵N]-phenylalanine, $P = 0.37$).

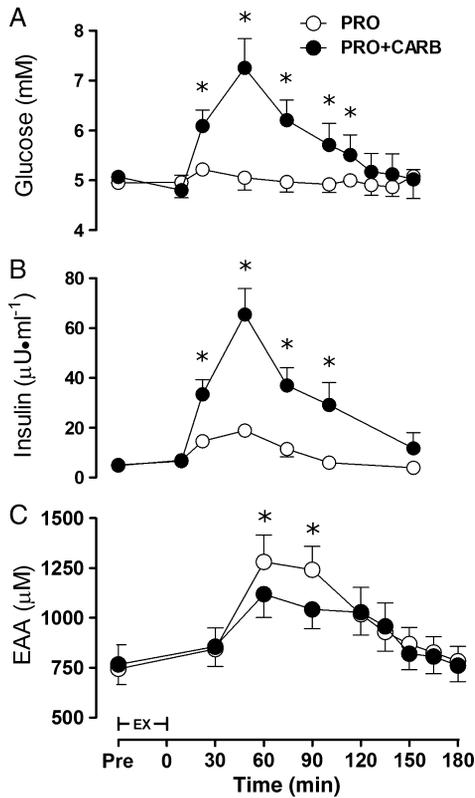


FIGURE 2—Plasma glucose (A), insulin (B), and EAA concentrations (C). *Significant group \times time interaction and a significant pairwise difference between values at the same time point in the other drink condition ($P < 0.05$). Values are means \pm SEM.

Femoral artery blood flow. Mean HR and mean arterial pressure were not different between drink conditions ($P = 0.49$ and 0.63 , respectively). The MBV was greater in the exercised than the nonexercised leg ($P < 0.05$), with no differences between the drinks ($P = 0.45$). No differences were found as a result of the drink ($P = 0.75$) or activity ($P = 0.18$) for mean arterial diameter. Femoral artery blood flow was elevated above rest from 15 to 60 min after exercise ($P < 0.05$), but there were no differences between drinks ($P = 0.49$; Fig. 3). No difference was detected in the nonexercised leg at any time point during the experimental trial (all, $P > 0.05$).

Mixed MPS and breakdown. Intracellular [*ring*- $^{13}\text{C}_6$]-phenylalanine enrichments were not different as a result of the different drinks (PRO = 4.0 vs PRO + CHO = 4.1 , $P = 0.38$). MPS was increased by $\sim 54\% \pm 14\%$ after exercise compared with the values in the nonexercised leg ($P < 0.05$), but there were no differences between the PRO and PRO + CARB trials in the nonexercised leg ($0.078\% \cdot \text{h}^{-1}$ vs $0.078\% \cdot \text{h}^{-1}$, $P = 0.99$) or in the exercised leg ($0.103\% \cdot \text{h}^{-1}$ vs $0.100\% \cdot \text{h}^{-1}$, $P = 0.99$; Fig. 4). MPB was increased by $\sim 37\% \pm 8\%$ after exercise compared with values from the nonexercised leg ($P < 0.05$), but there were no differences between the PRO and PRO + CARB trials in the nonexercised leg ($0.042\% \cdot \text{h}^{-1}$ vs $0.040\% \cdot \text{h}^{-1}$, $P = 0.76$) or after exercise ($0.053\% \cdot \text{h}^{-1}$ vs $0.055\% \cdot \text{h}^{-1}$, $P = 0.58$; Fig. 4). In this pro-

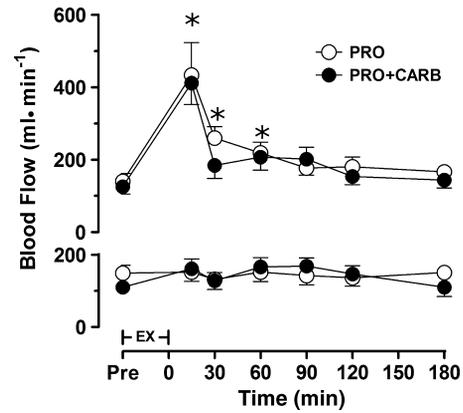


FIGURE 3—Femoral artery blood flow in the exercised leg (top panel) and the contralateral nonexercised leg (bottom panel). *Significant group \times time interaction and a significant pairwise difference between values at the same time point versus the rested leg ($P < 0.05$). Values are means \pm SEM.

col, we elected to take only postexercise biopsies because the effects were all within a single subject (i.e., an all-within design). In this manner, any resting preexercise values for MPS would have been relevant only if they differed between legs (i.e., intraleg variability). A power calculation suggested that given the variance in methods used (all $< 15\%$ and with population variances of a similar magnitude, giving a concatenated error of 20%), we would be able to detect any change $> 25\%$ of the mean with nine subjects. In fact, in previous studies from our laboratory (6,24,30,31) in which

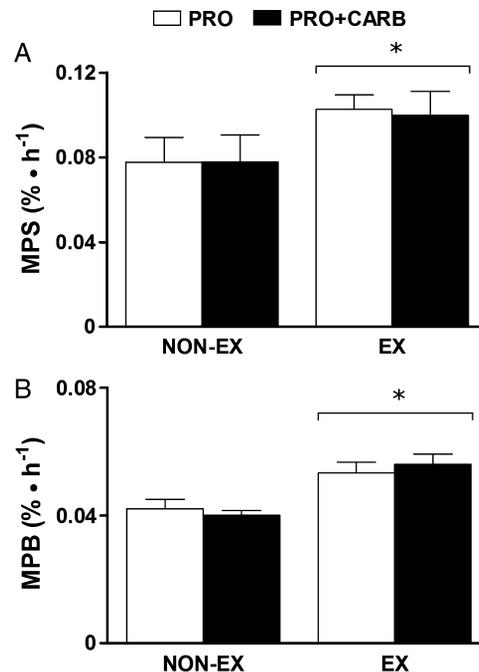


FIGURE 4—Mixed muscle protein fractional synthetic rate (MPS) and breakdown rate (MPB). *Significant main effect of exercised (EX) versus nonexercised (NON-EX; $P < 0.05$). The dashed line on the upper graph indicates a mean resting and fasted rate of MPS obtained from previous studies (6,24,30,31) of $0.041\% \cdot \text{h}^{-1} \pm 0.006\% \cdot \text{h}^{-1}$ to illustrate differences from the rested fasted state. Values are means \pm SEM.

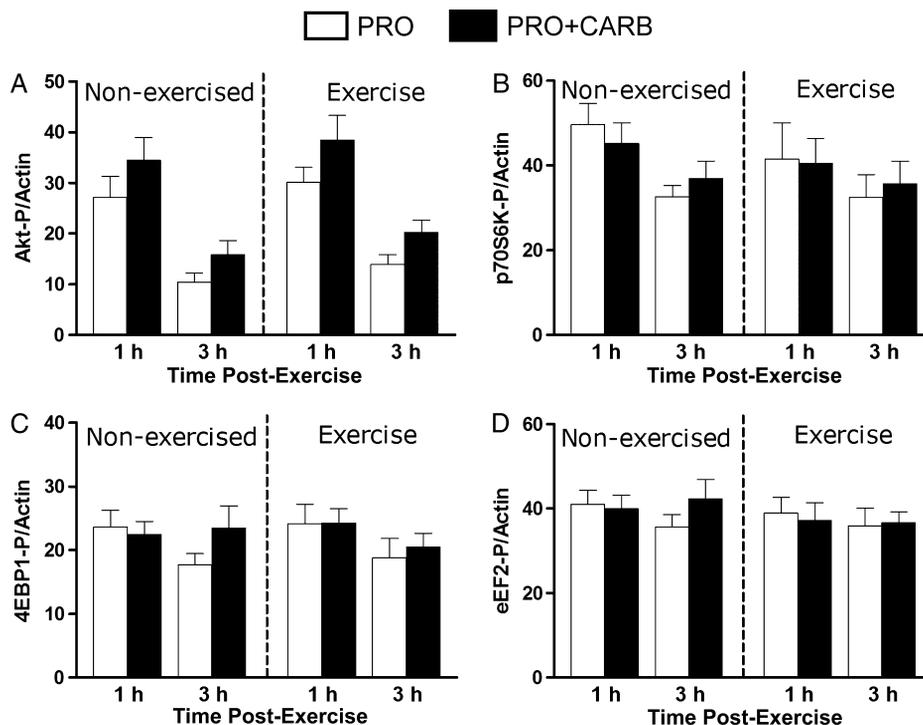


FIGURE 5—Phosphorylation of the anabolic signaling molecules: Akt (A), p70S6K (B), 4EBP1 (C), and eEF2 (D). For Akt, a significant main effect of PRO + CARB greater than PRO ($P < 0.05$) and a significant main effect of EX greater than NON-EX ($P < 0.05$) were observed. Values are means \pm SEM.

we used identical methods, we observed a coefficient of variation of resting MPS rate between legs $<10\%$ with pooled values of $0.041\% \cdot h^{-1} \pm 0.006\% \cdot h^{-1}$ ($N = 40$). Thus, our fed-state MPS estimates from the nonexercised leg (all $>0.07\% \cdot h^{-1}$) and the magnitude of difference between the nonexercised and exercised legs ($30\% \pm 3\%$) are effects attributable to exercise and/or feeding and thus represent real differences from a rested fasted state.

Muscle signaling molecule phosphorylation. Phosphorylation of Akt was greater in the PRO + CARB than the PRO trial ($P < 0.05$) and was increased in the exercised leg ($P = 0.05$; Fig. 5A). Phosphorylation of acetyl coA carboxylase- β , a surrogate marker for AMP-activated protein kinase, was greater after exercise than at rest ($P < 0.05$), but no differences were observed as a result of the different drinks ($P = 0.42$; data not shown). No differences were observed as a result of the different drinks or exercise for ERK1/2, 4EBP1, p70S6K, or eEF2 (Fig. 5B–D).

DISCUSSION

Our study is the first to report no augmentation of protein-stimulated rates of MPS and no further inhibition of MPB by hyperinsulinemia at rest or after resistance exercise after a bolus dose of whey coingested with CHO as compared with protein alone. Our data are supportive of the results of a previous study that used a repeated aliquot feeding pattern (17). In another study, in which a large dose of EAA

with low (30 g) or high (90 g) CHO was compared, the authors also observed no difference between conditions. In the current study, neither intramuscular signaling (4EBP1, p70S6K, eEF2) nor blood flow to the quadriceps was different between the PRO and PRO + CARB conditions. These results suggest that the plasma aminoacidemia and insulinemia in response to 25 g of whey protein alone were sufficient to maximize any insulin-mediated stimulation of MPS. Moreover, our observed lack of an insulin-stimulated inhibition of MPB is in accordance with recent evidence that the insulin-mediated inhibition of leg protein breakdown is fully manifested at plasma concentrations $>5 \mu U \cdot mL^{-1}$ but $<30 \mu U \cdot mL^{-1}$ (13). Clearly, any insulin-mediated mechanisms that normally stimulate MPS and/or inhibit MPB at rest (e.g., after a meal) are without any further effect after ingestion of protein at doses that have been shown to maximally stimulate rates of MPS (7,23).

Our results are consistent with those from Greenhaff et al. (13), who measured the dose–response of insulin on MPS during hyperaminoacidemia. They observed that low ($5 \mu U \cdot mL^{-1}$) concentrations of insulin are required to obtain a maximal amino acid–induced increase in MPS in agreement with other observations (28). It would be interesting to assess whether insulin could mediate an increase in MPS when amino acid delivery to the muscle is limiting, such as it would be after the ingestion of a quantity of protein (i.e., <20 g) that is insufficient to maximally stimulate rates of MPS (7,23). It has been reported that locally infused insulin increases blood flow, amino acid delivery, and intracellular

amino acid availability and can subsequently stimulate MPS above fasting rates in the absence of amino acid feeding or infusion (10). Therefore, insulin may be permissive or stimulatory for MPS depending on the quantity of protein ingested and the subsequent level of aminoacidemia.

The lack of an insulin-mediated augmentation of protein-stimulated MPS was reflected in the lack of differential phosphorylation of several muscle signaling molecules that are thought to be primary regulators of protein translation initiation and elongation (16). In particular, proteins downstream of the mammalian target of rapamycin such as 4EBP1, p70S6K, and eEF2 were not differentially phosphorylated up to 3 h after hyperinsulinemia, aminoacidemia, and/or exercise. The lack of differences in these particular signaling molecules is surprising given the exercise-stimulated increase in MPS observed in this study and the abundance of insulin- and exercise-mediated effects reported in the literature (8,16). This dissociation of signaling and MPS supports accumulating evidence that the extent of phosphorylation of many anabolic signaling molecules is not always as predictive of changes in rates of MPS as might be expected (13,23). We propose that, on the basis of the evidence from this and other studies, the component of the stimulus-to-product pathway for MPS that is rate controlling is not simply signaling molecule activation (i.e., phosphorylation) but can vary according to several factors, including the combination of stimuli, for example, feeding (10,13,23) and contractile stimuli (19,31).

Our results further extend the work by Greenhaff et al. (13) by refining the minimum concentration of plasma insulin that is required to induce maximal inhibition of MPB. Whereas a mean plasma insulin concentration of $5 \mu\text{U}\cdot\text{mL}^{-1}$ was insufficient and $30 \mu\text{U}\cdot\text{mL}^{-1}$ was sufficient to maximally inhibit leg protein breakdown in the study of Greenhaff et al. (13), a peak insulin concentration of

$\sim 19 \mu\text{U}\cdot\text{mL}^{-1}$ and a mean insulin concentration of $\sim 11 \mu\text{U}\cdot\text{mL}^{-1}$ were sufficient to inhibit MPB after protein feeding in this study. Interestingly, plasma insulin concentrations had returned to basal levels during the entire MPB measurement period in the protein feeding condition, suggesting that insulin may mediate an inhibition of MPB that is prolonged beyond the actual period of hyperinsulinemia. Preliminary evidence suggests that muscle atrophy F-box may be a suitable candidate of study because the decreased expression of this atrogene is coincident with the decrease in leg protein breakdown that occurred when insulin concentrations were increased from 5 to $30 \mu\text{U}\cdot\text{mL}^{-1}$ (13). Glynn et al. (12) showed no effect of low or high CHO on muscle RING-finger protein 1 mRNA expression, which was significantly elevated after exercise. The same authors observed no change in muscle atrophy F-box or caspase-3 mRNA levels. What is clear is that when sufficient protein is supplied to maximally stimulate MPS, the consequent plasma insulinemia and/or the hyperaminoacidemia are sufficient to inhibit MPB.

In summary, CHO coingestion with a single bolus of high-quality protein, previously shown to be maximally stimulatory for MPS after resistance exercise (23), does not further augment protein-stimulated MPS or protein-mediated inhibition of MPB at rest or after resistance exercise. Instead, the lack of differences in leg blood flow, intramuscular signaling molecule phosphorylation, and MPS after pronounced hyperinsulinemia suggests that insulin is not further stimulatory for MPS during hyperaminoacidemia.

Funding for this research was provided by the National Science and Engineering Research Council of Canada to S.M.P.

No authors have any conflicts of interest, financial or otherwise, to report.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

- Bell JA, Fujita S, Volpi E, Cadenas JG, Rasmussen BB. Short-term insulin and nutritional energy provision do not stimulate muscle protein synthesis if blood amino acid availability decreases. *Am J Physiol Endocrinol Metab.* 2005;289(6):E999–1006.
- Biolo G, Declan Fleming RY, Wolfe RR. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J Clin Invest.* 1995;95(2):811–9.
- Biolo G, Williams BD, Fleming RY, Wolfe RR. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes.* 1999;48(5):949–57.
- Burd NA, Holwerda AM, Selby KC, et al. Resistance exercise volume affects myofibrillar protein synthesis and anabolic signaling molecule phosphorylation in young men. *J Physiol.* 2010; 588(Pt 16):3119–30.
- Burd NA, Tang JE, Moore DR, Phillips SM. Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. *J Appl Physiol.* 2009;106(5):1692–701.
- Burd NA, West DW, Staples AW, et al. Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS One.* 2010;5(8):e12033.
- Cuthbertson D, Smith K, Babraj J, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J.* 2005;19(3):422–4.
- Drummond MJ, Dreyer HC, Fry CS, Glynn EL, Rasmussen BB. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *J Appl Physiol.* 2009; 106(4):1374–84.
- Fagan JM, Waxman L, Goldberg AL. Skeletal muscle and liver contain a soluble ATP + ubiquitin-dependent proteolytic system. *Biochem J.* 1987;243(2):335–43.
- Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. *Am J Physiol Endocrinol Metab.* 2006;291(4): E745–54.
- Gill RW. Measurement of blood flow by ultrasound: accuracy and sources of error. *Ultrasound Med Biol.* 1985;11(4):625–41.
- Glynn EL, Fry CS, Drummond MJ, et al. Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *Am J Physiol Regul Integr Comp Physiol.* 2010;299(2): R533–40.

13. Greenhaff PL, Karagounis LG, Peirce N, et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab.* 2008;295(3):E595–604.
14. Kasperek GJ, Conway GR, Krayski DS, Lohne JJ. A reexamination of the effect of exercise on rate of muscle protein degradation. *Am J Physiol.* 1992;263(6 Pt 1):E1144–50.
15. Kettelhut IC, Wing SS, Goldberg AL. Endocrine regulation of protein breakdown in skeletal muscle. *Diabetes Metab Rev.* 1988;4(8):751–72.
16. Kimball SR, Farrell PA, Jefferson LS. Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. *J Appl Physiol.* 2002;93(3):1168–80.
17. Koopman R, Beelen M, Stellingwerff T, et al. Coingestion of carbohydrate with protein does not further augment postexercise muscle protein synthesis. *Am J Physiol Endocrinol Metab.* 2007;293(3):E833–42.
18. Kumar V, Atherton P, Smith K, Rennie MJ. Human muscle protein synthesis and breakdown during and after exercise. *J Appl Physiol.* 2009;106(6):2026–39.
19. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol.* 2009;587(Pt 1):211–7.
20. Miller SL, Tipton KD, Chinkes DL, Wolf SE, Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. *Med Sci Sports Exerc.* 2003;35(3):449–55.
21. Moore DR, Atherton PJ, Rennie MJ, Tarnopolsky MA, Phillips SM. Resistance exercise enhances mTOR and MAPK signalling in human muscle over that seen at rest after bolus protein ingestion. *Acta Physiol (Oxf).* 2011;201(3):365–72.
22. Moore DR, Phillips SM, Babraj JA, Smith K, Rennie MJ. Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *Am J Physiol Endocrinol Metab.* 2005;288(6):E1153–9.
23. Moore DR, Robinson MJ, Fry JL, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr.* 2009;89(1):161–8.
24. Moore DR, Tang JE, Burd NA, Rericich T, Tarnopolsky MA, Phillips SM. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *J Physiol.* 2009;587(Pt 4):897–904.
25. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol.* 1997;273(1 Pt 1):E99–107.
26. Rasmussen BB, Fujita S, Wolfe RR, et al. Insulin resistance of muscle protein metabolism in aging. *FASEB J.* 2006;20(6):768–9.
27. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, Wolfe RR. An oral essential amino acid–carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J Appl Physiol.* 2000;88(2):386–92.
28. Svanberg E, Jefferson LS, Lundholm K, Kimball SR. Postprandial stimulation of muscle protein synthesis is independent of changes in insulin. *Am J Physiol.* 1997;272(5 Pt 1):E841–7.
29. West DW, Burd NA, Tang JE, et al. Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *J Appl Physiol.* 2010;108(1):60–7.
30. West DW, Kujbida GW, Moore DR, et al. Resistance exercise–induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. *J Physiol.* 2009;587(Pt 21):5239–47.
31. Wilkinson SB, Phillips SM, Atherton PJ, et al. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J Physiol.* 2008;586(Pt 15):3701–17.
32. Wilkinson SB, Tarnopolsky MA, Grant EJ, Correia CE, Phillips SM. Hypertrophy with unilateral resistance exercise occurs without increases in endogenous anabolic hormone concentration. *Eur J Appl Physiol.* 2006;98(6):546–55.
33. Zhang XJ, Chinkes DL, Sakurai Y, Wolfe RR. An isotopic method for measurement of muscle protein fractional breakdown rate *in vivo*. *Am J Physiol.* 1996;270(5 Pt 1):E759–67.