Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise[1–4]

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ABSTRACT

Background: Ingestion of whey or casein yields divergent patterns of aminoacidemia that influence whole-body and skeletal muscle myofibrillar protein synthesis (MPS) after exercise. Direct comparisons of the effects of contrasting absorption rates exhibited by these proteins are confounded by their differing amino acid contents.

Objective: Our objective was to determine the effect of divergent aminoacidemia by manipulating ingestion patterns of whey protein alone on MPS and anabolic signaling after resistance exercise.

Design: In separate trials, 8 healthy men consumed whey protein either as a single bolus (BOLUS; 25-g dose) or as repeated, small, "pulsed" drinks (PULSE; ten 2.5-g drinks every 20 min) to mimic a more slowly digested protein. MPS and phosphorylation of signaling proteins involved in protein synthesis were measured at rest and after resistance exercise.

Results: BOLUS increased blood essential amino acid (EAA) concentrations above those of PULSE (162% compared with 53%, $P < 0.001$) 60 min after exercise, whereas PULSE resulted in a smaller but sustained increase in aminoacidemia that remained elevated above BOLUS amounts later (180–220 min after exercise, $P < 0.05$). Despite an identical net area under the EAA curve, MPS was elevated to a greater extent after BOLUS than after PULSE early (1–3 h: 95% compared with 42%) and later (3–5 h: 193% compared with 121%) (both $P < 0.05$). There were greater changes in the phosphorylation of the Akt–mammalian target of rapamycin pathway after BOLUS than after PULSE.

Conclusions: Rapid aminoacidemia in the postexercise period enhances MPS and anabolic signaling to a greater extent than an identical amount of protein fed in small pulses that mimic a more slowly digested protein. A pronounced peak aminoacidemia after exercise enhances protein synthesis. This trial was registered at clinicaltrials.gov as NCT01319513. *Am J Clin Nutr* 2011;94:795–803.

INTRODUCTION

Protein ingestion elicits an increase in aminoacidemia that stimulates rates of muscle protein synthesis, which is an effect that is enhanced when resistance exercise is performed (1–3). The stimulation of muscle protein synthesis is driven primarily by EAAs[5] (4–8), appears to be triggered by leucine (9–13), and occurs in a dose-dependent manner at rest (14, 15) and postexercise (16). The digestion rate of proteins (and the resultant aminoacidemia) is an independent variable that also affects the amplitude of acute increases in muscle protein synthesis (12, 17). For example, ingestion of rapidly digested whey protein, compared with slowly digested micellar casein, results in a rapid transient aminoacidemia of greater amplitude than does a gradual prolonged aminoacidemia with casein (18). These stereotypical patterns of aminoacidemia have profound effects on whole-body protein turnover (18, 19), but far less is known about what happens in skeletal muscle, particularly after exercise.

Exercise-induced rates of muscle protein synthesis have been reported to be greater after ingestion of whey than after ingestion of casein during postexercise recovery (12), which may be stimulated by greater increases in blood leucine and other EAA concentrations that occur in close temporal proximity to the exercise bout; however, to our knowledge, this thesis remains untested. We propose that a rapid acute rise in postprandial circulating EAA, or leucinemia, is important for elevations in rates of muscle protein synthesis in response to food at rest and after resistance exercise (2, 12). Other lines of evidence support this thesis. For example, the enzymatic hydrolysis of casein protein, to yield more rapidly digested peptides, was shown (20) to yield a more pronounced aminoacidemia, which tended to elevate rates of mixed muscle protein synthesis at rest. With the use of the same protein source, these authors eliminated the

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[5] Abbreviations used: 4E-BP1, eukaryotic inititiation factor 4E binding protein 1; Akt, protein kinase B; BCAA, branched-chain amino acid; BOLUS, whey protein consumed as a single bolus; EAA, essential amino acid; eEF2, eukaryotic elongation factor 2; FSR, fractional synthetic rate; MPS, myofibrillar protein synthesis; mTOR, mammalian target of rapamycin; PRAS40, proline-rich Akt substrate of 40 kDa; PULSE, whey protein consumed as a repeated small pulse; rpS6, ribosomal protein S6; S6K1, 70 kDa ribosomal protein S6 kinase 1.

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confounding effects of differences in the amino acid composition of the protein to affect rates of muscle protein synthesis (20). However, in previous studies (12, 20), only the rate of mixed muscle protein synthesis was measured, and thus we have no knowledge of how the myofibrillar protein fraction is affected, which is a muscle protein fraction that is sensitive to both nutrients and exercise (21).

The aim of the current study was to determine how the pattern of aminoacidemia affected rates of postprandial MPS after resistance exercise. To remove the influence of differing amino acid compositions (22), we used whey protein consumed as a single bolus (BOLUS) or as a repeated small pulse (PULSE) to create a rapid transient aminoacidemia compared with a slower sustained aminoacidemia, respectively. We also examined Akt-mTOR pathway signaling to provide insight into the potential mechanisms for changes in MPS rates during postexercise recovery. We hypothesized that the BOLUS condition and the concomitant rapid aminoacidemia would stimulate greater rates of MPS (12, 20) and be associated with an increased activation of mTOR signaling (13, 23–26) during postexercise recovery than would the PULSE condition.

SUBJECTS AND METHODS

Participants and ethical approval

Eight recreationally active healthy young men [mean ± SEM age: 21.5 ± 1 y; height: 1.81 ± 0.02 m; weight: 80.1 ± 3.5 kg; BMI (in kg/m^2): 24.3 ± 0.8] volunteered to participate in the study. Participants were informed of the purpose of the study, the experimental procedure, and all potential risks involved and gave written consent to participate. The study was approved by the Hamilton Health Sciences Research Ethics Board and conformed to the standards for the use of human subjects in research as outlined in the most recent update of the Declaration of Helsinki as well as to standards established by the Canadian Tri-Council Policy on the ethical use of human subjects (Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, and Social Sciences and Humanities Research Council of Canada Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, 2010).

Experimental design

At least 1 wk before the experiment infusion trials, participants underwent a maximum strength test to determine their bilateral 10 repetition maximum on a standard leg-extension machine (Badger 2001 series; Magnum Fitness Systems). After strength testing, participants were randomly assigned to consume whey protein as either BOLUS or PULSE during infusion trial 1 or 2. The order of these trials (**Figure 1**) was randomized, and the average time period between crossover trials was 30 d (range: 8–72 d). Each participant was provided with a dietary log and instructed to maintain their regular diets and record their dietary intakes for 3 d before the first infusion trial. On completion of infusion trial 1, a copy of the dietary log was returned to participants who were instructed to maintain their previously logged dietary habits in the 3 d leading up to infusion trial 2. Participants were also asked to refrain from vigorous exercise for 2 d before the infusion trials and to eat no later than 2200 on the day before the infusion trials.

Infusion protocol

All participants reported to the laboratory at ~0600 in a 10–12-h postabsorptive state. A catheter was inserted into the hand, and a baseline blood sample was drawn before a 0.9% saline drip was started to keep the catheter patent for repeated blood sampling. A second catheter was placed in the opposite arm for a primed continuous infusion (0.05 μmol · kg^{-1} · min^{-1}; 2.0 μmol/kg prime) of [*ring*-$^{13}C_6$]phenylalanine (Cambridge Isotope Laboratories), which was passed through a 0.2-μm filter. We recently validated a method (27) in which the resting FSR of MPS was calculated from naturally abundant ^{13}C enrichments determined from a baseline preinfusion plasma sample taken from tracer-naive participants, and a single biopsy was taken after a period of tracer incorporation (12, 28–32). This method assumes that the ^{13}C enrichment of a mixed plasma protein fraction reflects the ^{13}C enrichment of the muscle protein (33). To minimize the number of biopsies taken, we obtained a single resting biopsy (trial 1 only) that was used to calculate a baseline rate of MPS. Participants performed an acute bout of resistance exercise that consisted of 8 sets of 8–10 repetitions of a bilateral leg extension (Badger 2001 series; Magnum Fitness Systems) at their previously established 10 repetition maximum with a 2-min rest between sets. After completion of the exercise bout, whey protein drinks were administered as either BOLUS or PULSE (1/10th of BOLUS per 20 min × 10), and biopsies were obtained at 1, 3, and 5 h of postexercise recovery. Arterialized blood samples were drawn from a hand vein that was warmed in a box heated to 60°C (34) every 60 min in the fasted state and

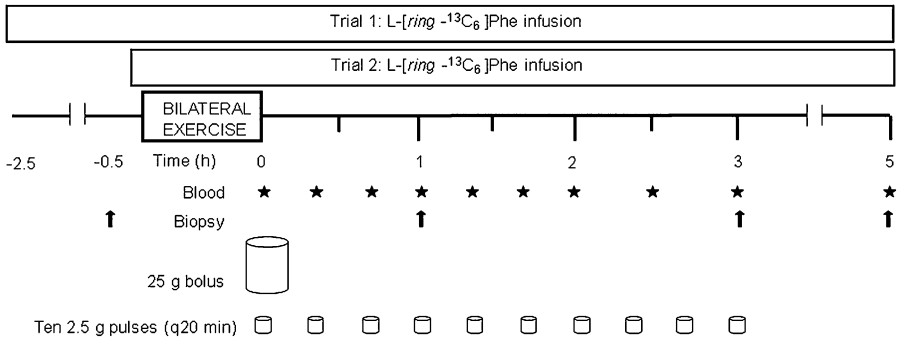

FIGURE 1. Schematic of the experimental protocol. Participants consumed whey protein as either a single bolus (1 × 25 g) or as a series of small pulsed drinks [10 × 2.5 g every 20 min (q20 min)] in a randomized order (n = 8 for both trials in a crossover design). A preexercise biopsy was taken at the first visit only (trial 1). Exercise consisted of 8 sets of 8–10 repetitions of a bilateral leg extension with 2 min of rest between sets. Asterisks indicate blood sample and upward arrows indicate biopsy.

the fed state (Figure 1). During trial 2, the infusion was initiated before commencement of the exercise to ensure the participant would be at an isotopic steady state at the time of the 1-h postexercise biopsy; this state was confirmed by similar plasma and intracellular enrichments during trials 1 and 2 (*see* Results). The exercise workload was matched to trial 1 such that the same load, number of sets, and repetitions were performed. Muscle biopsies were taken from the vastus lateralis with a 5 mm Bergström needle that was modified for manual suction under 2% xylocaine local anesthesia. Biopsy samples were freed from visible blood, fat, and connective tissue and rapidly frozen in liquid nitrogen for additional analyses as previously described (28, 35).

Drink composition

Participants consumed whey protein drinks in a randomized fashion (either trial 1 or 2) as either BOLUS (25-g dose) or PULSE (10 small 2.5-g drinks every 20 min). All drinks were prepared in water with no additives. The 25 g whey protein contained 12.8 g EAAs, 3.5 g leucine, and no carbohydrate or fat (Inbalance Nutrition; for a full listing of the amino acid content, *see* supplemental Table 1 under "Supplemental data" in the online issue). To minimize disturbances in isotopic equilibrium, drinks were enriched to 4% with tracer according to a phenylalanine content of 3.5% in whey protein. We recently validated this method of maintaining am isotopic steady state in the precursor pools (plasma free and muscle intracellular free pools) after protein ingestion and resistance exercise (27).

Analytic methods

After obtaining a blood sample, 100 μL whole blood was added to 0.6 mol perchloric acid/L and centrifuged at 10 000 $\times$ *g* for 3 min. The supernatant fluid was analyzed by HPLC to determine amino acid concentrations as previously described (17). Plasma was separated and collected from the remainder of the blood sample and stored at −20°C for analysis. Plasma insulin was measured with a commercially available immunoassay kit (ALPCO Diagnostics).

Muscle tissue was processed as previously described (21). Briefly, to determine the intracellular enrichment, ~20 mg muscle was homogenized in 0.6 mol perchloric acid/L. Free amino acids in the supernatant fluid were passed over an ion-exchange resin, converted to their heptafluorobutyric derivatives for analysis by using gas chromatography–mass spectrometry (models 6890 GC and 5973 MS; Hewlett-Packard) by monitoring ions 316 and 322 after electron ionization. A separate piece (~40 mg) of muscle was homogenized in a standard buffer that contained protease and phosphatase inhibitors. The supernatant fluid was collected for Western blot analysis, and the pellet was further processed to extract myofibrillar proteins by differential solubility as previously described (21). A mixture of all plasma proteins was extracted in acetonitrile from the preinfusion baseline plasma sample. Myofibrillar and plasma proteins were hydrolyzed overnight in 6 mol HCl/L, purified via an ion-exchange resin (Dowex 50WX8-200; Sigma-Aldrich Ltd), and converted to their *N*-acetyl-*n*-propyl ester derivatives for analysis by using gas chromatography combustion-isotope ratio mass spectrometry (model 6890 GC, Hewlett-Packard; IRMS model Delta Plus XP, Thermo Finnigan).

Changes in signaling protein phosphorylation were analyzed by Western blotting under conditions that were previously described in detail (36, 37). Briefly, cell lysate protein concentrations were determined (Pierce) and used to prepare working samples of equal concentration in Laemmli buffer. Equal amounts (50 μg) of protein were separated by SDS-PAGE and transferred to a polyvinylidene fluoride membrane for antibody incubation. Membranes were blocked in 5% fat-free milk, washed in Tris buffered saline with Tween 20 (0.02%) and incubated in primary antibody (1:1000) overnight at 4°C. Membrane-bound proteins were washed, incubated in a secondary antibody (1:2000), detected with chemiluminescence (Amersham Biosciences; Pierce Biotechnology) and quantified by using densitometry. Antibody details were as follows: Akt^{Ser473} (catalog no. 9271), Akt^{Thr308} (catalog no. 4056), $mTOR^{Ser2448}$ (catalog no. 2971), and ribosomal protein $S6^{Ser235/6}$ (catalog no. 4856) were from Cell Signaling Technology, $PRAS40^{Thr246}$ (catalog no. 05-988) and $p70S6K^{Thr389}$ (catalog no. 04-392) were from Millipore, and α-tubulin (catalogue no. T6074) was from Sigma-Aldrich Ltd. Samples were run within subject with both conditions on the same gel and quantified relative to α-tubulin (control).

Calculations

FSR (%/h) was calculated from [*ring*-$^{13}C_6$]phenylalanine enrichments according to the standard precursor-product equation as follows:

$$\text{FSR} = [(E_{2b} - E_{1b}) \div (E_{IC} \times t)] \times 100 \qquad (1)$$

where E_b is the enrichment of bound (myofibrillar) protein, E_{IC} is the average enrichment of the intracellular free amino acid precursor pool of 2 muscle biopsies, and t is the tracer incorporation time. The use of tracer nave subjects allowed us to use a preinfusion blood sample (ie, a mixed plasma protein fraction) as the baseline enrichment (E_{1b}) for the calculation of the fasted FSR (12, 30, 31).

Statistics

BOLUS and PULSE experimental trials were completed within subject on separate days. Blood amino acids, plasma insulin, MPS, and protein phosphorylation were analyzed by using a 2-factor (time × condition) repeated-measures ANOVA with Tukey's post hoc test. Precursor pool enrichments were analyzed by using a 2-factor (time × condition) repeated-measures ANOVA and linear regression. AUC was analyzed by a paired *t* test. The construction of normal probability plots revealed a better fit to a normal distribution of log-transformed data for phospho Akt^{Ser473}, Akt^{Thr308}, $S6K1^{Ser235/6}$, rps6, and $eEF2^{Thr56}$; therefore, statistical analyses were performed on transformed data for those phosphorylation sites to correct for skewness. All Western blot data were graphed as raw values with arbitrary units. Statistical analyses were performed with SigmaStat 3.1 software (Systat Software Inc). Values are expressed as means ± SEMs, and means were considered to be significantly different at $P < 0.05$.

RESULTS

Blood amino acid and plasma insulin concentrations

Blood EAA concentrations (**Figure 2**A) in BOLUS were greater than in the PULSE group at 60 and 80 min, whereas

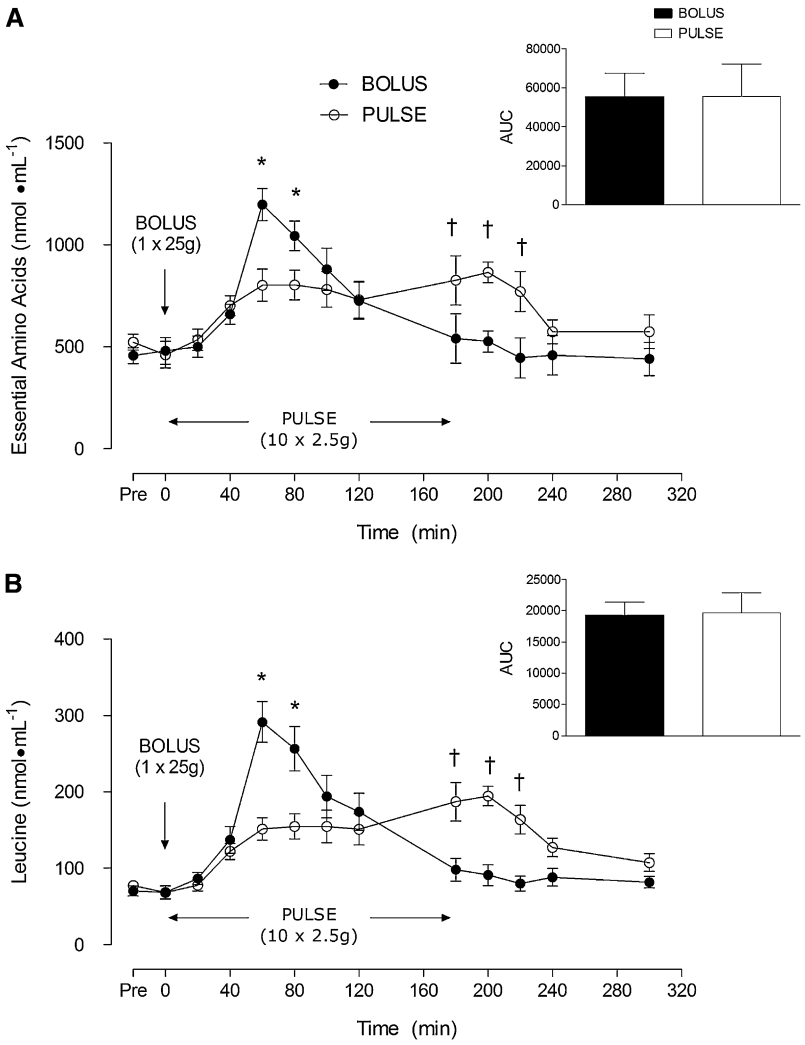

FIGURE 2. Mean (±SEM) blood concentrations of essential amino acids (A) and leucine (B) after ingestion of whey protein as a single bolus (BOLUS; 1 × 25 g) or as a repeated pulse (PULSE; 10 × 2.5 g every 20 min) (n = 8). Inset shows the AUC. *Significantly greater than PULSE, $P < 0.05$; †significantly greater than BOLUS, $P < 0.05$. Data were analyzed by using a 2-factor (time × condition) repeated-measures ANOVA with Tukey's post hoc test (time × condition interactions for A and B, $P < 0.001$). The AUC was analyzed by using a paired t test. Pre, preexercise and prior to protein consumption.

EAA concentration in the PULSE group were greater at 180, 200, and 240 min. The same pattern and differences between conditions occurred for blood leucine concentration (Figure 2B). The AUC for EAA and leucine concentrations was nearly identical (EAA: 99% similarity; leucine: 98% similarity) for the 2 protein ingestion conditions. There was no change in insulin concentrations from baseline in the PULSE group, whereas there was a pronounced rise in the BOLUS group such that insulin concentrations in the BOLUS group were greater than in the PULSE group at 20, 40, and 60 min (*see* supplemental Figure 1 under "Supplemental data" in the online issue).

Plasma and intracellular free phenylalanine enrichments

Plasma and intracellular free phenylalanine enrichments are shown in **Figure 3**, A and B, respectively. Tracer added to drinks did not substantially disturb the plasma pool, and slopes of enrichments over time were not significantly different from zero in BOLUS and PULSE groups (P = 0.38 and 0.12, respectively). Intracellular free phenylalanine enrichment was stable at the 1-, 3-, and 5-h time points (P = 0.76), which confirmed that measurements were made at the isotopic plateau; there were no differences between conditions (P = 0.93). There was no difference between trials 1 and 2 in intracellular free phenylalanine enrichment at the time of the 1-h postexercise biopsy (tracer-to-tracee ratio for trials 1 and 2 were 0.064 ± 0.001 and 0.061 ± 0.004, respectively; P = 0.58).

MPS

Exercise and protein consumption stimulated rates of MPS at 1–3 h (P = 0.026) and 3–5 h ($P < 0.001$) of recovery (**Figure 4**); however, this response was greater after BOLUS than after PULSE at 1–3 h (P = 0.01) and 3–5 h (P = 0.001) of exercise recovery. The aggregate (1–5 h) MPS response to exercise and protein ingestion was elevated above basal rates (P = 0.003) and to a greater extent with BOLUS (P = 0.003).

Muscle anabolic signaling

During BOLUS, there were greater changes in PRAS40^{Thr246}, S6K1^{Thr389}, and rpS6$^{Ser235/6}$ phosphorylation 1 h after exercise

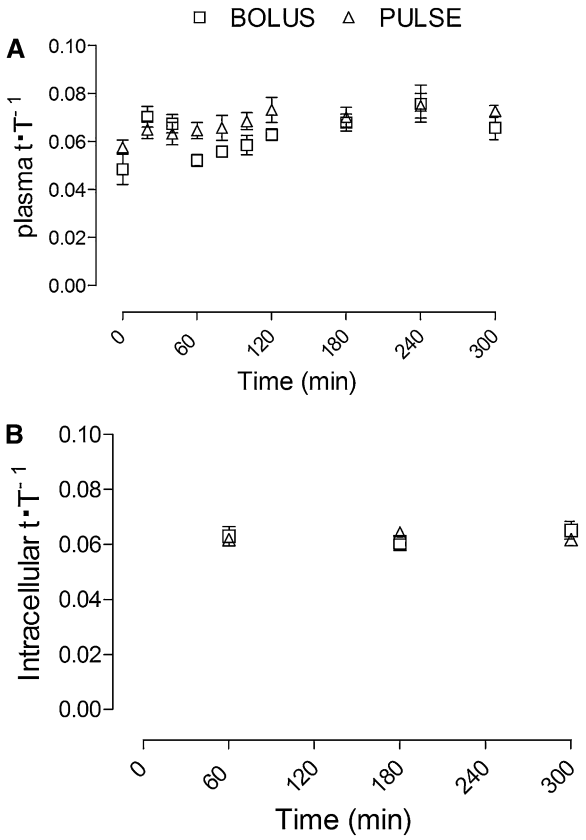

FIGURE 3. Mean (±SEM) plasma (A) and muscle intracellular free (B) phenylalanine enrichment [tracer-to-tracee ratio ($t \cdot T^{-1}$)] (n = 8). Time = 0 denotes the end of the exercise and the start of consumption. Data were analyzed by using linear regression (A and B: $P > 0.05$ for the difference of the linear regression slope from zero) and 2-factor (time × condition) repeated-measures ANOVA (B: main effect of time, P = 0.76; time × condition interaction, P = 0.15).

than after PULSE (**Figures 5** and **6**), whereas PULSE reduced $eEF2^{Thr56}$ phosphorylation (which indicated increased activation) at the same time point. There were no differences between BOLUS and PULSE groups for Akt^{Thr308}, Akt^{Ser473}, $mTOR^{Ser2448}$, or $4EBP1^{Thr37/46}$ phosphorylation (Figures 5 and 6).

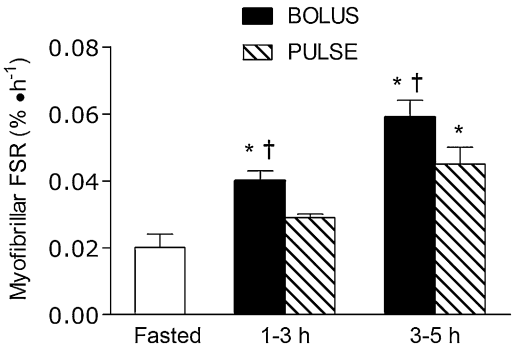

FIGURE 4. Mean (±SEM) myofibrillar protein synthesis [fractional synthetic rate (FSR)] in the fasted state (Fasted) and after a protein bolus (BOLUS; 1 × 25 g) and protein pulses (PULSE; 10 × 2.5 g every 20 min) after resistance exercise (n = 8). Data were analyzed by using a 2-factor (time × condition) repeated measures ANOVA with Tukey's post hoc test (time × condition interaction: P = 0.066). *Significantly greater than Fasted, $P < 0.05$; †significantly greater than PULSE at the same time point, $P < 0.05$.

DISCUSSION

To our knowledge, this was the first study to systematically manipulate the pattern of aminoacidemia by using a single protein source consumed by subjects after resistance exercise. In doing so, we eliminated the possibility of the amino acid content of the protein as a confounding factor. We showed that a rapid and pronounced hyperaminoacidemia (BOLUS) early postexercise stimulated MPS to a greater extent than a gradual and prolonged aminoacidemia (PULSE), which mimicked a slowly absorbed protein. In stark contrast to data from oral protein trials (38, 39), BOLUS stimulated greater rates of MPS in the 3–5-h period postexercise although blood amino acid concentrations had returned to basal amounts. This finding highlighted the potent effect that resistance exercise had in sustaining an elevation in MPS with protein consumption. The net exposure to amino acids (total, EAA, BCAA, and leucine) was identical between trials, which indicated that the pattern of aminoacidemia, and not the net amino acid exposure or total protein consumption, was the variable that defined our results.

Multiple lines of evidence suggested a primary role for leucine as an amino acid in stimulating muscle protein synthesis (40). We provided data (12) in the postexercise period in support of the hypothesis that blood leucinemia was important in maximizing the protein consumption–mediated rates of MPS (2); other authors had similar data with food consumption alone (41). Specifically, whey protein ingestion induced a rapid aminoacidemia and leucinemia postexercise that led to greater rates of muscle protein synthesis at rest and after resistance exercise (12). In contrast, a bolus dose of more slowly digested micellar casein protein, or soy protein, which was digested at a similar rate as whey but contained less leucine (12), stimulated MPS to a lesser degree at rest and postexercise. In addition, we observed a protein dose-response relation in muscle protein synthesis rates after resistance exercise that reached a plateau at 20 g (16). Collectively, these data (12, 16) suggested that, similar to amino acid infusions at rest (14), increases in blood EAA, BCAA, or leucine concentrations from dietary protein ingestion induced a graded MPS response that was based on a signal that is related to peak aminoacidemia or peak leucinemia after resistance exercise but that is also clearly saturable.

An intriguing and important divergence between our findings and reports in which aminoacidemia resulted in only a transient rise in MPS with infusion of amino acids (42) or with amino acid consumption (38, 39) is that our results were postexercise. It appears that a unique aspect of resistance exercise is to selectively sustain elevated synthetic rates of myofibrillar proteins after protein consumption (21). In contrast to the effects of protein consumption alone at rest, the current results and our earlier work (21) showed that the highest rates of MPS were observed at 3–5 h postexercise when aminoacidemia had subsided. It has not been determined when the prolonged elevation of MPS rates after resistance exercise and protein ingestion returns to baseline (preexercise); however, this response lasts much longer than the response to protein ingestion alone (38, 39, 42). Such a finding has important implications for eating patterns and/or protein choice in the postexercise period to maximize an anabolic

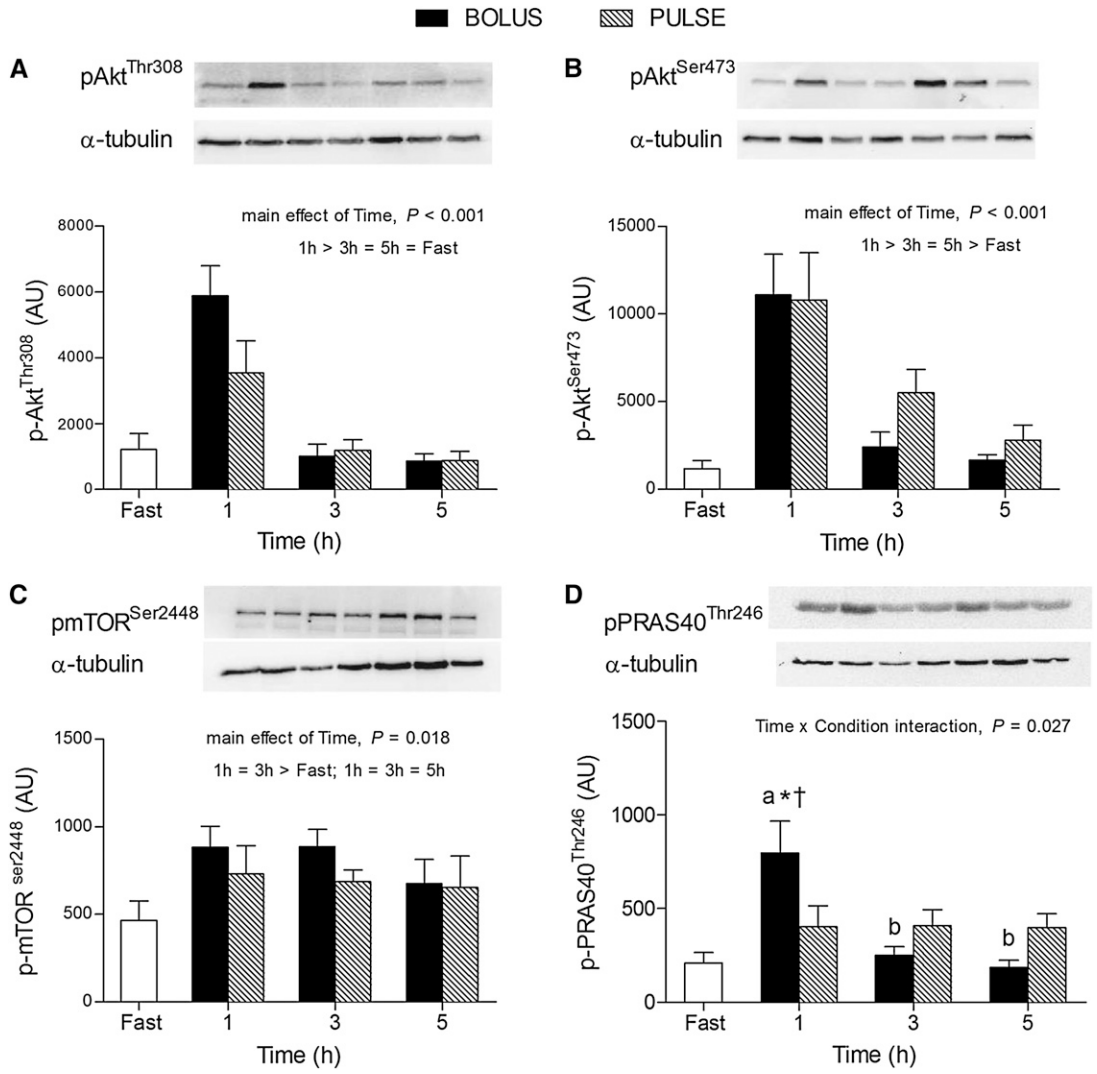

FIGURE 5. Mean (±SEM) phosphorylation of protein kinase B (p-Akt)^{Thr308} (A), p-Akt^{Ser473} (B), mammalian target of rapamycin (p-mTOR)^{Ser2448} (C), and phospho proline-rich Akt substrate of 40 kDa (p-PRAS40)^{Thr246} (D) in the rested-fasted state and after ingestion of whey protein as a single bolus (BOLUS; 1 × 25 g) or as a repeated pulse (PULSE; 10 × 2.5 g every 20 min) after resistance exercise (n = 8). Values are expressed relative to α-tubulin and presented in arbitrary units (AU). Phospho-protein bands on blots were ordered as follows: rested-fasted; 1, 3, and 5 h in trial 1; and 1, 3, and 5 h in trial 2, with the trial order counterbalanced for BOLUS and PULSE. Data were analyzed by using a 2-factor (time × condition) repeated-measures ANOVA with Tukey's post hoc test (time × condition interactions for A, B, C, and D: P = 0.098, 0.21, 0.89, and 0.027, respectively). *Different from Fast, $P < 0.05$; †different from PULSE at the same time point, $P < 0.01$. Times with different letters were significantly different from each other within the same condition, $P < 0.05$.

response that could be advantageous to maximize muscle maintenance or growth over the longer term.

The mechanisms that underpin the synergistic anabolic relation between amino acids and resistance exercise on muscle protein synthesis appeared to involve the independent and/or additive contraction- and nutrient-mediated activation of the Akt-mTOR pathway. We observed a prominent phosphorylation increase of upstream effectors (Akt^{Thr308} and PRAS40^{Thr246}) and downstream targets (S6K1^{Thr389} and rpS6$^{Ser235/6}$) of mTOR. Notably, the phosphorylation of S6K1 and rps6 were increased to a greater extent at 1 h postexercise after BOLUS than after PULSE, which was consistent with a greater acute stimulation of protein synthesis. However, changes in signaling, which reflected effects of exercise and protein consumption combined, were not enhanced after BOLUS in the case of eEF2 phosphorylation, which tended to be lower after PULSE at 1 h. Overall, our observation that greater rates of MPS after BOLUS were associated with an enhanced intramuscular signal for translation initiation supported the notion that acute signaling proteins in the Akt-mTOR pathway can be reflective of an anabolic response in skeletal muscle with resistance exercise (26, 43–45). Indeed, acute changes in mTOR signaling phosphorylation (Akt, p70S6K, and rps6) were significantly correlated with changes in MPS (data not shown).

We observed a differential stimulation of MPS between BOLUS and PULSE conditions, even though subjects ingested equal amounts of total protein, which resulted in an identical net exposure to EAAs during postexercise recovery. These data have a number of practical implications. First, the supply if the bulk of the amino acids immediately after exercise, as opposed to a slower delivery such as with small divided doses, appeared to be more beneficial to support muscle anabolism and presumably long-term muscle protein accretion. The consumption of slower digested proteins (ie, micellar casein) or use of large quantities of

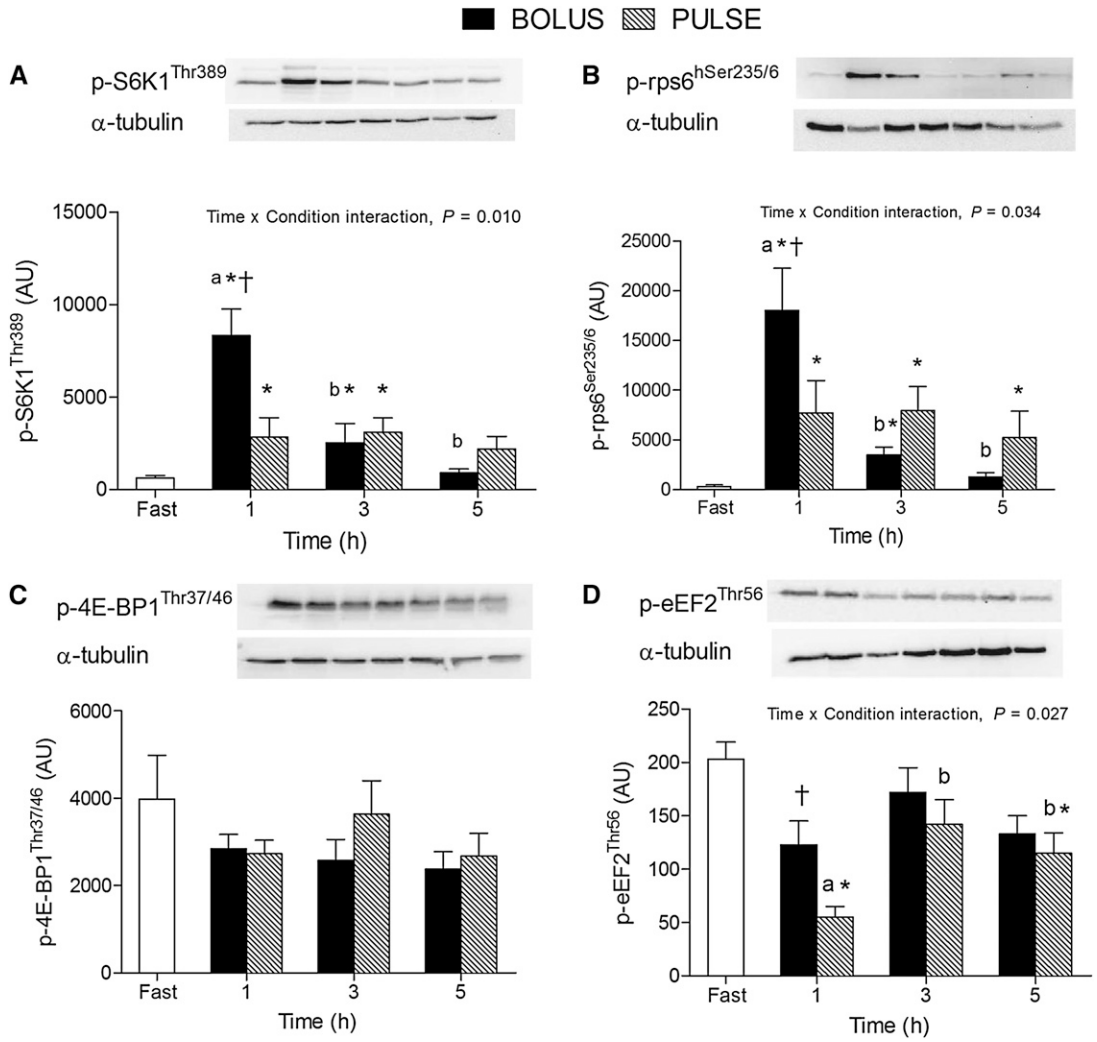

FIGURE 6. Mean (±SEM) 70-kDa ribosomal protein S6 kinase 1 (p-S6K1)^{Thr389} (A), ribosomal protein S6 (p-rps6)$^{hSer235/6}$ (B), eukaryotic inititiation factor 4E binding protein 1 (p-4E-BP1)$^{Thr37/46}$ (C), and eukaryotic elongation factor 2 (p-eEF2)^{Thr56} (D) phosphorylation in the rested-fasted state and after ingestion of whey protein as a single bolus (BOLUS; 1 × 25 g) or as a repeated pulse (PULSE; 10 × 2.5 g every 20 min) after resistance exercise ($n = 8$). Values are expressed relative to α-tubulin and presented in arbitrary units (AU). Phospho-protein bands on blots were ordered as follows: rest-fasted; 1, 3, and 5 h in trial 1; and 1, 3, 5 h in trial 2, with the trial order counterbalanced for BOLUS and PULSE. Data were analyzed by using a 2-factor (time × condition) repeated-measures ANOVA with Tukey's post hoc test (time × condition interactions for A, B, C, D: $P = 0.010$, 0.034, 0.73, and 0.027, respectively). *Different from Fast, $P < 0.05$; †different from PULSE at the same time point, $P < 0.05$. Times with different letters were significantly different from each other within the same condition, $P < 0.05$.

fat and/or carbohydrates, which would slow gastric emptying and protein absorption, would also likely reduce the rates of MPS during postexercise recovery. However, if a threshold of leucine needs to be exceeded to stimulate maximal oral protein–induced and/or exercise-induced rates of MPS (2, 11, 12), then the consumption of large quantities of slowly digested proteins will result in a leucinemia that will be sufficient to trigger an increase in MPS. Nonetheless, our data suggested that the ingestion of proteins that are more rapidly digested, which resulted in a pronounced aminoacidemia postexercise, were more likely to stimulate a greater rise in muscle protein synthesis. We speculated that, over time, the habitual practice of consuming rapidly digested proteins after resistance exercise would provide an anabolic advantage that leads to greater hypertrophy, which is a view that has support from longer-term supplementation trials (46). Data from the present study may also have applications to elderly populations (41) in that the higher blood leucine concentrations generated by a bolus of whey protein could be induced to overcome a reduced anabolic sensitivity to protein consumption (15, 47). An interesting question with respect to postexercise nutritional strategies to maximize muscle accretion is at what time someone could consume a second bolus of protein after resistance exercise to restimulate MPS because MPS becomes insensitive to frequent consumption (39, 42). Research is needed to determine the time at which skeletal muscle regains sensitivity to another protein dose (39) to maximize the repeated stimulation of MPS by protein consumption after resistance exercise throughout the rest of the day.

In conclusion, we report that BOLUS after resistance exercise is more effective in stimulating MPS than is PULSE. Our model allowed us to specifically address the question at the level of the muscle and eliminated limitations of previous studies in which observations of differences in rates of protein synthesis to intakes of different dietary proteins in combination with exercise were

ascribed to differences in rates of protein digestion. The greater MPS response after BOLUS was associated with greater acute phosphorylation of anabolic signaling proteins that regulate translation initiation. The rapid rise in extracellular EAA concentrations, or possibly of leucine alone (2, 11, 12), that occurred after BOLUS appears to underpin the greater signal activation and protein synthetic response that are observed after an acute bout of resistance exercise.

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