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# **Preexercise Aminoacidemia and Muscle Protein Synthesis after Resistance Exercise**

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<sup>1</sup>Sports Nutrition, Australian Institute of Sport, Canberra, ACT, AUSTRALIA; <sup>2</sup>Health Innovations Research Institute, School of Medical Sciences, RMIT University, Victoria, AUSTRALIA; <sup>3</sup>Nestlé Research Center, Nestec Ltd., Lausanne, SWITZERLAND; <sup>4</sup>Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, Ontario, CANADA; <sup>5</sup>School of Sport, University of Stirling, Stirling, SCOTLAND; and <sup>6</sup>School of Health Sciences, Deakin University, Burwood, Victoria, AUSTRALIA

#### ABSTRACT

BURKE, L. M., J. A. HAWLEY, M. L. ROSS, D. R. MOORE, S. M. PHILLIPS, G. R. SLATER, T. STELLINGWERFF, K. D. TIPTON, A. P. GARNHAM, and V. G. COFFEY. Preexercise Aminoacidemia and Muscle Protein Synthesis after Resistance Exercise. Med. Sci. Sports Exerc., Vol. 44, No. 10, pp. 1968–1977, 2012. Purpose: We have previously shown that the aminoacidemia caused by the consumption of a rapidly digested protein after resistance exercise enhances muscle protein synthesis (MPS) more than the amino acid (AA) profile associated with a slowly digested protein. Here, we investigated whether differential feeding patterns of a whey protein mixture commencing before exercise affect postexercise intracellular signaling and MPS. Methods: Twelve resistance-trained males performed leg resistance exercise 45 min after commencing each of three volume-matched nutrition protocols: placebo (PLAC, artificially sweetened water), BOLUS (25 g of whey protein + 5 g of leucine dissolved in artificially sweetened water;  $1 \times 500$  mL), or PULSE ( $15 \times 33$ -mL aliquots of BOLUS drink every 15 min). Results: The preexercise rise in plasma AA concentration with PULSE was attenuated compared with BOLUS (P < 0.05); this effect was reversed after exercise, with two-fold greater leucine concentrations in PULSE compared with BOLUS (P < 0.05). One-hour postexercise, phosphorylation of p70 S6K<sup>thr389</sup> and rpS6<sup>ser235/6</sup> was increased above baseline with BOLUS and PULSE, but not PLAC (P < 0.05); furthermore, PULSE > BOLUS (P < 0.05). MPS throughout 5 h of recovery was higher with protein ingestion compared with PLAC (0.037 ± 0.007), with no differences between BOLUS or PULSE (0.085  $\pm$  0.013 vs. 0.095  $\pm$  0.010% h<sup>-1</sup>, respectively, P = 0.56). Conclusions: Manipulation of aminoacidemia before resistance exercise via different patterns of intake of protein altered plasma AA profiles and postexercise intracellular signaling. However, there was no difference in the enhancement of the muscle protein synthetic response after exercise. Protein sources producing a slow AA release, when consumed before resistance exercise in sufficient amounts, are as effective as rapidly digested proteins in promoting postexercise MPS. Key Words: AMINO ACID DELIVERY, FAST AND SLOW PROTEINS, MUSCLE PROTEIN SYNTHESIS, LEUCINE

The combination of resistance exercise and increased amino acid (AA) availability is a potent stimulator of muscle protein synthesis (MPS) (5,28). This occurs via two general mechanisms: an increase in blood flow enhancing the delivery of AA for protein synthesis and the stimulation of signaling pathways that initiate mRNA translation. Changes in blood AA concentrations (6), in particular, leucine (15,16,25), are suggested to be a primary mechanism for increased muscle anabolism. This has led

0195-9131/12/4410-1968/0 MEDICINE & SCIENCE IN SPORTS & EXERCISE® Copyright © 2012 by the American College of Sports Medicine DOI: 10.1249/MSS.0b013e31825d28fa to considerable interest in determining the optimal feeding strategy to maximally enhance MPS after resistance exercise, with variables including the amount, type, and timing of intake of dietary protein sources (8).

The effect of consuming a dietary protein on plasma AA concentrations is characterized by its AA composition as well as the digestibility of the food or meal in which it was consumed (9,10). There is, however, some possible interplay between all of these variables that could be manipulated to produce optimal plasma AA responses. For example, different plasma AA patterns are produced by the consumption of the so-called fast or slow dietary proteins (7,12,13) but can also be manipulated by changing the timing and pattern of intake of a protein source (31) or the form (liquid vs. solid) in which it is eaten (10). If a specific threshold of leucine concentration is needed to stimulate maximal MPS (8,25), there may be several ways to achieve this from dietary intake: rapid achievement after the consumption of a "fast-digesting" leucine-rich protein or a slower achievement

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via the intake of a larger amount of a protein that is lower in leucine or more slowly digested, to compensate for a flatter AA profile. Full investigation of protein feeding strategies to maximize the response to resistance exercise requires research methodologies that can systematically isolate or combine different variables that affect plasma AA concentrations.

We have recently investigated the importance of the pattern of aminoacidemia associated with the intake of highquality proteins after resistance training on MPS. In that study (31), we systematically manipulated postexercise AA concentrations by feeding a single rapidly digested protein source, either as a large bolus to produce rapid and pronounced hyperaminoacidemia or as a series of small "pulsed" feedings that produced a gradual and prolonged aminoacidemia mimicking a slowly absorbed protein. This protocol was stimulated by the results of previous studies that have found different MPS after intake of slow (casein) and fast (whey) proteins at rest (27), but by eliminating the AA content of a protein as a confounding factor, we were able to focus on the pattern of aminoacidemia and the effects on MPS. Although our feeding protocols produced an identical net exposure to AA (total, essential, branched chain and leucine), the bolus condition, which elicited a pronounced aminoacidemia in the early postexercise period, was associated with a greater MPS for the 5 h of recovery (31). These results confirm that the best protein source for consumption soon after resistance exercise is the one that is quickly digested and able to promote a rapid increase in plasma leucine concentrations.

In real life, however, issues of poor appetite or food access may prevent an individual from consuming adequate protein sources after a resistance exercise session. In fact, guidelines to consume protein in close temporal relationship to resistance exercise include options to consume protein before the exercise session (8,30). In these circumstances, the choice of a slowly digested high-quality protein might not attenuate the response to an exercise stimulus if it allows the resultant increase aminoacidemia to better coincide with the postexercise period. The achievement of high plasma leucine concentrations might be further assisted by consuming a protein dose slightly higher than the previously determined optimal dose of 20 g of high-quality protein (24) and by ensuring a high content of leucine (232).

Accordingly, the aim of this study was to investigate the effects of manipulating patterns of aminoacidemia from protein sources consumed before a bout of resistance exercise bout. We used our successful model (31) whereby bolus and pulsed feedings of a rapidly digested high-quality protein source were consumed to simulate the aminoacidemia that would be achieved by consuming "fast" or "slow" protein foods before the exercise session. We hypothesized that this manipulation would achieve different patterns of aminoacidemia before, during, and after exercise, but that in these circumstances, the delayed response of the pulse feedings would be at least as good as the bolus protein intake in enhancing intracellular signaling and promoting greater rate of MPS after resistance exercise.

# METHODS

#### **Study Overview**

The protocol involved a double-blinded placebocontrolled design in which each subject undertook three trials in a randomized counterbalanced order with a 1- to 2-wk washout between trials. The study was undertaken at the Australian Institute of Sport and carried approval from its Human Ethics Committee. Subjects were informed of all experimental procedures and associated risks before their written informed consent was obtained. Each trial involved a session of intense single-leg resistance exercise  $(10 \times 8 - 10)$ repetitions of leg extension @ 80% 1 repetition maximum (RM)) with the contralateral leg being used in each subsequent trial and the starting order of legs being randomized between subjects. Muscle samples were collected at rest and 1 and 5 h after the resistance exercise bout for measurement of an array of signaling proteins involved in protein translation and to determine the fractional synthetic rate (FSR) of mixed muscle protein after exercise.

The dietary interventions consisted of a series of drinks commencing 45 min before the start of a resistance exercise bout (Fig. 1). The three treatments were placebo drink (PLAC), 30 g of protein representing a saturating protein dose (24) rich in leucine (25 g of whey protein plus 5 g of leucine) consumed in a single feeding (BOLUS) to simulate a fast protein, and the same protein consumed as a series of 15 small drinks (approximately 33 mL each) to simulate the absorption of a slow protein (PULSE). In anticipation of the flattened AA patterns with PULSE feeding and the potential for a leucine threshold, we provided the additional leucine in the drinks; indeed, others have reported benefits in adding leucine to a saturating dose of protein (21). Blood samples (5–10 mL) were collected throughout each trial to determine plasma AA concentrations.

#### **Subjects and Pretrial Preparation**

We recruited 12 resistance-trained males (mean  $\pm$  SEM; age, 27  $\pm$  1.3 yr; mass, 94.3  $\pm$  4.6 kg; 1 RM leg extension for single leg: 42.8  $\pm$  2.43 kg or 45.9%  $\pm$  2.3% body mass (BM)) with greater than 2 yr of experience of regular (at least twice per week) strength training. At least 1 wk before commencing the study, all subjects reported to the laboratory for measurement of BM and leg strength. A leg strength test was undertaken on a platinum leg extension machine (model P5018, Maxim Strength Fitness Equipment, Australia) to determine the 1 RM of each leg independently.

Subjects performed a standardized warm-up on the leg extension machine ( $6 \times 70\%$  perceived 1 RM;  $4 \times 80\%$  perceived 1 RM;  $2 \times 90\%$  perceived 1 RM with 3-min

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FIGURE 1—Schematic overview of study design.

recovery). Each subject was given six lifting attempts to achieve their 1 RM. A repetition was considered valid when the weight was lifted to a knee angle corresponding to full leg extension and then returned to its resting position of  $90^{\circ}$  of knee flexion. Subjects were instructed to rest for at least 3 min between repetitions to ensure a true maximal lift was achieved. After each successful lift, the load was increased by 2.5–5 kg until failure to complete one repetition. Subjects were given a maximum of two attempts to lift the weight, and the greatest amount of weight lifted successfully was recorded for both left and right legs. They were then familiarized with the resistance exercise protocol that would be performed during trials.

#### **Experimental Procedure**

Subjects reported to the laboratory for each trial in an overnight fasted state (10 h) having refrained from alcohol and resistance exercise for 48 h and caffeine and any other exercise for 24 h. For the 24 h before each trial, subjects were provided with a prepackaged diet with an energy content of 160 kJ·kg  $BM^{-1}$  composed of 4 g·kg  $BM^{-1}$  CHO (45% energy), 1.5 g·kg  $BM^{-1}$  fat (34% energy), and 2 g·kg  $BM^{-1}$  protein (21% energy). Compliance to the prescribed diet and standardized exercise instructions was determined from a food and exercise checklist. Subjects ingested only the prescribed protein for the trial on the trial day until completion of the experimental trial.

After subjects had rested quietly in a supine position for 10 min, an indwelling cannula was placed in a vein in the back of the hand, a resting blood sample (5–10 mL) was taken, and a 0.9% saline drip was attached to keep the line patent for the serial collection of blood samples as described below. A second cannula was placed in the vein of the opposite forearm, and a primed (2  $\mu$ mol·kg<sup>-1</sup>) continuous (0.05  $\mu$ mol·kg<sup>-1</sup>·min<sup>-1</sup>) infusion of L-[ring<sup>-13</sup>C<sub>6</sub>]-phenylalanine (CK Gas Products Ltd., England, UK) was commenced and continued until the end of the trial.

Sixty minutes after the start of the tracer infusion, a resting percutaneous muscle biopsy was taken using sterile techniques from the *vastus lateralis* of the leg that would subsequently undertake exercise in that day's trial. Local anesthesia (2–3 mL of 1% Xylocaine) was administered to the skin, subcutaneous tissue, and fascia of the *vastus lateralis* before the biopsy. Two further sites on the same leg, approximately 3 cm distal to the previous incision, were prepared for subsequent biopsies in the same trial. The muscle sample (approximately 100 mg) was collected by a medical practitioner using a 5-mm Bergstrom needle with modified suction. The sample was briefly blotted to remove excess blood and immediately frozen in liquid nitrogen before being stored at  $-80^{\circ}$ C until analysis was undertaken.

After the first muscle biopsy, subjects commenced the feeding protocol, which consisted of a series of drinks matched for color, flavor (artificially sweetened with Splenda<sup>®</sup>), and the volume of fluid (500 mL at first time point, followed

by  $14 \times 33$  mL of drinks, which were consumed at 15-min intervals thereafter). The treatments varied according to the absence or presence of high-quality protein plus leucine dissolved in the drinks. Beverage products were provided for all experimental conditions (Nestec, Lausanne, Switzerland). During BOLUS, 25 g of whey protein + 5 g of leucine was dissolved in the first (500 mL) feeding to provide rapid and maximal AA delivery to the muscle; thereafter, the 33-mL drinks consisted of an artificially sweetened placebo. During PULSE, ingestion of the 25 g of whey protein + 5 g of leucine was divided into equal doses across each of the feedings in an effort to maintain a steady supply of AAs. All placebo (PLAC) drinks consisted of artificially sweetened water.

A standard bout of single-leg resistance exercise was commenced 45 min after the consumption of the first feeding. This timing was chosen to allow the predicted peak leucine concentrations with the BOLUS trial to coincide with the start of exercise. This bout consisted of a standardized warm-up, followed by 10 sets of 8–10 repetitions of leg extension at a workload equivalent to 80% of the specific leg 1 RM with 2-min recovery between sets. The leg that performed exercise was alternated for each trial. The duration of the resistance bout was approximately 45 min. Following the completion of the resistance bout, subjects rested for 5 h. Further muscle samples were collected from the *vastus lateralis* of the exercised leg after 1 and 5 h of recovery.

#### **Blood Samples and Analysis**

All blood samples (5–10 mL) were arterialized by warming the hand with a heating blanket (50°C). The sampling protocol involved a resting sample at the commencement of the trial, one immediately before the start of the first feeding and all subsequent samples at 30- to 90-min intervals thereafter (Fig. 1). Samples were collected into lithium heparinized tubes and were centrifuged for 5 min at 4000 rpm to allow the collection of plasma. The plasma was stored at  $-20^{\circ}$ C until analysis.

Plasma insulin concentrations were determined using a solid phase, two-site chemiluminescent immunometric assay (Immulite; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Concentrations of free AAs (phenylalanine, leucine, and threonine) were determined by gas chromatography–mass spectrometry (GCMS) (Hewlett-Packard 5973, Palo Alto, CA) using an internal standard solution as previously described (27). Plasma <sup>13</sup>C<sub>6</sub> phenylalanine enrichments from arterialized blood samples were determined by GCMS (Hewlett-Packard 5973) as previously described (27).

#### **Muscle Samples—Signaling Proteins**

**Western blots.** We determined *a priori* from our previous work (31) that a sample size of eight subjects would provide sufficient power for determination of differences in muscle signaling proteins. Muscle samples were homogenized in buffer containing 50 mM Tris-HCl, pH 7.5, 1 mM ethylenediaminetetraacetic acid, 1mM Ethylene glycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid, 10% glycerol, 1% triton-X, 50 mM NaF, 5 mM Na pyrophosphate, 1 mM dithiothreitol 10  $\mu$ g·mL<sup>-1</sup> trypsin inhibitor, 2  $\mu$ g·mL<sup>-</sup> aprotinin, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride. After determination of protein concentration (Pierce Biotechnology, Rockford, IL), lysate was resuspended in Laemmli sample buffer, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride membranes blocked with 5% nonfat milk, washed with Tris-buffered saline and Tween (10 mM Tris-HCl, 100 mM NaCl, 0.02% Tween 20), and incubated with primary antibody (1:1000) overnight at 4°C. Membranes were incubated with secondary antibody (1:2000), and proteins were detected via chemiluminescence (Amersham Biosciences, Buckinghamshire, UK; Pierce Biotechnology) and quantified by densitometry. All samples (approximately 40  $\mu$ g) for each individual subject were run on the same gel and quantified relative to alpha tubulin protein. Polyclonal anti-phospho-mammalian target of rapamycin (mTOR) ser2448 (#2971), -p70 S6K thr421/ser424 (#9204), -eEF2K ser366 (#3691); monoclonal anti-phospho-Akt ser473 (#9271), -tuberin (TSC2) thr1462 (#3617), -S6 ribosomal protein ser235/6 (#4856), and -p70 S6K thr389 (#9206) were from Cell Signaling Technology (Danvers, MA). Anti-phospho-PRAS40 thr246 was from Millipore (#04-392; Temecula, CA), and monoclonal anti- $\alpha$ -tubulin control protein antibody was from Sigma-Aldrich (#T6074; St Louis, MO).

## FSR of Mixed Muscle Protein

Muscle biopsy tissue samples collected after 1 and 5 h of recovery were analyzed for mixed protein-bound (gas chromatography–combustion isotope ratio mass spectrometry) and free intracellular AA enrichment (GCMS) as previously described (27,28). Mixed muscle protein FSR was calculated from the determination of the rate of tracer incorporation into muscle protein using the muscle free intracellular enrichment as a precursor (27,28).

FSR  $(\% h^{-1}) = (EM2 - EM1)/(EPt) \times 60 \times 100$ , where EM1 and EM2 are the enrichments of the protein-bound L-[ring<sup>-1</sup>3C6]phenylalanine at the start (1 h) and end (5 h) of the sampling period, respectively, EP is the average intracellular L-[ring<sup>-1</sup>3C6]phenylalanine enrichment over the incorporation period, and *t* is the time in minutes.

Although we had planned to increase the statistical power of the study by undertaking muscle FSR measurements in 12 subjects, because of inadequate muscle sample sizes, data were achieved for n = 6, 10, and 11 for PLAC, PULSE, and BOLUS, respectively.

## **Data Analysis**

The data for plasma concentrations of AA and insulin (n = 12 subjects) and muscle cell signaling (n = 8) were



FIGURE 2—Plasma concentrations of selected AAs: phenylalanine (A), leucine (B), and threonine (C) at rest, throughout a bout of resistance exercise and for 5 h of recovery in response to a placebo feeding (PLAC), or the intake of 30-g high-quality protein fed as a bolus (BOLUS) or in a series of small feedings (PULSE). Values are mean  $\pm$  SEM (n = 12). \*, BOLUS different versus rest; #, PULSE different versus rest;  $\alpha$ , BOLUS different versus PLAC;  $\beta$ , BOLUS different versus PULSE;  $\chi$ , PULSE different versus PLAC (P < 0.05).

analyzed using a two-way (time  $\times$  trial) repeated-measures ANOVA using Statistica for Microsoft Windows (Version 6.0) (Microsoft Corporation, Redmond, WA). A Newman–Keuls *post hoc* analysis was performed to establish differences between treatments and across time. Pearson linear regression analysis was used to determine any potential linear relationship between plasma leucine area under the curve and phosphorylation of p70 S6K from muscle samples collected at 1 h of recovery. A single-factor ANOVA for independent samples was used to test for differences in estimates of FSR between trials. Statistical significance for these measures was established at the level of P < 0.05. All values are expressed as mean ± SEM.

# RESULTS

#### **Plasma AA and Insulin Concentrations**

Plasma concentrations of selected AAs (phenylalanine, leucine, and threonine) and insulin across the feeding, exercise, and recovery protocol are shown in Figures 2 and 3, respectively. Protein feedings were associated with an elevation in plasma AA concentrations, with BOLUS typically showing a significant increase in AA concentration immediately after the bolus feeding before the resistance bout but a return toward resting levels in the early phase of post-exercise recovery. By contrast, there was a smaller increase in AA concentrations after the commencement of PULSE. Thereafter, plasma concentrations were sustained and even increased after the cessation of the resistance bout in PULSE such that they were higher than the corresponding values from both BOLUS and PLAC in the early phase of recovery (P < 0.05).

In BOLUS, leucine concentrations were increased by the bolus feeding and remained above resting values and the corresponding values in the PLAC trial from the commencement of exercise until 0 and 1 h of recovery, respectively (P < 0.05), before returning to baseline. By contrast, in PULSE, there was a smaller but significant increase in plasma leucine with the commencement of feedings before exercise, which was sustained above resting and PLAC concentrations during the first half of the resistance bout. After the completion of the bout, there was a further increase in leucine concentrations in PULSE such that values were sustained at higher concentrations for the first 2 h of



FIGURE 3—Plasma insulin concentrations at rest, throughout a bout of resistance exercise and for 5 h of recovery in response to a placebo feeding (PLAC), or the intake of 30-g high-quality protein fed as a bolus (BOLUS) or in a series of small feedings (PULSE). Values are mean  $\pm$  SEM (n = 12). \*, BOLUS diff versus rest;  $\alpha$ , BOLUS diff versus PLAC;  $\beta$ , BOLUS diff versus PULSE (P < 0.05).

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recovery than that in BOLUS and PLAC (P < 0.05). Resistance exercise was associated with small rises in plasma insulin concentrations in all trials; the combination with protein feeding in the BOLUS trial resulted in a brief period of significantly elevated insulin concentrations at the onset of the resistance bout.

#### Cell Signaling

Akt-TSC2. Phosphorylation of Akt ser473 was elevated above rest for each trial after 1 h of recovery from resistance exercise (P < 0.05, Fig. 4A). Specifically, Akt phosphorylation increased approximately 225% in BOLUS, approximately 330% in PULSE, and approximately 135% in PLAC trials (P < 0.05). The largest magnitude of increase was observed in PULSE at the 1-h postexercise time point and was greater than either BOLUS or PLAC trials (P < 0.05). Moreover, although peak Akt phosphorylation dissipated 5 h postexercise, the increase above rest was sustained with BOLUS but not PULSE and PLAC (P < 0.05). There were modest changes in TSC2 thr1642 phosphorylation after resistance exercise (Fig. 4B). Phosphorylation of TSC2 increased approximately 45%-75% after 1 h of recovery from exercise but was only significantly elevated above rest in the PULSE trial (P < 0.05). TSC2 phosphorylation remained higher (approximately 30%-50%) but was not different from rest after 5 h of recovery (P > 0.05).

**mTOR–PRAS40.** There was a moderate difference in mTOR ser2448 phosphorylation between trials with increased mTOR phosphorylation 1 h postexercise that was higher compared with resting levels with PULSE (approximately 155%, P < 0.01) and PLAC (approximately 100%,

P < 0.05) but failed to reach significance with BOLUS (approximately 90%, Fig. 4C). Moreover, the increase in the PULSE trial was significantly different from BOLUS (P < 0.05) but not PLAC after 1 h of recovery. The phosphorylation of mTOR 5 h after exercise remained elevated above rest in PULSE (approximately 120, P < 0.05) but not BOLUS (approximately 85%) or PLAC (approximately 55%). After exercise, PRAS40 thr246 phosphorylation increased at 1 h postexercise with PULSE (approximately 65%, P < 0.05) but not BOLUS (approximately 25%) and PLAC (approximately 50%, Fig. 4D). In addition, the increase in PRAS40 phosphorylation after 1 h of recovery with PULSE was also higher than BOLUS at the equivalent time point (P < 0.05). The elevated PRAS40 phosphorylation across all trials after the 5 h of recovery period was not different from rest.

p70 S6K. There were similar responses in p70 S6K thr421/ser424 and thr389 phosphorylation after resistance exercise (Fig. 5A, B). Phosphorylation of thr421/ser424 increased above rest 1 h after exercise in PULSE (approximately 560%, P < 0.01) and PLAC (approximately 300%, P < 0.05) but failed to reach significance with BOLUS (approximately 370%, P = 0.07; Fig. 5A). p70 S6K thr421/ ser424 phosphorylation declined after 5 h of recovery and, despite remaining elevated (100%-200%) above baseline for each trial, was not different from rest. There were marked increases in thr389 phosphorylation above rest after 1 h of recovery from resistance exercise in BOLUS (approximately 650%, P < 0.05) and PULSE (approximately 1300%, P < 0.01) but not PLAC (approximately 250%, Fig. 5B). The greater thr389 phosphorylation with PULSE was also different compared with BOLUS and PLAC at the



FIGURE 4—Muscle Akt (A), TSC2 (B), mTOR (C), and PRAS40 (D) phosphorylation at rest and following 1 and 5 h of recovery from a bout of resistance exercise in response to a placebo feeding (PLAC) or the intake of 30-g high-quality protein fed as a bolus (BOLUS) or in a series of small feedings (PULSE). Values are mean  $\pm$  SEM (n = 8; PRAS40 n = 7) presented in arbitrary units relative to  $\alpha$ -tubulin. \*, diff versus rest;  $\ddagger$ , diff versus 5 h; #, diff versus BOLUS—1 h; §, diff versus PLAC—1 h (P < 0.05).

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FIGURE 5—Muscle p70 S6 kinase  $t^{tr421/ser424}$  (A), p70 S6 kinase  $t^{tr389}$  (B), S6 ribosomal protein (C), and eEF2 kinase (D) phosphorylation at rest and after 1 and 5 h of recovery from a bout of resistance exercise in response to a placebo feeding (PLAC) or the intake of 30-g high-quality protein fed as a bolus (BOLUS) or in a series of small feedings (PULSE). Values are mean ± SEM (n = 8) presented in arbitrary units relative to  $\alpha$ -tubulin. \*, different versus rest; ‡, different versus 5 h; #, different versus BOLUS— 1 h; § different versus PLAC—1 h (P < 0.05).

equivalent 1-h postexercise time point (P < 0.01). The elevated thr389 phosphorylation (approximately 80%–340%) after 5 h of recovery from exercise was not different from rest for any trial.

**rpS6–eEF2K.** There were substantial increases in rpS6 ser235/6 phosphorylation during recovery from resistance exercise that were significantly different from rest 1 h post-exercise in BOLUS (approximately 1300%, P < 0.05) and PULSE (approximately 1600%, P < 0.01) but not PLAC (approximately 660%, P = 0.08, Fig. 5C). Moreover, the increased ser235/6 phosphorylation following 1 h of recovery in PULSE was higher than PLAC (P < 0.05) and approached significance compared with BOLUS (P = 0.06). rpS6 phosphorylation after 5 h of recovery remained elevated above rest (approximately 350%–650%) but was not significantly different between trials.

There were corresponding changes in eEF2K ser366 phosphorylation regardless of trial during the 5-h recovery period after resistance exercise (Fig. 5D). Specifically, eEF2K phosphorylation was increased in BOLUS, PULSE, and PLAC (approximately 820%–1000%) 1 h after resistance exercise (P < 0.01). Despite a moderate decrease in magnitude, the enhanced ser366 phosphorylation above resting levels was sustained after 5 h of recovery in each trial (approximately 450%–860%, P < 0.05). Of note, there were no differences in eEF2K phosphorylation between trials for either postexercise time point.

**Muscle FSR.** The FSR for mixed muscle protein for 5 h of recovery after exercise are presented in Figure 6. Both protein feeding trials  $(0.085\% \cdot h^{-1} \pm 0.013\% \cdot h^{-1} \text{ vs.} 0.095\% \cdot h^{-1} \pm 0.010\% \cdot h^{-1}$  for BOLUS and PULSE, respectively) were associated with more than twofold higher

values for muscle FSR compared with PLAC (0.037% h<sup>-1</sup> ± 0.007% h<sup>-1</sup>, P < 0.05), although the difference between BOLUS and PULSE failed to reach statistical significance (P = 0.56).

#### DISCUSSION

In this study, we manipulated patterns of the ingestion of high-quality protein to deliberately alter the rate of appearance of plasma AA to mimic the preexercise ingestion of "fast" (rapid and maximal AA delivery) or "slow" (sustained AA release) digested proteins. In contrast to our previous work in which feeding patterns commenced after



FIGURE 6—The FSR for mixed muscle protein in the vastus lateralis during 5 h of recovery after a bout of resistance exercise in response to a placebo feeding (PLAC) or the intake of 30-g high-quality protein fed as a bolus (BOLUS) or in a series of small feedings (PULSE). Values are mean  $\pm$  SEM for n = 6, 10, and 11 for PLAC, PULSE, and BOLUS, respectively. \*BOLUS, PULSE > PLAC, < 0.05.

the resistance exercise (31), the pulse protocol mimicking a slow protein achieved higher AA and insulin concentrations after resistance exercise than a bolus ingestion of the same protein. The elevated postexercise blood profile associated with PULSE resulted in enhanced signal transduction of the Akt-mTOR-p70S6K pathway at 1 h postexercise compared with BOLUS, whereas there was only modest phosphorylation with a placebo treatment. As might be expected, MPS over the 5 h of recovery after resistance exercise was higher (approximately 250%) with protein ingestion as compared with PLAC, but we were unable to detect any differences between the protein treatments. Therefore, although a pulse feeding protocol mimicking the AA response associated with a slow protein produces an inferior MPS response to a fast protein when consumed after resistance exercise, when protein intake commences before the exercise bout, there is no disadvantage to consuming protein that produces a slower rise in plasma AA.

As in our previous study (31), resistance exercise was associated with increased phosphorylation of the upstream effectors (Akt and PRAS40) and downstream targets (S6K1 and rpS6) of mTOR. These changes were most pronounced in the case of p70S6K at 1 h postexercise in the PULSE feeding trial, supporting the interaction of exercise and nutrition, and in particular, the increase in plasma leucine concentrations, on anabolic pathways (1,15,26). These data add to previous work that has shown an additive effect of resistance exercise and protein intake on p70 S6K and rpS6 phosphorylation (4,19,21,22). Leucine appears to regulate phosphorylation of p70S6K Thr389, with a distinct dose– response relationship being shown in *in vitro* cell culture (3,14), *in vivo* rat models (11,25), and now human studies (31).

Unlike our previous observations (31), we observed similar rates of MPS after the protein feedings irrespective of divergent aminoacidemia and differences in the magnitude of signaling (Fig. 6). The discrepancy between the signaling response and MPS may be related to one or more of the following factors: i) the exercise stimulus and moderate postexercise aminoacidemia with BOLUS may have been sufficient to promote translation and supply adequate exogenous AAs for maximal protein synthesis during recovery, and/or ii) our study was limited to a single biopsy in the initial postexercise period while MPS reflects an average rate of protein synthesis over 4 h postexercise and precludes our ability to detect time-specific differences in peak rates of MPS (e.g., greater rates early in recovery with one feeding strategy balanced by greater rates late in recovery with the other). Indeed, in our previous study (31), we saw a temporal disassociation between signaling activation and MPS; the highest FSR values were seen 3-5 h after resistance exercise and postexercise feedings, whereas the phosphorylation of signaling proteins was greatest in the first hour of recovery and associated with the highest plasma leucine concentrations (31). This confirms the work of others who have noted the lack of association between intracellular signaling and MPS (20).

A novel feature of our study series is the application of bolus and pulse feeding protocols of a high-quality, quickly digested leucine-enriched protein source to mimic the plasma AA profiles of dietary proteins that might be called "fast" and "slow." Although this definition has most notably been applied to the milk protein subfractions, whey and casein, there are emerging data that the consumption of a range of dietary proteins creates robust differences in plasma AA responses. Indeed, we have recently investigated postprandial AA concentrations after the intake of various protein-rich foods providing a 20-g serve of protein. Although area under the curve values for plasma total AA were similar across protein sources, the pattern of aminoacidemia showed significant differences between foods in terms of the peak values and the time to reach peak concentration of plasma leucine (9). Specifically, liquid forms of protein (soy milk, skim milk) achieved peak concentrations twice as quickly after ingestion (e.g., approximately 50 min vs. approximately 100 min) than solid proteinrich foods (egg, steak, and protein bar), whereas skim milk achieved a significantly faster peak leucine concentration than all other foods (approximately 25 min). This is in agreement with the findings of Conley et al. who reported that a liquid meal replacement created earlier and sustained aminoacidemia compared with a solid supplement with the same nutritional composition (10). Thus, protein-rich foods and meals might be meaningfully classified as "fast" or "slow" in their effect on plasma AA responses, in the same way that carbohydrate-rich foods have been labeled as "high" or "low" in glycemic index on the basis of the resulting plasma glucose profiles (32).

This information is important because of the evidence that different dietary protein sources, even among those considered to be high in biological value, have differential effects on whole body protein synthesis in resting individuals (7,12,13) and MPS at rest and after resistance exercise (27). It is clearly unfeasible to undertake comparative studies of MPS in response to every type of dietary protein. However, it could be practical to investigate plasma AA responses to different types of dietary proteins and how these might change according to the form of the protein and meal characteristics; this information could be used to interpret the likely effect on MPS on the basis of an understanding of factors that are important. A specific issue in interpreting the finding of these previous studies (7,12,13,27) and in increasing the utility of the concept of "fast" and "slow" dietary proteins is the difficulty of determining the individual and interactive contributions of the different AA composition of protein-rich foods and the digestibility of proteins or protein-rich meals to the pattern of delivery of these AAs. Our protocol, in which the same (fast) protein was consumed to achieve its traditional AA profile or as a series of small divided feedings to replicate the plasma leucine response associated with a slow protein, provides an opportunity to differentiate these effects.

Our study also addresses a practical issue in sports nutrition of the optimal time to consume protein in relation to resistance exercise. This current literature shows contradictory findings on this theme. A previous investigation showed that the consumption of essential AA before resistance exercise achieved a greater muscle protein net balance than intake of the same AA after the session (29). However, a separate study involving whey protein feedings (28) failed to corroborate these benefits, and preexercise feedings of essential AA and carbohydrate did not enhance postexercise MPS compared with resistance exercise undertaken without nutritional provision (18). Indeed, it has been speculated that MPS is blunted during resistance exercise with this latent period extending for about an hour into recovery (2).

The present study shows that the type of protein that is ingested interacts with the timing of its intake. During the preexercise and exercise periods, our pulse and bolus feeding protocols mimicked the plasma AA profiles expected from the consumption of fast and slow protein foods. However, there was a reversal of this pattern after the resistance exercise was completed (Fig. 2B); we speculate that the postexercise hyperaminoacidemia in PULSE may be related to the restoration of normal rates of gastric emptying and intestinal absorption that were reduced during the period of exercise due to limb hyperemia in exercise (17). Of course, it is acknowledged that we assisted the PULSE protein feeding pattern to achieve a high plasma leucine concentration by consuming it in a modestly higher amount than the dose associated with maximal protein synthesis from a rapidly digested protein source (24) and by increasing its leucine content (23). Overall, these effects combined to stimulate MPS to a similar extent as the preexercise consumption of a BOLUS protein feeding, which we had previously shown to be superior in promoting MPS when consumed

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after exercise (31). This shows that optimal protein feeding related to resistance exercise is a complex issue and might be achieved by a variety of strategies including manipulating the timing, type, and amount of protein intake and, potentially, also the form of the food and other characteristics of a meal in which it is consumed.

In conclusion, we altered the intake of a protein source to mimic the aminoacidemia that would be associated with the consumption of fast-digested or slow-digested proteins before a bout of resistance exercise. We found that the pattern of protein ingestion before a bout of resistance exercise altered postexercise aminoacidemia and intracellular signaling but did not affect postexercise MPS. Importantly, we found that unlike the intake of proteins after resistance exercise where a rapidly digested protein produces a superior muscle protein synthetic response, when it is consumed before the exercise session, there is no disadvantage to consuming protein source that produces a slower AA response as long as the serve size is adjusted to achieve a high leucine. The achievement of optimal MPS associated with resistance exercise and protein intake involves a complex interaction of many characteristics of a protein source, including the timing and type of intake in relation to the exercise bout.

This study was supported by a grant from Nestec Ltd., Vevey, Switzerland, and authors Moore and Stellingwerff were employees of Nestec Ltd. during the period in which the study was conducted.

We acknowledge the technical assistance of Nikki Jeacocke, Graeme Allbon, Larissa Trease, and Richard Allen in completing this study and the oversight of Choo Hoffman. We thank the subjects for their time and commitment.

All other authors were signatories to a contract with Nestec Ltd. to undertake the research but have no other conflicts of interest.

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

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