Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise

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Glynn EL, Fry CS, Drummond MJ, Dreyer HC, Dhanani S, Volpi E, Rasmussen BB. 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 299: R533–R540, 2010. First published June 2, 2010; doi:10.1152/ajpregu.00077.2010.—Muscle protein breakdown (MPB) is increased following resistance exercise, but ingestion of carbohydrate during postexercise recovery can decrease MPB with no effect on muscle protein synthesis (MPS). We sought to determine whether a combination of essential amino acids (EAA) with low carbohydrate or high carbohydrate could effectively reduce MPB following resistance exercise and improve muscle protein net balance (NB). We hypothesized that higher levels of carbohydrate and resulting increases in circulating insulin would inhibit MPB and associated signaling, resulting in augmented NB. Thirteen male subjects were assigned to one of two groups receiving equivalent amounts of EAA (20 g) but differing carbohydrate levels (low carbohydrate or high carbohydrate). Groups ingested nutrients 1 h after an acute bout of leg resistance exercise. Leg phenylalanine kinetics (e.g., MPB, MPS, NB), signaling proteins, and mRNA expression were assessed on successive muscle biopsies using stable isotopic techniques, immunoblotting, and real-time quantitative PCR, respectively. MPB tended to decrease (P < 0.1) and MPS increased (P < 0.05) similarly in both groups following nutrient ingestion. No group differences were observed, but muscle ring finger 1 (MuRF1) protein content and MuRF1 mRNA expression increased following resistance exercise and remained elevated following nutrient ingestion, while autophagy marker (light-chain 3B-II) decreased after nutrient ingestion (P < 0.05). Forkhead box-O3a phosphorylation, total muscle atrophy F-box (MAFbx) protein, and MAFbx and caspase-3 mRNA expression were unchanged. We conclude that the enhanced muscle protein anabolic response detected when EAA + carbohydrate are ingested postexercise exercise is primarily due to an increase in MPS with minor changes in MPB, regardless of carbohydrate dose or circulating insulin level.

muscle protein breakdown; muscle protein metabolism; atrogin-1; MuRF1; LC3B

MUSCLE PROTEIN TURNOVER is a continuous cellular process, and protein net balance is dictated by the equilibrium between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). The effects of anabolic stimuli on MPS have been relatively well characterized, and it is established that resistance-type exercise alone or followed by ingestion of essential amino acids (EAA) with or without carbohydrate (CHO), increases MPI in humans (4, 9, 35, 39, 52). The increase in MPS corresponds to an increase in signal transduction through the mammalian target of rapamycin complex 1 (mTORC1) pathway in humans after resistance exercise (14–15) or after the ingestion of EAA + CHO (17). The combination of resistance exercise followed by ingestion of EAA or EAA + CHO elicits a greater increase in MPS and signaling through the mTORC1 pathway than to either stimulus alone (14–15, 17, 29, 40). However, additional carbohydrate content added to protein ingested postexercise exercise does not affect MPS (28).

On the other hand, there are multiple processes involved in skeletal muscle proteolysis including the ubiquitin-proteasome system (UPS), lysosomal and calcium-activated systems, and caspases. Within the UPS, the Forkhead box (FOX) family of transcription factors (FOXO1, FOXO3a, FOXO4) regulates the expression of E3 ubiquitin ligases muscle atrophy F-box (MAFbx), also known as atrogin-1, and muscle ring finger 1 (MuRF1) (8, 31, 47). FOXO3a is inhibited via phosphorylation by Akt/PKB (12), which can be activated by high levels of circulating insulin. The AMP-activated protein kinase (AMPK) is a cellular energy sensor that is activated in response to metabolic stress (15, 46, 50), inhibits anabolic processes, and stimulates catabolic, energy-producing pathways within the cell (20, 50), including MPB via the UPS (30, 42). In the lysosomal system, increased conversion of cytosolic microtubule-associated protein 1 light-chain 3 (LC3B-I) to the autophagosomal membrane-associated form, LC3B-II is a marker of enhanced autophagy (23, 25–27). Finally, caspases are a family of cysteine proteases generally activated in response to inflammation and/or cell injury. Caspase-3 (CASP3) is an effector caspase whose activation ultimately leads to increased proteolysis (43).

The effects of exercise and nutrient ingestion on proteolytic pathways and MPB have been less well defined, most likely due to the greater methodological difficulty of measuring MPB in vivo. However, several investigations have found MPB (4, 6, 44) and proteolytic gene expression (34) to be elevated following an acute bout of resistance exercise. Studies examining the role of postexercise exercise carbohydrate ingestion vary, but indicate lower levels of carbohydrate ingestion (less than ~40 g) are not sufficient to reduce postexercise exercise whole body protein breakdown (28) or MPB (45), whereas much higher levels (100 g carbohydrate) are sufficient (10). Additionally, postprandial concentrations of insulin (~30 μU/ml) can significantly reduce MPB at rest (19), suggesting that carbohydrate intake sufficient to elicit a similar insulin response may also reduce MPB.

In any event, protein net balance is dictated by both MPS and MPB, and thus will be further improved following resistance exercise if MPB is decreased. While carbohydrate and/or insulin release appear to affect MPB, EAA do not significantly

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alter MPB either alone or in combination with resistance exercise (5, 11, 48). Therefore, the purpose of this study was to determine whether a combination of EAA and low carbohydrate (EAA + LCHO), or EAA and high carbohydrate (EAA + HCHO), could effectively reduce MPB during postexercise recovery and improve net muscle protein balance. Specifically, we examined whether carbohydrate content sufficient to elicit a low-range postprandial insulin response (~30 μU/ml) or high-range insulin response (~70 μU/ml) would inhibit MPB and associated signaling. We hypothesized that higher circulating insulin concentrations (70 μU/ml vs. 30 μU/ml) would have a greater effect on inhibition of MPB and catabolic signaling, resulting in an augmented net muscle protein balance.

MATERIALS AND METHODS

Subjects. We studied 13 young healthy males who reported that they were not currently engaged in a resistance exercise training program during the screening interview. Seven subjects are from a subset of subjects that has been previously published (14); however, all tracer and signaling data in the current experiment is original aside from the few exceptions as noted in the table and figure legends. All volunteers were asked to refrain from performing vigorous physical activity for 24 h prior to participating in the study. All subjects gave informed written consent before participating in the study, which was approved by the Institutional Review Board of the University of Texas Medical Branch, and which is in compliance with the Declaration of Helsinki. Screening of subjects was performed with clinical history, physical exam, and laboratory tests including complete blood count with differential, liver and kidney function tests, coagulation profile, fasting blood glucose, and oral glucose tolerance test, hepatitis B and C screening, human immunodeficiency virus screening, thyroid-stimulating hormone level, lipid profile, urinalysis, drug screening, and ECG. The subjects' physical characteristics are summarized in Table 1.

Study design. Details of the cross-sectional study design have been previously published (14–15). Briefly, each subject's one-repetition maximum was determined on a leg extension machine (model VR2; Cybex, Medway, MA), which was located within the Institute for Translational Sciences Clinical Research Center’s Exercise Laboratory and used for each study. The one-repetition maximum values obtained were used to determine the starting weight (70% of one-repetition maximum) for the resistance exercise portion of our study. A dual-energy X-ray absorptiometry scan (QDR 4500W; Hologic, Bedford, MA) was performed to measure body composition and lean mass. Each subject was admitted to the Clinical Research Center of the University of Texas Medical Branch the day prior to the exercise study. The subjects were all fed a standardized meal (12 kcal/kg body wt; 60% carbohydrate, 20% fat, and 20% protein) prepared by the Bionutrition Division of the Clinical Research Center. Each subject was also offered a snack at 2200 h and did not eat again until after the study the following day.

The morning of the study, polyethylene catheters were inserted into a forearm vein for tracer infusion, in the contralateral hand vein that was heated for arterialized blood sampling, and in the femoral artery and vein (retrograde placement) of the leg for blood sampling. The femoral lines were placed in the same leg from which muscle biopsies were obtained. The arterial catheter was also used for the infusion of indocyanine green (ICG; Akorn, Buffalo Grove, IL) to determine blood flow.

After a background blood sample, a primed continuous infusion of either l-[ring-2H5]- or l-[13C6]phenylalanine (Cambridge Isotope Laboratories, Andover, MA) was begun (time 0) and maintained at a constant rate until the end of the experiment (Fig. 1). The priming dose for the labeled phenylalanine was 2 μmol/kg and the infusion rate was 0.05 μmol·kg⁻¹·min⁻¹.

Subjects in both groups were studied identically in three periods: a basal period (baseline), which was the hour prior to exercise; 1 h following exercise (1 h post-Ex); and 2 h following exercise (2 h post-Ex) in which the groups were separated into those receiving either EAA+LCHO or EAA+HCHO. For each period, all subjects rested comfortably in the semirecumbent position.

Marking the beginning of the basal period (baseline), and 2 h after starting the tracer infusion, the first muscle biopsy was obtained from the lateral portion of the vastus lateralis of the leg with the biopsy site between 15 and 25 cm from the midpatella. The biopsy was performed using a 5-mm Bergström biopsy needle under sterile procedure and local anesthesia (1% lidocaine). Once harvested, the muscle tissue was immediately blotted and frozen in liquid nitrogen and stored at −80°C until analysis. Immediately after the first biopsy, a continuous infusion of ICG was started in the femoral artery (0.5 mg/min) and maintained for 50 min. Blood samples were drawn four times, at 10-min intervals, from the femoral vein and the arterialized hand vein to measure ICG concentration (Fig. 1). In addition to the blood obtained for ICG measurement, blood samples were also taken from the femoral artery and vein and the arterialized hand vein to measure glucose, phenylalanine, and insulin concentrations. At the end of baseline, a second biopsy was obtained; however, the biopsy needle was inclined at a different angle so that the second biopsy was

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>EAA + LCHO</th>
<th>EAA + HCHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>30 ± 2</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176 ± 2</td>
<td>179 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79 ± 3</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>Body mass index, kg·(m²)⁻¹</td>
<td>26 ± 1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>61 ± 2</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>19 ± 2</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Leg lean mass, kg</td>
<td>11 ± 0.4</td>
<td>11 ± 0.5</td>
</tr>
<tr>
<td>EAA content in solution, g</td>
<td>22 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>CHO content in solution, g</td>
<td>31 ± 1</td>
<td>91 ± 4*</td>
</tr>
<tr>
<td>Total kcal consumed</td>
<td>210 ± 7</td>
<td>454 ± 20*</td>
</tr>
</tbody>
</table>

Values are means ± SE. The data of subjects in the EAA+LCHO are a subset of a group of subjects from a previously published study (14). EAA, essential amino acids; LCHO, low carbohydrates; HCHO, high carbohydrates.

*Significantly different from baseline (P < 0.05).
taken ~5 cm apart from the first. This method has been previously used by us (14–15, 17) and others (13, 24, 34).

Following the second biopsy, the subjects were seated in a Cybex leg extension machine to perform the exercise protocol. After a brief warm up (23 kg × 10 reps), each subject performed 10 sets of 10 repetitions of bilateral leg extension exercises at 70% of their predetermined one-repetition maximum. Each set was separated by 3 min.

During the period following exercise (1 h post-Ex), ICG was again infused continuously and blood was sampled as previously described. At the end of the first hour postexercise, a third muscle biopsy was obtained through a new incision site ~5 cm proximal to the first incision.

Marking the beginning of the final period (2 h post-Ex), subjects ingested either the EAA+LCHO solution or the EAA+HCHO solution (see below for solution composition). Blood samples were collected in the same manner as during the previous periods. At the end of the period (2 h post-Ex), a final muscle biopsy was collected from the second incision. Each biopsy was taken an average of 61 ± 1 min apart.

Composition of the EAA+CHO solutions. The composition of the EAA+CHO solutions consisted of ~20 g of the EAA in the following proportions: 8% histidine, 8% isoleucine, 35% leucine, 12% lysine, 3% methionine, 14% phenylalanine, 10% threonine, and 10% valine (Sigma-Aldrich, St. Louis, MO). Lean mass (LM), determined by dual-energy X-ray absorptiometry, was used to calculate the amount of EAA (0.35 g kg⁻¹ LM⁻¹) added to the nutrient solution. Similarly, carbohydrate was added at 0.5 g kg⁻¹ LM⁻¹ for the EAA+LCHO group and 1.4 g kg⁻¹ LM⁻¹ for the EAA+HCHO group. The EAA+CHO was mixed with a flavored, noncaloric beverage to aid in palatability (14, 17). To minimize the potential of tracer dilution with the addition of the amino acids, the appropriate phenylalanine tracer was added to the oral EAA solution at 6.5% of the total phenylalanine content. Average EAA and carbohydrate content are shown in Table 1.

Blood flow, glucose uptake, blood glucose, and insulin. Serum ICG concentration for the determination of leg blood flow was measured spectrophotometrically (Beckman Coulter, Fullerton, CA) at λ = 805 nm (22). Plasma glucose concentration was measured by using dual-energy X-ray absorptiometry, was used to calculate the amount of ICG (805 nm) and glucose from each sample (run in duplicate) was normalized to the internal control, GAPDH, after which relative fold changes were determined as described by Livak and Schmittgen (33).

Statistical analysis. All values are expressed as means ± SE. Comparisons were performed using repeated-measures ANOVA, the effects being group (EAA+LCHO or EAA+HCHO) and time (baseline, and EAA+LCHO 1 h post-Ex, and 2 h post-Ex periods). Post hoc testing was performed using the Bonferroni’s t-test for multiple comparisons vs. baseline. If a test of normality and/or equal variance failed, simple transformations were performed. Significance was set at P ≤ 0.05. While groups remained separated for statistical comparisons, for clarity, results are presented as pooled for baseline and 1 h post-Ex, because groups were treated identically through these time points and were not statistically different for any parameter. Individual group data (EAA+LCHO, EAA+HCHO) is presented for the time point following nutrient ingestion (2 h post-Ex).

RESULTS

Blood flow, glucose, and insulin. Blood flow increased during exercise (data not shown), but returned to baseline values during the first hour of postexercise recovery (Table 2). No differences were observed between groups during the second hour postexercise following the nutrient ingestion (P < 0.05, Table 2). Blood glucose concentrations were similar at baseline and 1 h post-Ex (5.3 ± 0.1 vs. 5.2 ± 0.1 mmol/l, P > 0.05). Glucose concentration was elevated at 2 h post-Ex in both groups and significantly higher in EAA+HCHO (6.2 ± 0.3 (LCHO) (P < 0.05 vs. baseline) vs. 7.8 ± 0.3 mmol/l
Table 2. Phenylalanine blood concentrations and kinetics across the leg

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 h Post-Ex</th>
<th>2 h Post-Ex</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>EAA + LCHO</td>
<td>EAA + HCHO</td>
</tr>
<tr>
<td>Blood flow (ml·min⁻¹·100 ml leg⁻¹)</td>
<td>3.4 ± 0.5</td>
<td>5.3 ± 0.8</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>Arterial Phe (μmol/l)</td>
<td>55 ± 2</td>
<td>54 ± 2</td>
<td>146 ± 13*</td>
</tr>
<tr>
<td>Intracellular Phe (μmol/l)</td>
<td>93 ± 17</td>
<td>78 ± 11</td>
<td>185 ± 9*</td>
</tr>
<tr>
<td>NB, net balance across the leg</td>
<td>-12 ± 3</td>
<td>-14 ± 5</td>
<td>107 ± 55*</td>
</tr>
<tr>
<td>F_{in}, delivery to the leg</td>
<td>189 ± 28</td>
<td>289 ± 47</td>
<td>561 ± 122*</td>
</tr>
<tr>
<td>F_{out}, release from the leg</td>
<td>201 ± 29</td>
<td>304 ± 49</td>
<td>454 ± 113*</td>
</tr>
<tr>
<td>R_d, rate of disappearance (synthesis)</td>
<td>64 ± 13</td>
<td>65 ± 11</td>
<td>157 ± 61*</td>
</tr>
<tr>
<td>F_{M,O}, release from proteolysis</td>
<td>88 ± 17</td>
<td>83 ± 12</td>
<td>51 ± 12‡</td>
</tr>
<tr>
<td>F_{O,M}, utilization for protein synthesis</td>
<td>76 ± 16</td>
<td>68 ± 12</td>
<td>158 ± 61*</td>
</tr>
</tbody>
</table>

Data for baseline are pooled from both groups and an hourly average of blood draws in the absence of nutrients and prior to performing exercise. Data for 1 h post-Ex are pooled and are an hourly average of blood draws in the absence of nutrients and during the 1st hour following exercise. EAA + LCHO and EAA + HCHO values are an hourly average of blood draws during the 2nd hour postexercise and 1 h following ingestion of either the EAA + LCHO or EAA + HCHO nutrient solution, respectively. Values for NB, F_{in}, F_{out}, R_d, F_{M,O}, and F_{O,M}, are expressed in mmol·min⁻¹·(100 ml leg volume)⁻¹. Phe, phenylalanine. All data are means ± SE.*Significantly different from baseline (P < 0.05); †P = 0.15; ‡P = 0.12. Blood flow, Phe concentrations, and NB data for EAA + LCHO are from a subset of a group of subjects that has been previously published (14). All other kinetic measures have not been previously published.

(HCHO) (P < 0.05 vs. baseline, P < 0.05 vs. LCHO). Insulin levels were similar at baseline and 1 h post-Ex and increased significantly in both groups following nutrient ingestion, but to a much larger extent in the EAA + HCHO group (Fig. 2A).

**Plasma and intracellular phenylalanine concentrations.** Arterial phenylalanine concentrations were not altered at 1 h post-Ex (P > 0.05) but were significantly elevated to a similar extent in both groups at 2 h post-Ex (P < 0.05, Table 2). Muscle intracellular phenylalanine concentrations remained constant from baseline to 1 h post-Ex (P > 0.05), but increased similarly following nutrient ingestion at 2 h post-Ex in both groups (P < 0.05, Table 2).

**Leg phenylalanine kinetics.** Leg phenylalanine kinetics are expressed per 100 milliliters leg volume and are reported in Table 2. There were no significant changes in any parameter at 1 h post-Ex (P > 0.05). Net balance across the leg was significantly increased in both groups following ingestion of either drink (P < 0.05). Phenylalanine delivery to and release from the leg increased to a similar extent following nutrient ingestion in both groups (P < 0.05). In the two-pool model, the rate of disappearance of phenylalanine, which is indicative of protein synthesis, increased at 2 h post-Ex in both groups (P < 0.05). Rate of appearance of phenylalanine, leg protein breakdown, tended to decrease in both groups similarly at 2 h post-Ex (Fig. 2D, P < 0.1). Three pool model calculations are consistent with the results from the two-pool model with a large and significant increase in protein synthesis (F_{M,O}) 2 h post-Ex in both groups (P < 0.05). Release from proteolysis, or breakdown (F_{O,M}), tended to decrease in both groups (EAA + LCHO, P = 0.15; EAA + HCHO, P = 0.12). No significant changes were detected between groups for any kinetic parameter.
Cellular signaling. Phosphorylation of Akt (Ser\textsuperscript{473}) significantly increased following exercise and remained elevated following nutrient ingestion in both groups (P < 0.05, Fig. 2B). Phosphorylation of AMPK\textalpha{} at Thr\textsuperscript{172} also increased in both groups 1 h post-Ex (P < 0.05) and remained significantly elevated in the EAA+LCHO group at 2 h post-Ex (P < 0.05), while returning to basal values in the EAA+HCHO group (P > 0.05 vs. baseline, P < 0.05 vs. EAA+LCHO; Fig. 2C). Phosphorylation of FOXO3a (Ser\textsuperscript{253} and Ser\textsuperscript{183/212}) did not change significantly over time and was not different between groups (P > 0.05, data not shown). Total MuRF1 was significantly elevated at 1 h post-Ex (P < 0.05) and there was a significant time effect at 2 h post-Ex; however, groups were not significantly different individually (Fig. 3B). There were no changes in total MAFbx protein content (Fig. 3D). LC3B-I (16 kDa) did not change significantly over time; however, LC3B-II (14 kDa) was decreased similarly in both groups following drink ingestion (P < 0.05 vs. baseline, Fig. 4).

mRNA expression. MURF1 mRNA expression was significantly elevated at 1 h post-Ex and remained similarly elevated in both groups following nutrient ingestion (P < 0.05, Fig. 3A). MAFbx (Fig. 3C) and CASP3 mRNA levels were unchanged across time and between groups (P > 0.05, CASP3 data not shown).

DISCUSSION

This study was designed to determine whether differing levels of carbohydrate, combined with EAA, could effectively reduce MPB during postexercise recovery and improve net muscle protein balance. Specifically, we were interested in determining whether a postprandial insulin response (~30 µU/ml) was sufficient, or a higher physiological response (~70 µU/ml) was required to reduce MPB and associated signaling. The primary and novel finding from our study was that low and high levels of carbohydrate added to an EAA solution in the postresistance exercise recovery period, altered MPB, associated signaling, and gene expression similarly. Concurrently with significant increases in circulating insulin levels and signaling through Akt, measures of MPB were modestly (though not significantly) improved following nutrient ingestion. Our findings are consistent with other reports that indicate postexercise MPS is not enhanced when large amounts of carbohydrate (>40 g) are added to a protein solution (28); however, our data do show that a EAA+CHO nutrient solution may be capable of attenuating and/or reducing MPB when ingested following resistance exercise regardless of carbohydrate dose (LCHO or HCHO).

The present study was intended to elicit a maximal increase in MPS, to exclude even minor changes in synthesis between the groups as a confounding variable. Still, contrary to our hypothesis regarding breakdown, leg phenylalanine kinetics showed no significant differences between groups. Leg protein breakdown (LPB), represented as phenylalanine rate of appearance, decreased similarly in both groups though neither reached significance compared with basal values (time effect at 2 h post-Ex, P < 0.1). Another measure of LPB, F_{\text{LCHO}}\text{O}, also declined following nutrient ingestion (time effect at 2 h post-Ex, P = 0.03; EAA+LCHO, P = 0.15; EAA+HCHO, P = 0.12). In a previous study by Biolo et al. (4) in which subjects were on a weight-maintaining diet (15–20% protein for ≥1 mo prior to each study condition, cross-over design) and reported to the Clinical Research Center following an overnight fast, results indicated a 50% increase in MPB over 3 h of postresistance exercise recovery. We believe our results, in which measures of MPB fall below basal levels following nutrient ingestion, suggest that the nutrients were able to alleviate the exercise-induced increase in breakdown. Nevertheless, MPB appears to be playing a minor role in the overall muscle protein...
anabolic response that occurs postexercise following EAA + CHO ingestion, accounting for < 30% of the improvement in net protein balance. It remains to be determined whether the addition of carbohydrate to an EAA or protein solution ingested following resistance exercise (with the subsequent minor changes in MPB) is capable of enhancing protein accretion (as compared with EAA supplementation alone) during resistance exercise training.

Similar to previous findings (15), we report an increase in AMPKα phosphorylation 1 h following exercise. AMPKα phosphorylation returned to baseline levels only when larger amounts of carbohydrate were ingested (EAA + HCHO), implying the additional energy available in the HCHO solution was able to suppress the exercise-induced activation of AMPK. Although we did not measure glycogen levels in the present study, it is possible that the higher carbohydrate level in EAA + HCHO restored depleted glycogen levels from resistance exercise more rapidly than the lower amount of carbohydrate, thus contributing to the attenuated AMPKα phosphorylation response. While we can only speculate as to the mechanism by which the additional carbohydrate attenuated AMPKα signaling, decreased AMPKα activation would hypothetically allow for increases in MPS and decreased MPB. However, we did not observe either effect 1 h following nutrient ingestion, although the possibility of a longer time course revealing such differences cannot be ruled out.

AMPK activation can stimulate autophagy (32, 36, 38, 49); however, we did not see changes in the autophagy marker LC3B-II (27) at 1 h post-Ex. This could be due to the timing of our sample collection, such that we missed peak processing of LC3B-I to LC3B-II. Nonetheless, LC3B-II was reduced similarly and significantly in both groups following nutrient ingestion. Insulin signaling through class I phosphatidylinositol 3-kinase to Akt, and signal transduction through the mTORC1 pathway, are potent inhibitors of autophagy (21, 37, 49). The large increases in insulin and Akt phosphorylation, as well as previous evidence from our lab showing large increases in mTORC1 signaling following EAA + CHO ingestion postexercise (14), provide a potential mechanistic explanation for the observed reduction of LC3B-II. While the UPS is believed to be the main pathway for bulk protein degradation in skeletal muscle, these findings raise the possibility that the lysosomal system may play more of a role in the regulation of MPB in response to nutrients.

Of the potential UPS markers we examined (p-FOXO3a Ser253, Ser318/321; MAFbx and MuRF1 protein, and mRNA expression), only MuRF1 showed any significant alterations. MuRF1 total protein and MURF1 mRNA expression levels were increased following exercise and remained elevated following ingestion of the nutrients. These findings are consistent with a previous report by Louis et al. (34) in which a bout of resistance exercise in trained subjects resulted in increased mRNA expression of MURF1 at 1 and 2 h post-Ex, with no changes in MAFbx or FOXO3a expression at these time points. The lack of changes to CASP3 mRNA expression connotes there is also little contribution from protease-dependent breakdown. Taken together, these results provide further evidence to support the hypothesis that the lysosomal system may play the most significant role, of the proteolytic pathways, in the regulation of MPB following nutrient ingestion. In fact, Mordier et al. (41) has shown autophagy is an essential cellular response involved in increasing protein breakdown in muscle following food deprivation, which would support the role of the lysosomal system in regulation of the response to nutrients. However, this is mere conjecture at this point and future studies are required to determine whether the inhibition of autophagy following feeding is responsible for the minor reduction in the rate of MPB following exercise. In any event, collectively there were only small changes in the breakdown markers examined. This is in agreement with the relatively small variation we observed in phenylalanine kinetic measures of MPB (P < 0.1) and supports the idea that breakdown is playing a minor role in the overall protein anabolic response to EAA + CHO ingestion following resistance exercise.

We do acknowledge a few limitations with the current experiment. For example, we did not include a fasting exercise group. Our rationale for this was that an acute bout of resistance exercise (without nutrients) has been previously shown to increase the rate of MPB by ~50% (4). We also did not include...
a group that only ingested EAA following exercise because only a high amount of carbohydrate ingestion following resistance exercise is capable of inhibiting MPB (10), and EAA ingestion (without carbohydrate) results in minor increases in circulating insulin concentrations and does not alter the rate of MPB (5, 11, 48). In addition, we have only selected a few of the most well-known markers of proteolysis, and our signaling data is rather correlative in nature. However, since relatively little is known about how MPB is regulated in human skeletal muscle, we feel like our approach of combining tracer techniques (to assess breakdown rates) with the assessment of proteolytic markers does provide novel information about potential regulatory processes governing MPB in human skeletal muscle.

In summary, we show that the ingestion of an anabolic nutrient solution (EAA + CHO) containing a higher amount of carbohydrate (i.e., 90 vs. 30 g) decreased phosphorylation of AMPKα, but this did not further decrease MPB as similar trends for decreases in breakdown were observed in both groups. In addition, the E3 ubiquitin-ligase MuRF1 was significantly increased (both protein and mRNA expression) following exercise and remained elevated following drink ingestion, while the autophagy marker LC3B-II was not altered by exercise but decreased following nutrient ingestion in both groups. The observed improvement in MPB may be regulated largely by the lysosomal system, though additional studies are required to confirm the contribution of each proteolytic system in response to exercise and nutrition. We conclude that the enhanced muscle protein anabolic response detected when EAA + CHO are ingested following resistance exercise is primarily due to enhanced protein synthesis (accounting for >70% of improved net balance), with relatively minor changes in protein breakdown, regardless of the level of carbohydrate ingestion. Therefore, in the design of nutrient solutions to promote improved muscle recovery and growth following resistance exercise, it is not necessary to include large quantities of carbohydrate since a nutrient solution containing a sufficient amount of EAA with modest amounts of carbohydrate is capable of producing a maximal protein anabolic response. However, future studies are needed to determine whether the insulin release and reduction in MPB resulting from modest carbohydrate intake can generate significant improvements in net muscle protein accretion over time.

GRANTS

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DISCLOSURES

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

REFERENCES