Peri-exercise co-ingestion of branched-chain amino acids and carbohydrate in men does not preferentially augment resistance exercise-induced increases in PI3K/Akt-mTOR pathway markers indicative of muscle protein synthesis

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Abstract

The effects of a single bout of resistance exercise (RE) in conjunction with peri-exercise branched chain amino acid (BCAA) and carbohydrate (CHO) ingestion on skeletal muscle signaling markers indicative of muscle protein synthesis (MPS) were determined. It was hypothesized that CHO + BCAA would elicit a more profound effect on these signaling markers compared to CHO. Twenty-seven males were randomly assigned to CHO, CHO + BCAA, or placebo (PLC) groups. Four sets of leg presses and leg extensions were performed at 80% 1RM. Supplements were ingested 30 min and immediately prior to and after RE. Venous blood and muscle biopsy samples were obtained immediately prior to supplement ingestion and 0.5 hr, 2 hr, and 6 hr after RE. Serum insulin and glucose and phosphorylated levels of muscle insulin receptor substrate 1 (IRS1), protein kinase B (Akt), mammalian target of rapamycin (mTOR), p70S6 kinase (p70S6K), and 4E binding protein 1 (4E-BP1) were assessed. Data were analyzed by two-way repeated measures ANOVA. Significant group x time interactions were observed for glucose and insulin ($p < 0.05$) showing that CHO and CHO + BCAA were significantly greater than PLC. Significant time main effects were observed for IRS1 ($p = 0.001$), Akt ($p = 0.031$), mTOR ($p = 0.003$), and p70S6K ($p = 0.001$). CHO and CHO + BCAA supplementation significantly increased IRS-1 compared to PLC ($p = 0.002$). However, peri-exercise co-ingestion of CHO and BCAA did not augment RE-induced increases in skeletal muscle signaling markers indicative of MPS when compared to CHO.

Key words. Branched-chain amino acids, carbohydrate, muscle protein synthesis, insulin, men

Abbreviations

1-RM; one repetition maximum

4E-BP1; 4E binding protein 1

Akt; protein kinase B

ANOVA; analysis of variance

BCAA; branched-chain amino acids

BSA; bovine serum albumin

CHO; carbohydrate

ELISA; enzyme-linked imunoabsorbent assay

HSD; honestly significant difference

IRS-1; insulin receptor substrate 1

MPS; muscle protein synthesis

mTOR; mammalian target of rapamycin

P70S6K; phosphorylated ribosomal S6 kinase 1

PI3K; phosphatidylinositol 3 kinase

PMSF; phenylmethanesulphonylfluoride

RE; resistance exercise

RNA; ribonucleic acid

SD; standard deviation

TOP; terminal oligopyrimidine

1. Introduction

The provision of nutrients, primarily protein and carbohydrate (CHO), peri-exercise is considered a beneficial strategy in improving muscle performance because ingesting nutrients during this time helps increase muscle protein synthesis (MPS) while concomitantly decreasing muscle proteolysis. The provision of CHO increases circulating insulin levels, which can subsequently increase MPS. Additionally, BCAA can also increase insulin levels but also have a direct effect on MPS.

The disparate signals of amino acids, insulin, and resistance exercise (RE) promote muscle protein synthesis (MPS) through up-regulation of the PI3K/Akt-mTOR signaling pathway [1]. In humans, relatively low doses of exogenous essential amino acids (e.g., 10 g) stimulate MPS at physiological insulin concentrations, and are associated with enhanced mammalian target of rapamycin (mTOR), phosphorylated 70S6 kinase (p70S6K), and 4E binding protein 1 (4E-BP1) phosphorylation [2,3]. Without maintenance of blood amino acid concentrations, carbohydrate (CHO) alone apparently does not increase MPS synthesis. When CHO are consumed following RE, the resultant hyperinsulinemia does not increase MPS, rather it is associated with a reduction in skeletal muscle protein breakdown [4]. Conversely, protein and/or amino acid ingestion following RE has been shown to stimulate MPS during the recovery period [5,6].

Besides providing amino acids as precursors for MPS, co-ingestion with CHO can elicit a strong insulinotropic response [7].

Branched-chain amino acids (BCAA), particularly leucine, have a positive effect on muscle protein accretion [8], likely through the permissive effects of insulin on increased essential amino acid supply [9-12]. The co-ingestion of protein and leucine has been shown to stimulate MPS and optimize whole-body protein balance in the post-exercise period from RE when compared to CHO only [13]. Moreover, unlike insulin the BCAAs are nutrient effectors of mTOR activity, independent of the upstream signaling aspects of the mitogenic phosphatidylinositol-3-kinase (PI3K) signaling pathway [1,14]. As such, BCAA supplementation when provided during and after RE has been shown to significantly increase p70S6K phosphorylation up to two hours post-exercise when compared to placebo [15]. Similarly, it has also been demonstrated that BCAA supplementation provided before, during, and after RE resulted in significant increases in p70S6K phosphorylation at rest and after one hr following RE when compared to placebo [16].

Based on the concept of peri-exercise nutrient provision and the subsequent possibility of enhancing muscle performance, it has been suggested that protein ingestion before, as opposed to after, RE could further augment MPS due to a greater supply of amino acids to the muscle during the acute stages of post-exercise recovery [17]. Furthermore, it is also possible that protein and/or amino acid ingestion before and/or during RE already

stimulates MPS during exercise which creates a greater time frame for MPS to be elevated [18]. However, a number of studies suggest that protein ingestion post-exercise is more effective [7,10,11]. It is apparent that the timing of nutrient ingestion appears to have a differential response in regulating MPS and/or muscle accretion. However, there does not appear to be a consensus as to the most appropriate nutrient timing protocol.

As with whey protein and essential amino acids, the timing of BCAA ingestion appears to also have a differential response in regulating MPS. Few studies have been performed using BCAA and RE, and none appear to exist involving the co-ingestion of BCAA with CHO. Therefore, the purpose of this study was to investigate the impact of peri-exercise supplementation of CHO or CHO + BCAA on markers indicative of muscle protein synthesis within the PI3K/Akt-mTOR signaling pathway in human skeletal muscle following a single bout of RE. Because of the possible augmented insulogenic response from the co-ingestion of CHO and BCAA, it was hypothesized that the provision of CHO + BCAA would elicit activation of the signaling markers IRS-1, Akt, mTOR, and p70S6K to a greater extent than CHO in the post-exercise period.

2. Methods and materials

2.1 Experimental approach

In a randomized, double-blind, parallel design, non-resistance-trained males participated in three separate testing sessions involving the ingestion of one of three nutritional supplements and a single bout of RE. The three nutritional supplements were CHO, BCAA + CHO, and energy-free placebo control (PLC). Each testing session involved four blood sampling and muscle biopsy time points (pre-supplement/exercise and 0.5 hr, 2 hr, and 6 hr post-exercise). A schematic presentation of the experimental protocol is presented in Figure 1.

2.2 Participants

Thirty-seven prospective participants met initial phone screening criteria and were invited to familiarization sessions. Of these, 32 met entrance criteria and were eligible; however, five chose not to participant due to hesitation with the blood draw and muscle biopsy. Therefore 27 active, but non-resistance-trained [no consistent resistance training (e.g., thrice weekly) for one year prior to the study] men between the ages of 18 to 30 yr began and completed the study. Enrollment was open to men of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine

(ACSM), and who had not consumed any nutritional supplements (excluding multi-vitamins) three months prior to the study were allowed to participate. All eligible subjects signed university-approved informed consent documents and approval was granted by the Institutional Review Board for Human Subjects of Baylor University. Additionally, all experimental procedures involved in the study conformed to the ethical considerations of the Helsinki Code.

2.3 Pre-testing and dietary analyses

A pre-testing session was performed to acquaint participants and to determine the one-repetition maximum (1-RM) for the angled leg press and knee extension exercises based upon our previous procedures [19,20]. As a warm-up, an estimated 50% 1-RM was utilized to complete 10 repetitions. After a 2.5 min rest period, a load of 70% of estimated 1-RM was utilized to perform five repetitions. At this point, the weight was gradually increased until a 1-RM was reached, with a 2.5 min rest period in between each successful lift. Test-retest reliability of performing these strength assessments on subjects within our laboratory has demonstrated low mean coefficients of variation and high reliability for the angled leg press (2.1%, intra-class $r = 0.92$) and knee extension (0.81%, intra-class $r = 0.93$), respectively.

Participants were instructed by a registered dietician to record their dietary intake for a 48 hr period, without changing their usual dietary consumption patterns. The dietary data were analyzed with the ESHA Food Processor dietary assessment software (Salem, OR, USA) for determination of the average intake of total food energy and intake of the macronutrients.

2.4 Supplementation protocol

The three dietary supplements were CHO (n = 9), CHO + BCAA (n = 8), and PLC (n = 10). The BCAA supplement (Now Foods, Bloomingdale, IL, USA) was comprised of 50% leucine, 25% isoleucine, and 25% valine and the total ingested dose was 120 mg/kg body mass (60 mg/kg leucine, 30 mg/kg isoleucine, and 30 mg/kg valine; ~5 gm leucine/participant). The CHO supplement provided 1.4 g sugars (dextrose, fructose, and sucrose) per 1.5 g CHO supplement (DGC, AST Sports Science, Golden, CO, USA). The placebo (PLC) consisted of an energy-free flavored beverage (Crystal Light®). Based on previously published guidelines, participants consumed a total dose of 1.5 g/kg body mass (approximately 120 g per person) [15]. The CHO and CHO +BCAA supplements were dissolved in 450 mL of the placebo solution, for a total supplement ingestion volume of 450 mL. Participants ingested *per os* 1/3 (150 ml) of their assigned supplement dose at each of the following time points: fasting blood draw, 10 min pre-exercise, and 5 min post-exercise.

2.5 Resistance exercise protocol

Following the baseline blood sample, muscle biopsy, and immediately after ingestion of the second supplement dose, participants rode a stationary bicycle at low resistance for 2 min. Participants then performed a warm-up using the angled leg press and knee extension exercise, each involving 2 sets of 10-12 repetitions at 50% of 1-RM with 2.5 min of rest between sets. After a 2.5 min rest period, participants performed the exercise bout which consisted of 4 sets of 8-12 repetitions at 75%-80% of 1-RM on the plate-loaded, angled leg press (Nebula Fitness, Versailles, OH, USA) followed by 4 sets of 8-12 repetitions at 75%-80% of 1-RM on the selectorized leg extension (Cybex, Medway, MA, USA). During the exercise bout, a rest period of 2.5 min was required between sets and exercises.

2.6 Blood sampling and muscle biopsies

Venous blood samples were collected from the antecubital vein in vacutainer tubes using standard phlebotomy protocol. Blood samples were allowed to sit at room temperature

for 10 min, and then centrifuged for 15 min at 2,500 rpm (Cole Parmer, Vernon Hills, IL, USA). Serum was stored at -80C for subsequent analysis.

Muscle biopsies were taken from the vastus lateralis of the dominant leg using the percutaneous biopsy technique with suction [2,21]. Under local anesthesia using 1% Lidocaine, a sterile 5 mm Bergstrom needle was used to extract samples from the vastus lateralis, midway between the patella and the greater trochanter, at a depth of 1-2 cm. For subsequent biopsies, the needle was re-introduced into the incision site and rotated in a clock-wise fashion relative to the prior sample (e.g., 1200 hr; 1500 hr; 1800 hr; 2100 hr) to obtain a new sample. Muscle samples were immediately frozen in liquid nitrogen and stored at -80C for future analysis. Blood and muscle samples were obtained prior to supplement ingestion and pre-exercise and at 0.5 hr, 2 hr, and 6 hr post-exercise.

2.7 Serum glucose and insulin analyses

Serum glucose concentrations were determined with a clinical chemistry analyzer (Dade-Behring Dimension RXL, Siemens Healthcare, Malvern, PA, USA) using an adaptation of the hexokinase-glucose-phosphate dehydrogenase method. Serum insulin concentrations were analyzed by a commercially-available enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic Systems Labs, Webster, TX, USA). The

sensitivity of this assay is reported by the manufacturer to be 0.26 μIU/mL. Absorbances for each sample were determined in duplicate at 450 nm with a microplate reader (iMark, Bio-Rad, Hercules, CA, USA). A set of standards of known concentrations for insulin was utilized to construct a standard curve by plotting the net absorbance values of the standards against their respective protein concentrations. Using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA, USA), the overall intra-assay percent coefficients of variation were 6.2% and 7.6, respectively, for glucose and insulin.

2.8 Cell extraction and total protein content

Approximately 20 mg of each muscle sample was homogenized using a commercial cell extraction buffer (Biosource, Camarillo, CA, USA) and a tissue homogenizer. The cell extraction buffer was supplemented with phenylmethanesulphonylfluoride (PMSF) and a protease inhibitor cocktail (Sigma Chemical Company, St. Louis, MO, USA) with broad specificity for the inhibition of serine, cysteine, and metallo-proteases [2]. Total protein remaining from the cell extraction process of the muscle tissue was isolated by repeatedly incubating the protein in 0.01% SDS at 50˚C. Total protein content was determined spectrophotometrically at a wavelength of 595 nm and using bovine serum albumin (BSA) as the standard [2,22]. All assays were performed in duplicate and total protein content was expressed relative to muscle wet-weight.

2.9 mTOR and 4E-BP1 analysis

Extracted muscle homogenates were analyzed for phosphorylated mTOR (Ser^{2448}) and 4E-BP1 (Thr^{46}) using commercially-available phosphoELISA kits (Invitrogen, Carlsbad, CA, USA). The sensitivity of these assays is reported by the manufacturer to be less than 1 U⁄mL. The absorbances, which are directly proportional to the concentration in the samples, were determined at 450 nm with a microplate reader (iMark, Bio-Rad, Hercules, CA). A set of standards of known concentrations for each phosphorylated muscle variable was utilized to construct standard curves by plotting the net absorbance values of the standards against their respective protein concentrations. Using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA, USA), the concentrations in the muscle samples were appropriately calculated and expressed relative to total protein content. The overall intra-assay percent coefficients of variation were 8.4% and 7.8%, respectively, for mTOR and 4E-BP1.

2.10 IRS-1, Akt, and p70S6K analyses

Muscle homogenates were analyzed in duplicate for IRS-1 (Ser^{636}/Ser^{639}), Akt (Ser^{473}), and p70S6K (Thr^{421}/Ser^{424}) with the Bio-Plex multiplex assay (Bio-Rad, Hercules, CA,

USA), which simultaneously detects the relative quantity of all targeted phosphoproteins in a similar fashion as a capture sandwich immunoassay using the Luminex 100 fluorometric/colorimetric system. Phosphoprotein concentrations were expressed relative to total protein content. The overall intra-assay percent coefficients of variation were 6.5%, 6.9%, and 7.2% respectively, for IRS-1, Akt, and p70S6K.

2.11 Statistical analyses

A one-way analysis of variance (ANOVA) was performed to assess for baseline differences among the dependent variables. Data from blood and muscle samples were analyzed a 3 x 4 [group (CHO, CHO + BCAA, PLC) x time (PRE, 0.5 hr, 2 hr, and 6 hr post-exercise)] two-way ANOVA. Significant main effects were further evaluated using Bonferroni adjusted pairwise contrasts. Significant differences among groups were identified by a Tukey HSD post-hoc test. However, to protect against Type I error, the conservative Hunyh-Feldt Epsilon correction factor was used to evaluate observed within-group F-ratios. The index of effect size utilized was partial Eta squared ($\eta 2$), which estimates the proportion of variance in the dependent variable that can be explained by the independent variable. Partial Eta squared effect sizes were determined to be: weak = 0.17, medium = 0.24, strong = 0.51, very strong = 0.70 [23]. Analyses were performed with SPSS for Windows version 20 statistical package (Microsoft Corporation, Chicago, IL, USA) with the *a priori* alpha set at $p \leq 0.05$. A *posteriori*

power calculation on p70S6K showed that 10 participants per group was adequate to detect a significant difference between groups given a type I error rate of 0.05 and a power of 0.80.

3. Results

3.1 Descriptive statistics

Descriptive data are reported as mean ± SD. At baseline, there were no significant differences among groups in regards to height (180 ± 1.52 cm), body mass (81.8 ± 2.65 kg), or age (20.85 ± 0.51 yr). Analysis of the food records did not reveal differences among groups for intakes of energy (9,678.0 ± 475.33 kJ/d), fat (90.2 ± 6.09 g/d), CHO (278.4 ± 15.96 g/d), PRO (98.2 ± 6.38 g/d), or dietary fiber (14.9 ± 0.91 g/d). Similarly, RE volumes for each bout were 17,784 ±6,600 kg, 17,054 ±8,160 kg, and 15,547 ±3,384 kg, respectively, for PLC, CHO, and CHO + BCAA, and did not differ significantly among groups. Further analysis revealed no significant baseline differences between groups for serum glucose and insulin as well as any of the skeletal muscle phosphoproteins ($p > 0.05$).

3.2 Serum glucose and insulin

A significant group x time interaction was observed for serum glucose [F(3.7)=7.4, p=0.001, effect size = 0.38]. Pair-wise comparisons of the significant main effect for time indicated a significant increase in glucose at 0.5 hr post-exercise. However, post-hoc testing indicated that the CHO and CHO + BCAA, while significantly increased at 0.5 hr post-exercise, were statistically different from PLC (Table 1).

For insulin, a statistically significant group x time interaction was observed [F(3.3) = 9.52, p = 0.001, effect size = 0.44]. Pair-wise comparisons showed that insulin levels were significantly increased 0.5 hr after ingestion of both experimental supplements. Post-hoc tests indicated that both CHO and CHO + BCAA groups, while not different from each other, were statistically different from PLC (Table 1).

3.3 Skeletal muscle phosphoproteins

While the results revealed no significant interactions for any of the phsophoproteins, there was a significant group main effect for IRS-1 (F = 6.77, p = 0.002, effect size = 0.12). Pair-wise comparisons revealed CHO and CHO + BCAA to be significantly

greater than PLC. There were also significant time main effects for IRS-1 ($F = 5.62$, $p = 0.001$, effect size = 0.15), Akt ($F = 4.12$, $p = 0.031$, effect size = 0.13), mTOR ($F = 4.88$, $p = 0.003$, effect size = 0.13), and p70S6K ($F=8.27$, $p = 0.001$, effect size = 0.21). Pair-wise comparisons demonstrated that IRS-1, Akt, and mTOR were significantly increased at 0.5 and 2 hr post-exercise, whereas p70S6K activity was significantly increased at 6 hr post-exercise. No significant main effects for Time were observed for 4E-BP1 ($F = 1.65$, $p = 0.18$, effect size = 0.05).

4. Discussion

The purpose of this investigation was to evaluate the effects of the peri-exercise co-ingestion of CHO and BCAA on the phosphorylation status of intermediates contained within the PI3K/Akt-mTOR signaling pathway in response to a single bout of RE. Emerging literature suggests there is synergism between BCAAs and insulin's effects on cell signaling pathways; thus, optimized translation initiation may require a combination of BCAAs and insulin [24-27]. Hormonal, nutritional, and mechanical factors have been suggested to stimulate cell signaling pathways associated with increased MPS. Therefore, we wanted to explore whether the peri-exercise co-ingestion of CHO and BCAA (to stimulate insulin secretion while providing amino acids suggested to stimulate MPS) in response to RE would optimize cell signaling markers indicative of MPS. We hypothesized that the augmented insulogenic response from the co-ingestion CHO and

BCAA ingestion in the peri-exercise period would be associated with enhanced activation of signaling markers indicative of MPS in the post-exercise period when compared to CHO or an energy-free PLC. We failed to demonstrate an augmented insulogenic response from the co-ingestion of CHO and BCAA and that the effects of signaling markers of the PI3K/Akt-mTOR pathway were not preferentially affected compared to CHO. Therefore, based on our findings, we must reject our hypothesis.

A previous study found that 6 g of amino acids and/or 35 g of CHO consumed one hr after resistance exercise had no differential effect on MPS [28]. The present study demonstrates similar results; however, our main findings suggest that provision of CHO or CHO + BCAA results in no preferential increase in activity of signaling intermediates of the PI3K/Akt-mTOR pathway above that elicited by a single bout of RE. Interestingly, we observed these outcomes despite significant increases in insulin in both groups occurring at 30 min post-exercise. Since leucine is an insulin secretagogue, we hypothesized that insulin would permissively elicit a strong response in PI3K/Akt-mTOR activity due to the co-ingestion of CHO and BCAA in the post-exercise period. Instead, our hypothesis cannot be accepted since we report an increase in insulin that was not different between CHO and CHO + BCAA groups, thereby removing insulin as a potential anabolic trigger mechanism in which MPS may be augmented due to the co-ingestion of CHO and BCAA.

In the present study, in the CHO and CHO + BCAA groups, we discovered IRS-1 activity to be greater than PLC, and to also be significantly increased at 0.5 and 2 hr post-exercise. In regard to the signaling intermediates downstream of IRS-1, Akt, and mTOR were significantly increased at 0.5 and 2 hr post-exercise, and p70S6K at 6 hr post-exercise. However, the increases in these signaling intermediates occurring in CHO and CHO + BCAA were no different than those observed with PLC, suggesting a RE-dependent increase in the activity of markers indicative of MPS. We used the same BCAA dosing protocol as previous studies [15,16]. However, unlike our present study in both prior studies they found that BCAA, compared only to a non-CHO placebo, increased P70S6K phosphorylation one hour after a single bout of RE. Consequently, in agreement with our present study, one of these studies showed that mTOR activity exhibited a significant increase post-exercise, independent of BCAA supplementation [16].

In the presence of elevated insulin levels in response to CHO and CHO + BCAA ingestion, our findings demonstrate that CHO and CHO + BCAA effectively, but equally, increased activation of IRS-1. However, in regard to the downstream signaling intermediates of IRS-1, there was only a RE-induced activation suggesting that the up-regulation of the tyrosine kinase-mediated insulin receptor, along with the subsequent activation of other downstream signaling pathway intermediates (i.e., Akt, mTOR, and p70S6K) associated with MPS, can occur independent of CHO and/or BCAA ingestion.

The lipid kinase PI3K acts downstream of the receptor tyrosine kinases to mediate signals from insulin to promote cell growth. Serine phosphorylation of proteins, such as mTOR and p70S6K, downstream of IRS-1 are implicated in feedback inhibition serving to terminate insulin signaling [29]. We found that that RE, but not CHO and/or BCAA ingestion was associated with mTOR activation at 0.5 and 2 hr post-exercise. This is not entirely surprising since we also observed both upstream effectors of mTOR (IRS-1 and Akt) to also be activated by RE. Furthermore, we also observed RE-induced activation of the downstream effector p70S6K, but not 4E-BP1.

Akt is a putative regulatory interface between mechanical loading and downstream activity of signaling-related enzymes [30]. We found Akt phosphorylation to be RE-responsive and was significantly increased at 0.5 and 2 hr post-exercise. Phosphorylation of this serine-threonine kinase is typically associated with translation of extracellular inputs to regulate a range of biological responses [31] including TOP mRNA and global mRNA translation in skeletal muscle tissue. Akt has been shown to rapidly and transiently increase in phosphorylation following exercise [32]; although, Akt phosphorylation in response to RE may or may not correlate with the intensity of exercise [33].

Our findings demonstrate a significant effect of CHO supplementation on Akt phosphorylation, regardless of BCAA and RE. Since we also observed an increase in mTOR activity at 0.5 and 2 hr post-exercise that was dependent on RE, but not CHO and/or BCAA ingestion, our findings provide support for the observation that the PI3K signaling pathway, which is associated with MPS, but upstream of Akt-mTOR, may be Akt-independent in response to RE. The mechanical stress of RE has the potential to override exogenous nutritional signals in the post-exercise period. Muscle damage associated with eccentric contractions can impair insulin stimulation of IRS-1, thereby negatively impacting PI3K-Akt signaling in skeletal muscle tissue through transient systemic insulin resistance [34-36]. Therefore, negative regulation of IRS-1 and Akt during physiological perturbations is associated with uncoupling of insulin signaling and its effectors resulting in insulin resistance [29]. Based on this possibility, our RE bout was sufficiently intense and/or the recovery periods assessed appropriately-timed to note increases in Akt-mTOR signaling, even though we did not observe an increase in 4E-BP1 phosphorylation. This is an important consideration given that the PI3K/Akt-mTOR pathway can be temporally evaluated and has been associated with rapid changes within the first few hours post-exercise [25,37] followed by longer term changes (e.g., six hr post-exercise) associated with increased ribosomal capacity [38-40]. In humans, increased MPS has been reported between 24 hr [41] and 48 hr after a single bout of RE [42], but as early as two to three hr post-exercise [43].

Glover et al. recently demonstrated neither RE nor mixed-meal feeding impacted the phosphorylation status of mTOR, while increased p70S6K phosphorylation was observed up to six hr following RE, which was augmented by feeding [44]. Others have also not shown mTOR activation after a single bout of RE [45,46] or with mixed meal feeding [47]. However, there is evidence that mTOR and 4E-BP1 signaling is discrepant in the period following a single bout of RE as the phosphorylation status of 4E-BP1 has been shown to decrease immediately post-exercise [43,47]. However, from two to six hr post-exercise 4E-BP1 phosphorylation increases, with either no change [47] or increases [43], in mTOR activity. Increased mTOR phosphorylation in the one to two hr post-exercise period corresponds to observed increases in fractional synthesis rates [43].

It is also important to bear in mind some of the limitations of our study. For example, there are data to support the hypotheses that BCAA stimulate molecular markers of translation initiation in human skeletal muscle tissue, and that insulin secretion (as elicited by CHO ingestion) potentiates this response [9-13]. That we were unable to demonstrate such a response may have been due to having an insufficient number of participants subjects in each group to allow detection of between-group differences. We did perform a *posteriori* power analysis and discovered that our study was slightly underpowered. With such a small n-size, there is an increased likelihood of committing a type II error. Another limitation is that we used signaling pathway intermediates indicative of MPS, rather than directly assessing fractional synthesis rate. As a result,

one should exercise caution when using markers of MPS to interpret actual changes in MPS in humans, as these events are not tightly coupled [48].

Peri-exercise nutrient provision can be a beneficial component to recovery from RE regarding its ability to assist in increasing MPS and decreasing muscle proteolysis, thereby improving muscle performance during successive bouts of resistance training. However, MPS is preferentially impacted by the co-ingestion of CHO and BCAA compared to CHO alone, is not well known. In summary, the results of the current investigation demonstrate that the ingestion CHO or CHO + BCAA preferentially augments RE-induced IRS-1 phosphorylation, but not of Akt, mTOR, and p70S6K as we hypothesized, despite a significant increase in insulin levels 0.5 hr post-exercise in the CHO-containing groups. Therefore, we conclude that the peri-exercise co-ingestion of BCAA and CHO does not preferentially augment resistance exercise-induced increases in skeletal muscle signaling markers indicative of muscle protein synthesis when compared to CHO alone.

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The authors declare that they have no competing interests.

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