Co-ingestion of branched-chain amino acids and carbohydrate stimulates myofibrillar protein synthesis following resistance exercise in trained young men

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Running head: Stimulation of protein synthesis with BCAA and carbohydrate

Abstract

Branched-chain amino acids (BCAA) and carbohydrate (CHO) are commonly recommended postexercise supplements. However, no study has examined the interaction of CHO and BCAA ingestion on myofibrillar protein synthesis (MyoPS) rates following exercise. We aimed to determine the response of MyoPS to the co-ingestion of BCAA and CHO following an acute bout of resistance exercise. Ten resistance-trained young men completed two trials in counter-balanced order, ingesting isocaloric drinks containing either 30.6g CHO plus 5.6g BCAA (B+C) or 34.7g CHO alone (CON) following a bout of unilateral, leg resistance exercise. MyoPS was measured postexercise with a primed, constant infusion of L-[ring$^{13}C_6$]phenylalanine and collection of muscle biopsies pre and 4h post drink ingestion. Blood samples were collected at time-points before and after drink ingestion. Serum insulin concentrations increased to a similar extent in both trials ($p>0.05$), peaking at 30 min post drink ingestion. Plasma leucine (514±34 nmol/L), isoleucine (282±23 nmol/L) and valine (687±33 nmol/L) concentrations peaked at 0.5h post drink in B+C and remained elevated for 3h during exercise recovery. MyoPS was ~15% greater (95%CI [-0.002, 0.028], $p=0.039$, Cohen's $d=0.63$) in B+C (0.128±0.011%/h) than CON (0.115±0.011 %/h) over the 4h post-exercise period. Co-ingestion of BCAA and CHO augments the acute response of MyoPS to resistance exercise in trained young males.

Keywords: leucine, fractional synthetic rate, muscle anabolism

Introduction

Nutritional modulation of the muscle anabolic response to exercise is underpinned by changes in muscle protein turnover at the metabolic level (Tipton & Wolfe, 2001). Ingesting an amino acid source following resistance exercise stimulates muscle protein synthesis (MPS), leading to a positive net muscle protein balance (Biolo et al., 1997) and muscle hypertrophy. An exogenous source of essential amino acids (EAA) is necessary for stimulation of MPS (Tipton et al., 1999), i.e. endogenous nonessential amino acids are sufficient to support increased rates of MPS stimulated by exercise and exogenous EAA. The branched-chain amino acids (BCAA), especially leucine, are known to stimulate MPS, as evidenced by *in vitro* (Atherton et al., 2010) and *in vivo* rodent (Anthony et al., 1999) and human (Jackman et al., 2017; Wilkinson et al., 2013) studies. Thus, BCAA supplementation is a popular nutritional approach to enhance muscle anabolism, relevant to both athletic and clinical populations (Attlee et al., 2018; Ruano & Teixeira, 2020).

We previously demonstrated that ingestion of BCAA alone enhanced the MPS response to resistance exercise (Jackman et al., 2017). However, the MPS response to BCAA following exercise (Jackman et al., 2017) appeared to be inferior, at least in qualitative terms, to the response to intact protein containing the same amount of BCAA measured in a separate study using identical methods (Witard et al., 2014). The lack of sufficient EAA for substrate to sustain the MPS response during the latter stages of postexercise recovery has been proposed to explain this reduced anabolic response (Jackman et al., 2017; Stokes et al., 2018). Direct evidence for this idea stems from a study in which the stimulation of MPS by BCAA ingestion alone was directly compared to ingestion of a source of intact protein (Fuchs et al., 2019). The early (0-2h) response of MPS was similar between conditions, but as the

postprandial period progressed (2-5h), MPS was not sustained following ingestion of BCAA alone. Thus, the efficacy of postexercise BCAA supplementation to maximise muscle anabolism has been questioned (Plotkin et al., 2021).

Carbohydrate (CHO) ingestion is commonly recommended as a postexercise nutritional strategy. Muscle glycogen is decreased by resistance exercise (Koopman et al., 2006) and postexercise CHO is often recommended to stimulate the resynthesis of muscle glycogen during recovery (Roy & Tarnopolsky, 1998; Slater & Phillips, 2011). CHO supplementation also has been shown to elevate the release of potentially anabolic hormones such as insulin and stimulate a net muscle protein synthesis during recovery from resistance exercise (Borsheim, Cree, et al., 2004) . Given the popularity of BCAA and CHO supplementation after training, it is important to understand the interaction between these nutrients on muscle anabolism. Co-ingestion of CHO and an amino acid source have been recommended to enhance the muscle anabolic response following resistance exercise (Borsheim, Aarsland, et al., 2004; Miller et al., 2003), attributed to an increased stimulation of MPS and relatively minor attenuation of muscle protein breakdown (Glynn et al., 2010). However, to date no study has reported the impact of adding BCAA (i.e., no other EAA) to CHO ingestion on the muscle anabolic response to resistance exercise in humans.

It is not clear whether ingestion of BCAA in addition to CHO following resistance exercise enhances muscle anabolism. Elevating insulin concentrations increases mixed MPS rates, while potentially reducing circulating amino acid availability for MPS in the exercised muscle (Borsheim, Cree, et al., 2004). Since the response of MPS to BCAA ingestion is limited by EAA availability (Fuchs et al., 2019; Jackman et al., 2017), a further reduction in EAA availability due to CHO ingestion

125 may not be desirable. Thus, the aim of this study was to determine the response of
126 myofibrillar protein synthesis (MyoPS) to the addition of BCAA to CHO ingestion
127 following resistance exercise in trained, young men.
128

Methods

Participants and study design

Eleven healthy (body mass: 86.9±9.5 kg; percent lean mass: 69±5%) resistance-trained (≥ 2 sessions/wk for ≥ 6 months) young (21±1y) males were recruited for this crossover, double-blind, randomised, and counterbalanced study. Due to an unrelated skin condition that caused issues with biopsy healing only 10 volunteers completed both trials. A power calculation (Gpower v3.1) conducted a priori based on Jackman et al (2017) suggested that a sample size of 10 participants (effect size: 0.97; power: 0.85) would be sufficient to detect a difference in MPS between conditions.

Following screening, informed consent and preliminary testing, participants reported to the laboratory on five occasions, including two isotope infusion trials. Trials were separated by ~3 wk. Prior to trials, body composition was assessed using dual-energy x-ray absorptiometry (DEXA) and maximal strength, i.e., one repetition maximum (1RM), was predicted for each leg individually. Approximately 1 wk later, each participant returned to the laboratory to confirm their single leg 1RM. Two or three days later, participants performed their first blinded trial in which they consumed either a BCAA and CHO containing beverage (B+C) or a CHO only (CON) beverage (GlaxoSmithKline, Brentford, UK; Table 1). Participants performed a unilateral bout of resistance exercise prior to consuming the test drink. MyoPS was determined by measurement of the incorporation of L-[ring-$^{13}C_6$] phenylalanine into myofibrillar proteins during a primed continuous infusion. The infusion protocol was repeated on the contralateral leg ~3 wk after the first trial. Trial and exercised leg order were counter-balanced and randomised. All trials were conducted in

accordance with the Declaration of Helsinki and following ethical approval by the National Research Ethics Service ethics board (Warwickshire, UK) and registered as a clinical trial (ISRCTN98737111).

INSERT TABLE 1 HERE

Preliminary testing

Strength testing

Prediction of 1RM was completed using a published method (Verdijk et al., 2009). Briefly, following a warm up on each leg, participants completed as many repetitions of leg press or leg extension as possible with an applied resistance equating to their self- predicted 80% 1RM. Values for load and repetitions were inserted into equation 1 to estimate 1RM (Mayhew et al., 1995).

$$1RM = \frac{load}{(1.0278 - 0.0278) \bullet reps} \quad (1)$$

Confirmation of each participant's 1RM was completed 2-3 day prior to each trial day (Kraemer, 1995). Briefly, volunteers completed the maximum number of repetitions possible at a load of 90% from equation 1. The load was then increased by 5-10% until only one repetition could be completed. A rest period of 3 min was provided between each effort.

Diet and physical activity:

Prior to the experimental trial, participants completed a 3 day diet diary that represented their habitual daily intake. The average energy intake (2816±701 kcal)

178 and macronutrient composition (protein: 1.8±0.7 g·kgBM^{-1}·day^{-1}; carbohydrate:
179 3.9±1.5 g·kgBM^{-1}·d^{-1}; fat: 1.2±0.4 g·kgBM^{-1}·d^{-1}) from
180 the diet diary was used to calculate the participant's diet before the experimental
181 trial. Food parcels that matched each participant's habitual energy and
182 macronutrient intakes were supplied for 48 h before the experimental trial.
183 Participants were instructed to consume only food and drink sources provided by
184 investigators and to consume their final meal no later than 22:00 on the evening
185 before the experimental trial. Participants also were asked to refrain from alcohol
186 consumption and exercise during this 2 day period. Diet analysis was performed
187 using commercially available software (Wisp v3.0, Tinuviel software). The final meal
188 prior to infusion day was consumed prior to 22:00.
189
190 *Body Composition:*
191 Whole-body and segmental body composition was assessed using DEXA (Hologic
192 Discovery W, Massachusetts), as described previously (Jackman et al., 2017).
193
194 **Experimental Protocol**
195 The experimental protocol is summarised in Figure 1. On the morning of the trial,
196 each participant reported to the laboratory following an overnight fast. Height and
197 body mass were recorded. Next, a cannula was inserted in a forearm vein and a
198 resting blood sample was obtained. Participants were fed a standardised breakfast
199 (30 kJ/kg body mass (BM)) with 30% total energy provided by protein. Participants
200 rested for 75 min before a primed (2 μmol/kgBM) constant infusion (0.050 μmol·min^{-1}·kgBM^{-1}) of L-[*ring*-$^{13}C_6$] phenylalanine (Cambridge Isotope Laboratories Inc,
202 Massachusetts) was started. A hand or wrist vein of the contralateral arm was then

cannulated and heated (~55°C) for frequent arterialised blood sampling throughout the remainder of the protocol. Participants then performed a single bout of unilateral leg resistance exercise 105 min after initiating the infusion that lasted ~25 min. A warm-up on the leg press machine (Cybex International, Medway, Massachusetts, USA) was performed as previously described (Jackman et al., 2017). After 2 min rest, the exercise protocol was completed consisting of 4×10 repetitions at 70% and 75% 1RM on leg press and leg extension machines, respectively. A rest period of 2 min was provided between sets and participants were verbally encouraged throughout the exercise routine. In the event of a failed lift, load was decreased by 4.5 kg. Rating of perceived exertion (RPE) was recorded after each set (Borg, 1982). Arterialised blood samples and a muscle biopsy from the exercised leg were collected within 5 min of exercise cessation (t=0). Arterialised blood samples also were collected at t = -145, -85, -25, 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240 min. A second muscle biopsy was collected at t=240 min.

INSERT FIGURE 1 HERE

Muscle biopsy collection and analysis

Biopsies were obtained from the *vastus lateralis* of the exercised leg under local anaesthesia (1% lidocaine) using a 5mm Bergstrom needle with suction. Different incisions (~1cm apart) were used for each biopsy to minimise the impact of local inflammation on the muscle tissue. Muscle samples were immediately rinsed, blotted of excess blood, visible fat and connective tissue were removed and the biopsy was divided, before being frozen in liquid nitrogen, and stored at -80°C until later analysis. Muscle samples were analysed for enrichment of L-[*ring*-$^{13}C_6$] phenylalanine in the intracellular pool and bound myofibrillar protein fractions.

Blood collection and analysis

Blood was collected in Lithium heparin, ethylene diamine tetra acetic acid and serum separator tubes and centrifuged at 3500 revs·min^{-1} for 15 min at 4°C. Plasma and serum were frozen at -80°C for subsequent analysis. Plasma glucose and urea concentrations were analysed using an automated blood metabolite analyser (Instrumentation Laboratory, Cheshire, UK). Serum insulin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (DRG Diagnostics, USA).

Amino acid concentrations and enrichments

$^{13}C_6$ tyrosine and phenylalanine enrichments were determined by GCMS (model 5973; Hewlett Packard, California), as previously described (Jackman et al., 2017). Phenylalanine, leucine, threonine, isoleucine and valine concentrations were measured using an internal standard method (Jackman et al., 2017).

Myofibrillar protein enrichment

The enrichment of L-[*ring*-$^{13}C_6$] phenylalanine was analysed in the myofibrillar protein fraction. Myofibrillar proteins were isolated from ~30 mg of tissue as previously described (Jackman et al., 2017).

Intracellular protein enrichment

Approximately 20 mg of muscle tissue was used to obtain intracellular $^{13}C_6$ phenylalanine enrichments. Frozen tissue was powdered under liquid nitrogen using a mortar and pestle and 500 µL of 1 M perchloric acid (PCA). The mixture was centrifuged at 10,000 g for 10 min. The supernatant was then neutralized with 2 M

253 potassium hydroxide and 0.2 M PCA and combined with 20 µL of urease for removal
254 of urea. The free amino acids from the intracellular pool were purified on cation-
255 exchange columns as described above. Intracellular amino acids were converted to
256 their N-Methyl-Ntert-butyldimethylsilyltrifluoroacetamide derivative and $^{13}C_6$
257 phenylalanine enrichment was determined by monitoring at ions 234/240 using gas
258 chromatography mass spectrometry (GCMS; model 5973; Hewlett Packard, Palo
259 Alto, CA).
260
261 ***Calculations***

262 *Fractional synthetic rate*
263 The fractional synthetic rate (FSR) of myofibrillar proteins was calculated using the
264 standard precursor-product method:

265 $$FSR\ (\% \cdot h^{-1}) = \left(\frac{\Delta E_m}{E_P \cdot t}\right) \cdot 100 \qquad (2)$$ (Biolo et al., 1997)

266

267 Where ΔE_m is the change in bound $^{13}C_6$ phenylalanine enrichment between two
268 biopsy samples, E_p is the intracellular precursor enrichment and *t* is the time
269 between muscle biopsies.
270
271 *Phenylalanine kinetics*
272 Phenylalanine was chosen as the tracer as it is not oxidised in the muscle or
273 synthesized in the body. Therefore, the appearance rate of phenylalanine in the
274 blood can be attributed to protein breakdown. Whole-body phenylalanine
275 appearance was calculated according to equation 3 (Tipton et al., 1996).

276 $$Ra = \frac{i}{E_p} \qquad (3)$$

where i is the infusion rate of the tracer ($\mu mol \cdot h^{-1} \cdot kgBM^{-1}$) and E_p is the enrichment of phenylalanine.

Data presentation and statistical analyses

Area under the curve (AUC) was calculated for serum insulin concentrations and phenylalanine R_a using Graphpad Prism V9.5.0 (Graphpad software, San Diego, California). Baseline was set at the insulin concentration measured at t=0 (immediately pre drink) resulting in incremental AUC (iAUC). Baseline was set at 0 $\mu mol \cdot min^{-1} \cdot kgBM^{-1}$ when calculating AUC for phenylalanine Ra resulting in total AUC (tAUC).

Plasma and serum concentrations of glucose, insulin, amino acids, and urea were analysed using a two-way repeated measures ANOVA. Where significance was detected, a least significant difference correction was applied for post hoc analysis. A paired samples t-test was used to analyse differences in exercise variables, MyoPS and AUC of serum insulin concentrations and phenylalanine kinetics during the postexercise period. Significance for all analyses was set at $p<0.05$ and effect sizes (η_p^2 or Cohen's d) and 95% confidence intervals (CI) were reported where appropriate. All statistical tests were completed using statistical package for social sciences version 28.0. All values are presented as means ± standard deviation unless otherwise stated.

Results

Exercise variables

There were no differences in RPE (>18) or total weight lifted throughout the exercise protocol (including warm-up) between trials (B+C: 10,698±756 kg; CON: 10,236±801 kg, p>0.050).

Blood metabolites

Plasma glucose concentrations (Figure 2A) increased following breakfast and drink ingestion in both trials (p=0.179) and there was a significant time × trial interaction effect (p<0.001, η_p^2 = 0.462) such that greater glucose concentrations were detected in CON *vs*. B+C at 15 (95%CI [0.069, 0.89], p=0.027), 30 (95%CI [0.732, 2.388], p=0.002), and 45 min (95%CI [0.201, 0.559], p<0.010) post drink. At 60 min post exercise (95% CI [0.201, 0.0559], p=0.034), glucose concentrations were greater in B+C than CON.

Serum insulin concentrations changed over time (p<0.001, η_p^2 = 0.859), however there were no differences between trials (Figure 2B). The iAUC for insulin over 240 min following drink ingestion was similar (p=0.091) between B+C (38±11 µIU/mL × 240 min) and CON (46±12 µIU/mL × 240 min).

INSERT FIGURE 2 HERE

Amino acid concentrations

Main effects of time and trial, and a time × trial interaction was observed for all BCAA concentrations (Figure 3). Peak leucine (514±34µM), isoleucine (282±23µM) and

valine (687±33μM) concentrations were observed at 30 min post drink ingestion. From 15 min post drink until the end of measurement period, leucine ($p<0.002$) and valine ($p<0.001$) concentrations were higher in B+C than CON. Isoleucine concentrations were higher in B+C than CON from 15 min post drink to 120 min post drink ($p<0.005$). Threonine concentrations were increased following breakfast ($p<0.05$); however, following exercise there was a significant decrease in threonine concentrations compared to pre-exercise levels. There were no differences in threonine concentrations between trials.

There was a significant time × trial interaction for phenylalanine concentrations ($p=0.001$). In both trials, phenylalanine concentrations were elevated at 150 min following breakfast ($p<0.001$) compared with baseline, and decreased below baseline from 60 min following exercise until 180 min post exercise. Relative to pre-exercise levels, there was a decrease in phenylalanine concentration from 60 min post drink ingestion until the end of the testing period in both trials. Phenylalanine concentrations were significantly lower in B+C than CON a 75, 90, 150, 180 and 240 min postexercise (all $p<0.050$).

INSERT FIGURE 3 HERE

Urea Concentrations

Plasma urea concentrations declined ($p=0.002$, $\eta_p^2 = 0.437$) from t=0 (end of exercise; B+C: 6.27±1.23 mmol/L; CON: 6.59±1.18 mmol/L) trials at 180 (B+C: 5.98±1.08 mmol/L CON: 6.06±1.23 mmol/L, $p<0.05$) and 240 min (B+C: 5.78±0.99 mmol/L; CON: 5.73±0.89 mmol/L, $p<0.05$) post exercise in both trials. No main effect

of trial (p=0.749) or time × trial interaction (p=0.356) was observed between B+C and CON.

Phenylalanine kinetics

$^{13}C_6$ phenylalanine enrichments

Plasma $^{13}C_6$ phenylalanine enrichment remained stable for the duration of tracer incorporation in both B+C and CON (Figure 4). Intracellular $^{13}C_6$ phenylalanine enrichments remained stable over the tracer incorporation period for B+C and CON (2.7±1.2 t/T and 2.8±1.2% t/T, respectively for both timepoints combined). Trial order did not influence plasma or muscle intracellular $^{13}C_6$ phenylalanine tracer enrichments.

INSERT FIGURE 4 HERE

Phenylalanine R_a

A decrease in phenylalanine R_a was observed during the post drink period compared to baseline in both trials (time effect: $p<0.001$, $\eta_p^2 = 0.847$) (Figure 5A). The tAUC of phenylalanine R_a expressed as tAUC over the entire 4 h post drink period was ~7% lower (95%CI [-20.9, -7.8], p<0.001, Cohen's d=1.6) in B+C than CON (Figure 5B).

INSERT FIGURE 5 HERE

Myofibrillar protein synthesis

Mean myofibrillar FSR was ~15% greater (95%CI [-0.0018, 0.0280], p=0.039, Cohen's d = 0.63) over the 240 min recovery period in B+C than CON (Figure 6).

INSERT FIGURE 6 HERE

Discussion

We investigated the response of MyoPS to the addition of BCAA to CHO ingestion following resistance exercise. Our results indicate that adding BCAA to CHO following resistance exercise increases MyoPS during acute recovery. In qualitative terms, this increase (15%) was similar to the 22% increase in MyoPS with BCAA ingestion alone compared to a nonenergetic placebo reported previously (Jackman et al., 2017). Thus, no additive effect of BCAA co-ingestion with CHO is observed with regards to the post-exercise stimulation of MyoPS. Collectively, these results suggest that despite the popularity of BCAA supplements (Attlee et al., 2018;

Ruano & Teixeira, 2020), BCAA ingestion, with or without CHO, is not sufficient to achieve the maximal anabolic response following resistance exercise.

The effectiveness of combining an amino acid source with CHO to provide a maximal muscle anabolic response following resistance exercise is controversial. Hyperinsulinemia from CHO ingestion presumably is responsible for any anabolic response to CHO. Previous work demonstrates that hyperinsulinemia using local insulin infusion eliminates the systemic decrease in amino acid concentrations and thus availability of amino acids to skeletal muscle, resulting in increased stimulation of MPS (Biolo et al., 1995; Biolo et al., 1999). The modulatory role of hyperinsulinemia in stimulating MPS is less clear following exercise (Biolo et al., 1999). Nevertheless, in a more physiological situation, increasing insulin concentration with the addition of CHO to ingested protein does not potentiate rates of MPS at rest (Glynn et al., 2013) or following exercise (Koopman et al., 2007; Miller et al., 2003; Staples et al., 2011). However, the efficacy of adding a source of amino acids, including BCAA, to CHO is less well studied. The addition of EAA to CHO administered in two boluses following resistance exercise resulted in an increased stimulation of MPS compared to CHO alone (Miller et al., 2003). Moreover, Koopman and colleagues (2005) compared the response of MPS to CHO alone, with CHO plus protein (casein hydrolysate) and CHO plus protein and supplemental leucine after exercise. Whereas mixed-MPS was not increased with ingested protein in addition to CHO, MPS was greater than CHO alone when leucine was ingested in addition to CHO and protein. These results are generally consistent with our previous findings, i.e. adding a sufficient source of leucine to CHO following exercise increases MPS (Jackman et al., 2017). Moreover, the increase in MPS when BCAA are ingested in addition to CHO relative to ingesting CHO alone (~15%) is not dissimilar to the

422 increase observed when BCAA alone are ingested compared to a placebo (~22%)
423 (Jackman et al., 2017). Indeed, a retrospective statistical comparison of the
424 differences in MyoPS between respective BCAA and control conditions in past
425 (Jackman et al., 2017) and present studies revealed no significant differences in the
426 magnitude of increased stimulation of MyoPS with BCAA ingestion. Hence, taken
427 together, these results suggest no clear interaction exists between BCAA and CHO
428 that impacts MPS following exercise. Hence, while combining CHO and BCAA
429 following exercise is often recommended, the efficacy of such a strategy for
430 stimulation of muscle anabolism does not appear to be supported.
431 Our results indirectly support the notion that BCAA ingestion stimulates
432 MyoPS but, without a source of exogenous EAA, the amplitude of the acute post-
433 exercise increase in MyoPS is not maximised. We acknowledge that caution must
434 prevail when comparisons are made studies that did not use identical methods to
435 measure MPS. Nonetheless, this observation is consistent with previous data
436 (Koopman et al., 2005; Miller et al., 2003) that demonstrated ingestion of a source of
437 amino acids in addition to CHO stimulated a robust increase in MPS that was, at
438 least qualitatively, greater than observed in the present study with combined BCAA
439 and CHO ingestion. Participants in both Miller et al. (2003) and Koopman et al.
440 (2005) ingested an amino acid source that provided all EAA in addition to BCAA,
441 thus preventing the decline in EAA availability observed in our previous (Jackman et
442 al., 2017) and current (Figure 3) study. This observation supports the notion that
443 BCAA ingestion without co-ingestion of a full complement of amino acids may not
444 result in an optimal muscle anabolic environment following resistance exercise.
445 Both insulin and BCAA modulate protein breakdown as well as protein
446 synthesis. Hyperinsulinemia decreases protein breakdown on both whole-body

(Denne et al., 1991; Shangraw et al., 1988) and muscle (Biolo et al., 1995; Biolo et al., 1999; Meek et al., 1998) levels at rest. BCAA ingestion decreases whole-body (Louard et al., 1990; Nair et al., 1992) and muscle protein breakdown at rest (Ferrando et al., 1995) and following resistance exercise (Borsheim, Cree, et al., 2004). Muscle protein breakdown was not directly measured in the present study, but our results indicate that whole-body protein breakdown was decreased by adding BCAA to CHO. Indicators of whole-body protein breakdown, i.e., urea concentration and phenylalanine R_a, decreased with BCAA ingestion in addition to CHO. Since the insulin response was identical between trials, the response of whole-body protein breakdown appears to be due to BCAA ingestion, as indicated in our previous study (Jackman et al., 2017). Thus, as with MPS, these collective results suggest no interaction exists between BCAA and insulin on whole-body protein breakdown. However, our results should not be interpreted to indicate that BCAA ingestion reduced myofibrillar protein breakdown since the responses of whole-body and muscle metabolism to various stimuli do not necessarily match (Tipton & Wolfe, 1998). Moreover, based on recent findings generated within a home-based resistance exercise setting (Waskiw-Ford et al., 2022), we speculate that the provision of all EAA rather than BCAA alone would be required to further attenuate the decline in protein breakdown following exercise.

Novelty statement: To conclude, our results demonstrate for the first time that the addition of BCAA to CHO ingestion results in an increased stimulation of MyoPS following resistance exercise.

Practical application statement: The combined ingestion of BCAA and CHO supports greater myofibrillar protein synthesis RATES after exercise than CHO alone.

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Funding:
Supported by GlaxoSmithKline Healthcare (research grant to KDT)

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TABLES

Table 1: Test drink composition

	B+C	CON
Calories (Kcal)	141	140
Leucine (g)	2.8	0
Isoleucine (g)	1.4	0
Valine (g)	1.9	0
Carbohydrate (g)	30.6	34.7
Fat (g)	0.1	0.1
Sodium (mg)	277	276

Tests drinks were consumed after the unilateral bout of resistance exercise and were either a branched-chain amino acid containing beverage (B+C) or a carbohydrate only beverage (CON).

FIGURES

Figure 1: Schematic diagram of the infusion protocol. A baseline blood sample was collected before participants consumed an energy-rich, high-protein breakfast. A bout of unilateral leg-resistance exercise was performed 3 h after breakfast. Muscle biopsies (*vastus lateralis*) were collected from the exercised leg immediately prior (0 h), and 4 h-post drink ingestion. Drink ingestion was either a branched-chain amino acid and carbohydrate containing beverage (B+C) or carbohydrate only (CON). Multiple blood samples were collected throughout the protocol.

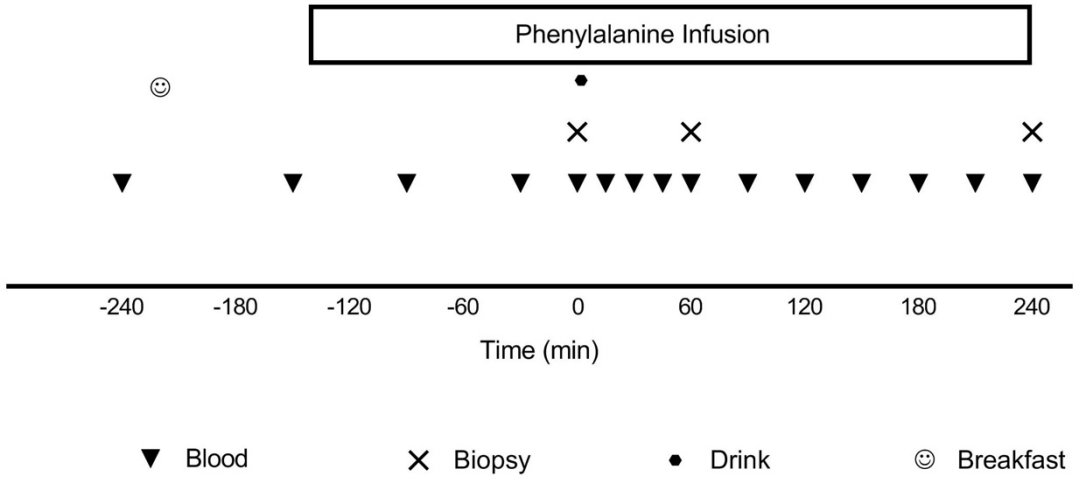

Figure 2: Plasma glucose (A) and serum insulin (B) concentrations in response to consumption of pre-exercise breakfast (-240 min) and ingestion of a branched-chain amino acid plus carbohydrate (B+C, closed circles) or carbohydrate (CON, open circles) beverage following resistance exercise (0-240 min). Data are presented as means ± SEM.

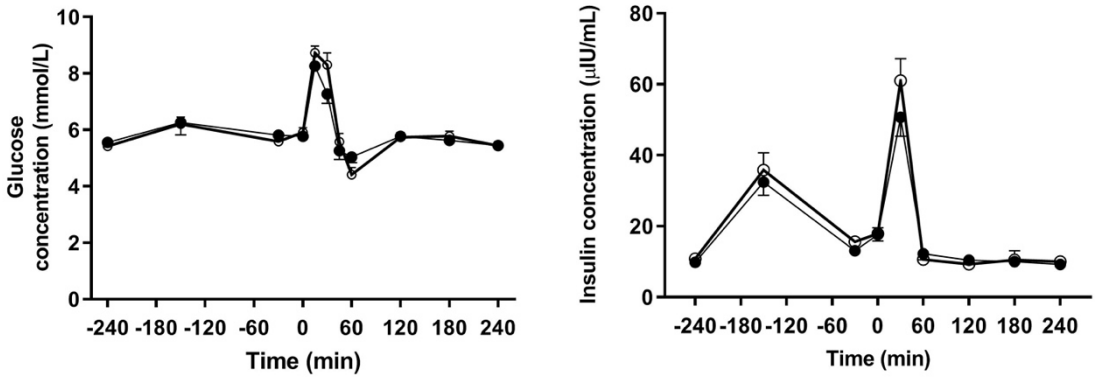

Figure 3: Plasma concentrations of A) leucine, B) isoleucine, C) valine, D) threonine and E) phenylalanine following consumption of pre-exercise breakfast (Time -240 min) and pre and post ingestion of either a branched-chain amino acid and carbohydrate (B+C, closed circles) or carbohydrate (CON, open circles) beverage following intense resistance exercise (Time 0). Data are presented as means ± SEM.* significant difference between trials.

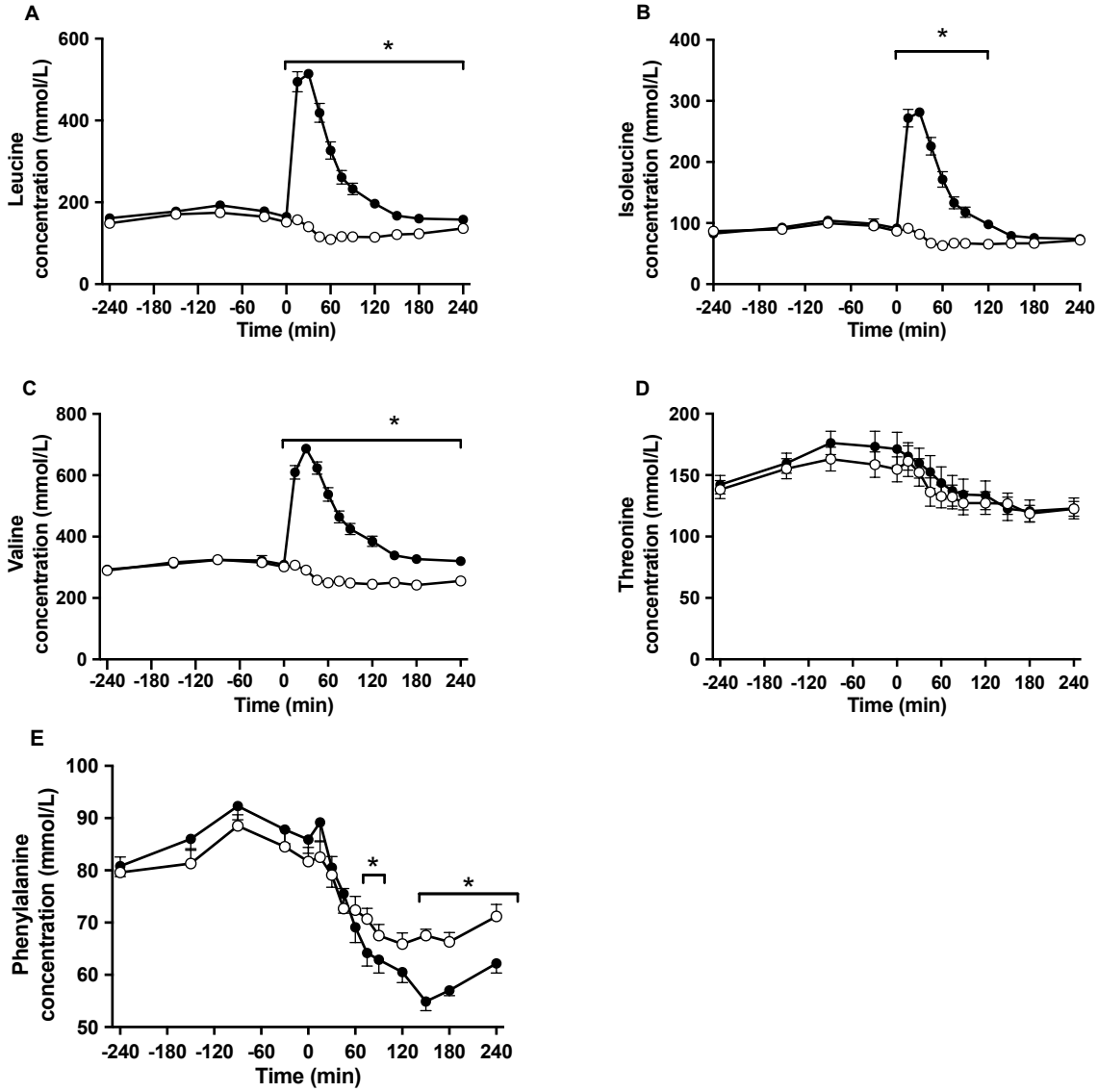

Figure 4: Plasma $^{13}C_6$ enrichments following consumption of pre-exercise breakfast (Time -240 min) and pre and post ingestion of either a branched chain amino acid and carbohydrate (B+C, closed circles) or carbohydrate (CON, open circles) beverage following resistance exercise (Time 0). Data are presented as means ± SEM.

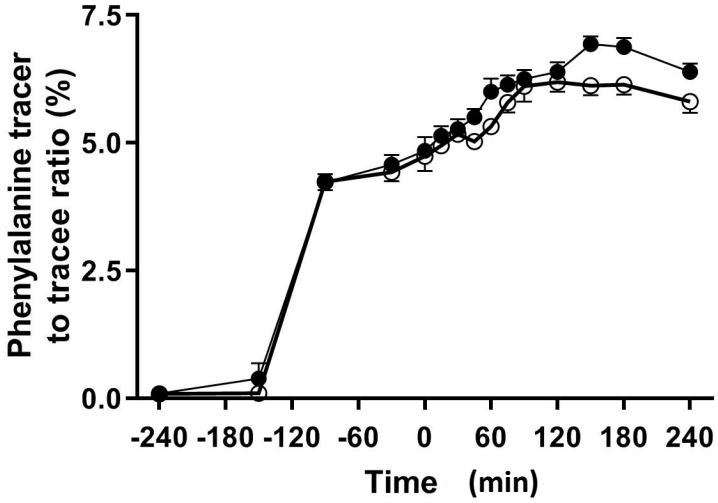

Figure 5: Phenylalanine rate of appearance (Ra) (following ingestion of a branched-chain amino acid plus carbohydrate (B+C, closed circles) or carbohydrate only (CON, open circles) beverage following resistance exercise (Time 0 min). Data (means ± SEM) are expressed over time (A) and as area under the curve (B). * significant difference compared to CON.

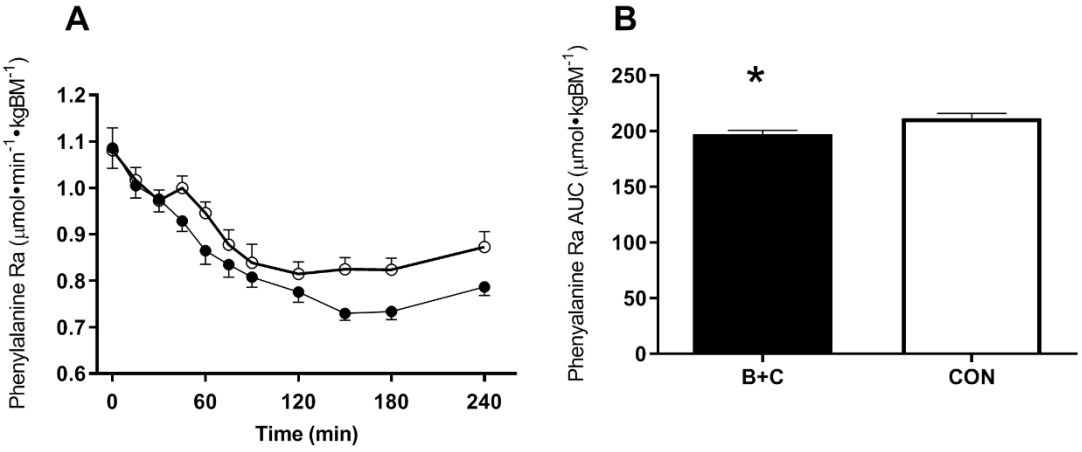

Figure 6: Fractional synthesis rate (FSR) of myofibrillar protein following ingestion of either a branched-chain amino acid plus carbohydrate (B+C) or carbohydrate alone (CON) beverage following intense resistance exercise. Data are presented as means and individual data points.* significantly higher compared to CON