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The effects of branched-chain amino acids on muscle protein synthesis, muscle protein breakdown and associated molecular signalling responses in humans: an update

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Abstract

Branched-chain amino acids (BCAA: leucine, isoleucine and valine) are three of the nine indispensable amino acids, and are frequently consumed as a dietary supplement by athletes and recreationally active individuals alike. The popularity of BCAA supplements is largely predicated on the notion that they can stimulate rates of muscle protein synthesis (MPS) and suppress rates of muscle protein breakdown (MPB), the combination of which promotes a net anabolic response in skeletal muscle. To date, several studies have shown that BCAA (particularly leucine) increase the phosphorylation status of key proteins within the mechanistic target of rapamycin (mTOR) signalling pathway involved in the regulation of translation initiation in human muscle. Early research in humans demonstrated that BCAA provision reduced indices of whole-body protein breakdown and MPB; however, there was no stimulatory effect of BCAA on MPS. In contrast, recent work has demonstrated that BCAA intake can stimulate postprandial MPS rates at rest and can further increase MPS rates during recovery after a bout of resistance exercise. The purpose of this evidence-based narrative review is to critically appraise the available research pertaining to studies examining the effects of BCAA on MPS, MPB and associated molecular signalling responses in humans. Overall, BCAA can activate molecular pathways that regulate translation initiation, reduce indices of whole-body and MPB, and transiently stimulate MPS rates. However, the stimulatory effect of BCAA on MPS rates is less than the response observed following ingestion of a complete protein source providing the full complement of indispensable amino acids.

Keywords: **Branched-chain amino acids: Skeletal muscle: Muscle protein synthesis: Muscle protein breakdown: Exercise training: Molecular signalling**

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Introduction

Amino acids are the fundamental building blocks of skeletal muscle and other bodily proteins. In total, there are twenty proteinogenic amino acids that when combined via peptide bonds produce proteins that are incorporated into various tissues. Nine of the twenty amino acids are deemed 'indispensable' or essential amino acids (EAA); the human body is unable to synthesize them endogenously in quantities sufficient to meet requirements[(1)], and they therefore must be obtained exogenously via dietary intake. The branched-chain amino acids (BCAA) represent three of the nine EAA that together account for ~14% of the amino acids found in skeletal muscle proteins[(2)]. They are neutral (nonpolar and hydrophobic) amino acids and are unique in that they contain a non-linear (branched) aliphatic side chain. At the whole-body level, BCAA physiology can be divided into a tissue pool and a circulating pool. BCAA that are derived from dietary intake or liberated from protein via protein breakdown appear in the circulation. BCAA are then taken up from the circulation into body tissues (e.g. skeletal muscle) where they can be either oxidized or incorporated into proteins

Abbreviations: 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; Akt, protein kinase B; A-V, arteriovenous; BCAA, branched chain amino acids; BCAT, branched-chain amino transferase; BCKDH, branched chain amino acid dehydrogenase; CASTOR1, cellular arginine sensor for mTORC1; EAA, essential amino acids; eEF2, eukaryotic elongation factor 2; eIF4E, eukaryotic translation initiation factor 4E; ERK1/2, extracellular signal-regulated kinases 1/2; FOXO3, forkhead box O3; FSR, fractional synthesis rate; GATOR1, GAP activity towards the Rags 1; GATOR2, GAP activity towards the Rags 2; IAAO, indicator amino acid oxidation; LAT1, L-type amino acid transporter 1; LAT2, L-type amino acid transporter 2; LNAA, large neutral amino acid; MAFbx, muscle-atrophy F-box; MAPK, mitogen-activated protein kinase; MPS, muscle protein synthesis; MPB, muscle protein breakdown; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; mTORC2, mechanistic target of rapamycin complex 2; MuRF1, muscle RING-finger protein-1; MyoPS, myofibrillar protein synthesis; NEAA, non-essential amino acids; NPB, net protein balance; p70S6K1, p70 S6 kinase 1; PRAS40, proline-rich Akt substrate of 40 kDa; Ra, rate of appearance; Rd, rate of disappearance; Rheb, Ras homolog enriched in brain; rpS6, ribosomal protein S6; SAMTOR, *S*-adenosylmethionine sensor upstream of mTORC1; SNAT, sodium-dependent neutral amino acid transporter 2.

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via protein synthesis[3]. The tissue distribution of enzymes involved in BCAA catabolism and oxidation (i.e. branched chain amino transferase (BCAT) and branched chain amino acid dehydrogenase (BCKDH) complex) differs between humans and rats[4,5]. Specifically, total activity of both BCAT and BCKDH enzymes is lower in humans than rats, and distribution of BCKDH (oxidative) capacity among body tissues in humans is greatest in skeletal muscle[4–6]. In addition to serving as amino acid substrates for protein synthesis, the BCAA have long been thought to have a unique role in the regulation of skeletal muscle protein turnover. Testing of the effects of BCAA on muscle protein synthesis (MPS) and muscle protein breakdown (MPB) was initially carried out on isolated rodent muscle[7,8]. These early *in vitro* studies found that BCAA stimulated rates of MPS and reduced MPB. However, when the individual BCAA were examined, the stimulatory effect on MPS was attributed primarily to leucine (not isoleucine or valine)[7,9]. In contrast, perfusion of isolated rodent muscle with an amino acid mixture devoid of BCAA has no effect on MPS[10]. In support of these findings, subsequent research has determined that, of the three BCAA, leucine appears to be a key regulator of the mechanistic target of rapamycin (mTOR)[11], a pivotal multi-subunit complex recognized for its key role in the regulation protein synthesis and cellular growth[12]. Therefore, while there are many commonalities amongst the BCAA in terms of their structure and metabolism, leucine appears to have a unique signalling role amongst the BCAA.

Given the importance of BCAA, and leucine in particular, in regulating protein turnover and mTOR signalling, it is perhaps not surprising that dietary BCAA supplements have become a commercially popular means of nutritional support to enhance the response of skeletal muscle to exercise. For example, a recent study[13] on dietary supplement use amongst fitness club members reported that BCAA were one of the most commonly used dietary supplements, consumed by ~37% of the sampled population. The popularity of BCAA supplements is largely predicated on the notion that they can stimulate rates of MPS and suppress rates of MPB in response to exercise, the combination of which promotes a net anabolic response in skeletal muscle. However, although the role of BCAA (leucine) as a growth-regulatory signal has been known since the 1970s[7,8,10], the specific effect of BCAA on skeletal muscle protein turnover (i.e. the simultaneous processes of MPS and MPB) in humans is less clear. A now seminal review by Wolfe[14] published in 2017 concluded that BCAA alone are insufficient to stimulate MPS rates in humans. However, since publication of this review, additional research has emerged providing evidence that BCAA are in fact capable of stimulating rates of MPS in humans.

The purpose of this evidence-based narrative review is to provide an update and critical appraisal of the available research literature pertaining to studies examining the effects of BCAA on MPS and MPB (i.e. muscle protein turnover), as well as associated molecular signalling responses implicated in the regulation of these processes. Emphasis is placed on studies performed in healthy humans, both at rest and in response to exercise, where the collective provision of isolated free BCAA (i.e. the provision of isoleucine, leucine and valine together as free amino acids independent of other amino acids) has been utilized within the research study design. Readers specifically interested in the role of leucine as a nutrient regulator of mTOR signalling and skeletal muscle protein turnover can refer to recent reviews on the topic[15,16].

Overview of the regulation of muscle protein turnover in response to amino acids and exercise

The purpose of this section is to provide a brief overview of the regulation of muscle protein turnover in response to amino acids and acute exercise to provide context for subsequent sections of the review examining the specific effects of BCAA on MPS, MPB and associated molecular signalling responses in humans. For more extensive reviews on the effects of protein/EAA and/or exercise on muscle protein turnover and its molecular regulation, the reader is referred to the following articles[17–21].

Muscle protein synthesis in response to amino acids and exercise

Increases in muscle mass (i.e. muscle anabolism) are determined by the balance between MPS and MPB; two ongoing, dynamic and highly regulated metabolic processes. When the rate of MPS exceeds the rate of MPB (MPS > MPB), the result is a state of positive net protein balance (NPB) and muscle protein accretion. Alternatively, when the rate of MPB exceeds the rate of MPS (MPS < MPB), NPB becomes negative, leading to loss of muscle protein. In general, the principle regulators of muscle protein turnover in adult humans are nutrient availability and exercise[22]. In terms of nutritional factors, dietary protein-derived amino acids are the key nutrients that support anabolic processes via their uptake and incorporation into skeletal muscle proteins via the process of MPS. The postprandial stimulation of MPS following protein ingestion is transient, lasting only a few hours[23,24], and serves to replace protein that is lost in the fasted state. Of the proteinogenic amino acids, the EAA, not the non-essential amino acids (NEAA), appear largely responsible for stimulating MPS rates in humans[25–29]. The provision of a complete mixture of EAA in young adults stimulates MPS rates in a dose-dependent manner up to 10 g EAA[30]; however, older adults display 'anabolic resistance' which manifests as a decreased sensitivity and responsiveness of MPS to EAA intake[30]. As noted previously, early work in rodents[7,10] demonstrated that the independent provision of leucine enhanced MPS to a similar extent as supplying a complete mixture of all three BCAA. Similarly, some[31,32] but not all[33,34] studies in humans have shown that the independent provision of leucine is able to stimulate MPS rates. Other studies have also demonstrated that leucine supplementation of a protein-containing meal[35–38] or leucine-enriched EAA intake[39] can further stimulate MPS rates in humans. Stimulation of postprandial MPS rates in response to EAA ingestion in humans appears contingent upon mTOR complex 1 (mTORC1) activation since administration of the mTORC1 inhibitor rapamycin blunts the postprandial stimulation of MPS rates in response to EAA ingestion[40].

An acute bout of resistance exercise can stimulate MPS rates for 48 h[41]; however, NPB remains negative in the absence of

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exogenous amino acid provision following exercise due to a concomitant stimulation of MPB[42,43]. The consumption of protein or EAA following resistance exercise further stimulates post-exercise MPS rates as compared with resistance exercise alone, and results in a positive NPB[42,43]. Therefore, acute exercise sensitizes skeletal muscle to the anabolic effects of protein/EAA feeding[43], an effect that lasts for at least 24 h[44]. In young adults, protein ingestion stimulates MPS rates in a dose-dependent manner up to ~20 g (containing ~10 g EAA) following resistance exercise[45,46]. The contraction-induced stimulation of MPS in humans is blunted in response to the mTORC1 inhibitor rapamycin[47], highlighting the importance of this pathway in regulating muscle anabolism in humans. However, studies in rodent muscle suggest that mTORC1 inhibition does not fully prevent the contraction mediated stimulation of MPS[48–52], suggesting mTORC1-independent mechanisms may also stimulate MPS after anabolic stimuli, potentially involving the mitogen-activated protein kinase/extracellular signal-regulated kinases 1/2 (MAPK/ERK1/2) pathway[18]. In addition to amino acid availability and exercise, reduced energy availability has a strong influence on muscle protein turnover[53]. For example, reduced energy availability reduces both basal and postprandial MPS rates[54,55] and increases whole-body amino acid oxidation[56,57]. However, a bout of resistance exercise performed in an energy deficit can restore MPS rates to values observed at rest in a state of energy balance[54]. Furthermore, dietary protein ingestion after resistance exercise performed in an energy deficit can further stimulate MPS above resting MPS rates in energy balance in a dose-dependent manner[54].

As previously indicated, stimulation of MPS in response to anabolic stimuli is primarily regulated by mTOR, an evolutionary conserved serine/threonine kinase[58]. mTOR constitutes the catalytic subunit of two structurally different multiprotein complexes known as mTORC1 and mTOR complex 2 (mTORC2) that ultimately serve two different functions[59]. mTORC1 serves as a central hub responsible for integrating signals derived from nutrients, contractile activity (e.g. exercise) and growth factors and is a key regulator of protein synthesis and cellular growth[12]. Alternatively, mTORC2 activates several pro-survival pathways and governs cytoskeletal behaviour[60]. The kinase activity of mTORC1 and its downstream targets are dynamically regulated by protein–protein interactions and intracellular translocation and colocalization (for review see Ref.[61]). Once activated, mTORC1 stimulates MPS by phosphorylating the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and p70 S6 kinase 1 (p70S6K1). Phosphorylation of 4E-BP1 via mTORC1 results in the release of eukaryotic translation initiation factor 4E (eIF4E) from 4E-BP1 and an increase in 5′ cap-dependent translation of mRNA, while phosphorylation of p70S6K1 by mTORC1 results in the phosphorylation ribosomal protein S6 (rpS6)[18]. For example, Fujita and colleagues[62] reported that EAA-carbohydrate co-ingestion stimulated MPS rates concomitant with an increase in Akt$^{(Ser473)}$ and mTOR$^{(Ser2448)}$ phosphorylation, along with an increase in the phosphorylation of downstream effectors p70S6K1$^{(Thr389)}$ and 4E-BP1$^{(Thr37/46)}$, and a decrease in eukaryotic elongation factor 2$^{(Thr56)}$ (eEF2) phosphorylation in human skeletal muscle. For an overview of key proteins and phosphorylation events implicated in the regulation of MPS in response to amino acids, exercise, and their combination, the reader is referred to the following article[18].

Exactly how amino acids activate mTORC1 is an area of intense research interest. *In vitro* studies have demonstrated that increases in amino acid availability enhance mTORC1 recruitment to the lysosome via the Rag GTPases, thereby allowing lysosomal Ras homolog enriched in brain (Rheb) to stimulate mTORC1 kinase activity[63]. In human skeletal muscle, translocation of mTORC1 to the cell periphery (i.e. sarcolemmal membrane) appears important to support its activation[64,65]. In support of this notion, Hodson and colleagues[66] reported that protein–carbohydrate co-ingestion resulted in mTOR translocation to the cell periphery human muscle 1 h after intake, which coincided with elevated S6K1 kinase activity. Similarly, isolated leucine ingestion (2 g) has recently been demonstrated to promote mTOR translocation to the cell periphery and enhance mTOR localization with the lysosome in human skeletal muscle at 30 and 60 min post-ingestion[12]. *In vitro* studies have identified the protein complexes GAP activity towards the Rags (GATOR1 and GATOR2), Sestrin2, cellular arginine sensor for mTORC1 (CASTOR1), and *S*-adenosylmethionine sensor upstream of mTORC1 (SAMTOR) as amino acid sensors (i.e. they sense amino acid sufficiency or lack thereof) that ultimately act to regulate mTORC1 activity[12]. Of particular interest within the context of the present review is Sestrin2, a cytosolic leucine sensor that inhibits GATOR2, preventing lysosomal translocation of mTORC1 during leucine insufficiency. Alternatively, increased leucine availability results in binding of leucine to Sestrin2, dissociating the protein from GATOR2 to relieve mTORC1 inhibition[67,68]. It is important to highlight that our understanding of how amino acids activate mTORC1 largely comes from *in vitro* studies performed in various several cell types. The stimulation of mTORC1 activity by amino acids in human skeletal muscle is still poorly understood.

MicroRNA are also emerging as potentially important regulators of anabolic process in skeletal muscle[69]. MicroRNA are short (twenty to twenty-two nucleotides) non-coding RNA that recognize the 3′-untranslated regions of their target mRNA substrates and silence their expression by blocking translation or inducing transcript degradation[69]. MicroRNA expression has been shown to be acutely altered in response to exercise[70], and both EAA[71–73] and protein[74] ingestion in human muscle. Furthermore, the expression of some microRNAs (i.e. miR-206 and miR-499) has been reported to be inversely correlated with MPS rates during exercise (i.e. increased MPS rates (%/h) are associated with reduced miR-206 and miR-499 expression)[73]. While microRNA expression may be altered in response to anabolic stimuli, the specific effect of BCAA ingestion on muscle microRNA expression has not yet been explored.

Muscle protein breakdown in response to amino acids and exercise

In addition to MPS, changes in MPB play a critical role in muscle remodelling and may influence the overall anabolic response.

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Proteins throughout the body undergo continuous turnover, which is necessary to prevent the accumulation of damaged proteins, prevent cellular dysfunction and maintain proteostasis. Although muscle anabolism is conventionally thought to be the result of stimulation of MPS rates, it may also result from a suppression of MPB[75]. A number of studies have demonstrated that an acute bout of resistance exercise stimulates MPB rates[41,42], an effect that can be sustained for 24 h post-exercise[41]. In support of these observations, a number of studies[76–82] have reported increases in the mRNA expression patterns of the ubiquitin ligase muscle RING-finger protein-1 (MuRF1), a known regulator of proteolysis, in the early post-exercise recovery period. In addition to exercise, some[83] but not all studies[84] have demonstrated an increase in MPB rates in response to energy restriction. Theoretically, nutritional strategies that are able to suppress exercise-induced increases in MPB may contribute to a more positive NPB, and therefore facilitate the accretion of muscle mass in response to resistance exercise. While protein/EAA intake and the resulting post-prandial hyperaminoacidaemia stimulate MPS rates[22], amino acids can also stimulate an increase in circulating insulin concentration (i.e. hyperinsulinaemia)[85]. Insulin is a powerful regulator of protein turnover in humans, primarily through its capacity to supress MPB[86], even at low circulating concentrations (i.e., ~15–30 mU/l)[87,88]. The stimulation of MPB that occurs in response to resistance exercise in the postabsorptive (i.e. fasted) state[42] may be prevented when amino acids are administered after exercise[43]. A common claim associated with BCAA supplements within the context of exercise is that they are 'anti-catabolic' (i.e. they are able to attenuate exercise-induced increases in protein breakdown), and therefore are able to better support the achievement of a positive NPB after exercise[89].

The breakdown of muscle proteins occurs via the coordination of several systems/pathways: the ubiquitin–proteasome pathway, autophagy-lysosome system, $Ca2^{+}$-dependent calpains and the cysteine protease caspase enzymes. In the ubiquitin–proteasome pathway, proteins are tagged for breakdown by ubiquitin, leading to recognition by the 26S proteasome that digests ubiquitinated proteins to smaller peptides that are ultimately degraded to amino acids by peptidases[90,91]. In the autophagy-lysosome system, lysosomal machinery degrades intracellular protein and organelles. This system is activated in muscle cells during catabolic conditions (e.g. disuse[92] and caloric restriction[93]), and is physiologically induced by both endurance[94] and resistance exercise[95]. Calpains are Ca^{2+}-dependent cysteine proteases that target myofibrillar, cytoskeletal and sarcolemmal proteins[96]. Sustained increases in $[Ca^{2+}]$ are thought to be a mechanism that prevents excessive calpain driven proteolysis[7]. Caspases are a family of proteases that have been proposed to play a role in the initial steps of MPB[97]. In skeletal muscle, Forkhead box O3 (FOXO3), a transcriptional regulator of the ubiquitin ligases MuRF1 and muscle atrophy F-box (MAFbx) involved in proteasome-dependent muscle atrophy[98], is linked to the expression of autophagy-related genes *in vivo* and in C_2C_{12} myotubes[99,100], and regulates both these systems[100]. These protein degradation systems are thought to work concurrently to regulate MPB in response to a variety of conditions including exercise and nutrition[96].

Dietary BCAA requirements and general recommendations

BCAA represent ~15–25% of the amino acids found in common food sources, with protein from milk (26%), eggs (22%) and maize (21%)[101] representing foods particularly rich in BCAA. The current World Health Organization/Food and Agriculture Organization/United Nations University guidelines[102,103] set the total requirement for the BCAA at 85 mg kg^{-1} d^{-1} (leucine: 39 mg kg^{-1} d^{-1}; isoleucine: 20 mg kg^{-1} d^{-1}; valine: 26 mg kg^{-1} d^{-1}) for healthy adult populations. Alternatively, the mean requirement and population-safe level (upper limit of 95% confidence interval) for total BCAA, based on data obtained in healthy sedentary young men using tracer L-[1-^{13}C]phenylalanine and the indicator amino acid oxidation (IAAO) technique, has been reported to be substantially higher; 144 and 210 mg kg^{-1} d^{-1}, respectively[104]. However, the mean requirement for total BCAA is likely to be even higher amongst athletes and those who habitually engage in exercise training, as overall daily IAAO-derived recommended protein intake estimates appear to be higher in younger endurance (females: ~1·71 g kg^{-1} d^{-1}[105]; males: ~1·83 g kg^{-1} d^{-1}[106]) and resistance-trained (females: ~1·53 g kg^{-1} d^{-1}[107]; males: ~2 g kg^{-1} d^{-1}[108]) populations. In support of this notion, a recent IAAO study[109] demonstrated that BCAA are the primary rate-limiting amino acids in the greater daily protein requirement of endurance-trained young men. This increased requirement may reflect the need to replace amino acids (BCAA in particular) that are oxidized during exercise[110], and provide amino acid substrates to support whole-body and skeletal muscle protein remodelling[111]. Using the IAAO technique, Kato and colleagues[106] reported a recommended protein intake of 1·83 g kg^{-1} d^{-1} after exercise in endurance-trained young men ($n = 6$), corresponding to a BCAA intake of ~396 mg kg^{-1} d^{-1}. Based on the overall daily recommended protein intakes reported above derived from IAAO methodology, this would equate to a BCAA intake of ~376 mg kg^{-1} d^{-1} for endurance-trained young females[105], ~337 mg kg^{-1} d^{-1} for resistance-trained young females[107], and ~440 mg kg^{-1} d^{-1} for resistance-trained young males[108]. Although the IAAO technique has many practical advantages as a method to determine dietary protein requirements in humans[112], there are some potential limitations of the method. For example, the IAAO method typically involves testing the response to mixture of free amino acids (not intact protein) modelled after the composition of egg protein[113]. Compared with intact protein, free amino acids result in more rapid amino acid absorption and greater postprandial plasma amino acid availability[114]. The IAAO method applies a repeated sip-feeding protocol (i.e. hourly protein/amino acid ingestion) that does not reflect typical food intake patterns[113]. Finally, the IAAO method does not account for the possible role of increased dietary protein intake on changes in protein breakdown[115]. Overall, while additional research is required to confirm these findings, individuals who regularly engage in either resistance or endurance exercise

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training, and who are seeking to optimize post-exercise recovery and adaptation, may benefit from higher dietary protein (and therefore BCAA intake) relative to their sedentary counterparts in order to meet elevated whole-body metabolic requirements due to training.

Branched-chain amino acid transport and availability in circulatory and intramuscular pools

Like all dietary amino acids, the BCAA are absorbed by the small intestinal epithelial cells, via discrete amino acid carriers/transporters, transported to the liver via the portal vein, and then released into systemic circulation where they can be delivered and transported into skeletal muscle[116,117]. The large neutral amino acid (LNAA) transporter, a heterodimer composed of L-type amino acid transporter 1 (LAT1), L-type amino acid transporter 2 (LAT2) and its molecular chaperone CD98 (SLC7A5, SLC7A8 and SLC3A2, respectively) is responsible for the transport of BCAA and other large neutral amino acids[118–121] across the intestinal basolateral membrane. However, the primary BCAA transporter in the gut is LAT2[118–121]. Following uptake across the intestinal basolateral membrane, the BCAA are transported in the portal blood to the liver, a major site of metabolism for most amino acids. Results from a number of studies[6,122] suggest that the BCAA largely escape first pass hepatic metabolism relative to other amino acids, and are instead heavily catabolized within skeletal muscle. This observation may relate to the low hepatic expression of the mitochondrial BCAT isozyme in humans[5]. In a now seminal study, Wahren and colleagues[122] determined that in response to protein (lean beef) ingestion in men, BCAA exceeded all other amino acids regarding their escape from the splanchnic bed, arterial concentration, and uptake by peripheral tissues (e.g. leg muscle). Specifically, it was demonstrated that, in response to a protein-rich meal, isoleucine, leucine and valine accounted for >50% of the splanchnic output of amino acids while accounting for only 20% of the protein source ingested. Therefore, ingested BCAA appear to largely escape splanchnic catabolism and become predominantly available in the circulation for uptake into peripheral tissue (i.e. skeletal muscle). The influx of BCAA into skeletal muscle is largely mediated by LAT1, which is dependent on the glutamine gradient generated by the sodium-dependent neutral amino acid transporter 2 (SNAT2)[123,124].

A common observation following the provision of BCAA alone is a decline in the plasma concentration of other amino acids including methionine and the aromatic amino acids[125,126]. Similarly, the provision of leucine alone results in a decline in the plasma concentrations of isoleucine and valine[125,127,128], as well as other amino acids (tyrosine, phenylalanine and methionine)[125]. However, a similar reduction in plasma amino acid concentrations is not observed in response to the isolated provision of isoleucine or valine[125]. For example, Alvestrand and colleagues[129] reported that a continuous intravenous infusion of L-leucine (300 μmol min^{-1}) to twelve healthy females over 2·5 h decreased the concentration of most other amino acids by 17–79% in plasma, and by 17–48% in the muscle intracellular free pool, when compared with the other BCAA and the aromatic amino acids. This reduction in the plasma and intramuscular concentration of select amino acids has been taken to suggest that provision of isolated BCAA (or leucine) suppresses protein breakdown (and therefore the rate at which protein-bound amino acids are released into the intracellular free pool and circulation), and/or stimulates protein synthesis[130].

The effect of branched-chain amino acids on muscle protein synthesis and associated molecular signalling in humans

Protein synthesis is an extremely energy- and resource-intensive process in growing cells[131]. The effects of BCAA on MPS, MPB and associated molecular signalling responses may be influenced by the nutritional state of the participant when BCAA are administered, and differ depending on whether BCAA are ingested individually (e.g. leucine intake alone) or collectively (e.g. isoleucine, leucine and valine) or are co-ingested with other amino acids (e.g. as part of a complete protein or BCAA-enriched protein supplement)[132]. As the current review is primarily focused on studies evaluating the effects of the provision of a complete mixture of isolated BCAA, the studies discussed in this review have provided BCAA as free amino acids. It is important to highlight that the included studies vary in that some have provided BCAA via intravenous infusion[133–136] or oral consumption[2,35,76,137–145], studied participants at rest or the response in non-exercised (i.e. rested) muscle[2,35,76,133–137,139,145], studied participants under post-exercise conditions[35,76,138–145], studied younger adults[2,35,76,133–136,138–145] and studied older adults[137]. The included studies also vary in the BCAA dose and amount of energy provided, the corresponding ratio of isoleucine, leucine and valine within a given dose, nature of the control/comparator treatment(s), and timing of sample collection. All these factors may have implications for the study results obtained and any subsequent conclusions drawn. A detailed overview of studies in this review examining the effects of BCAA on MPS, MPB and/or associated molecular signalling responses in humans is presented in Supplementary Table 1.

Studies performed under resting conditions

A purported benefit of BCAA supplements is that they stimulate MPS rates and promote a net anabolic effect in muscle, particularly when coupled with resistance exercise. However, this claim remains a contentious issue as only a limited number of studies[133–138,141,144] have been performed in humans addressing the effect of a complete mixture of isolated BCAA on MPS rates. To the best of our knowledge, Louard and colleagues[134] performed the first investigation in humans (men and women aged 18–34 years) examining the effects of isolated BCAA on whole-body and MPS in the overnight post-absorptive state under resting (i.e. non-exercise) conditions. In a parallel-group design, the BCAA were provided to one group of participants via intravenous infusion, and their effects were compared against another group of participants who were infused with saline as a control. The researchers found that infusion of BCAA for 3 h led

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to a marked increase in indices of whole-body protein synthesis (i.e. based on measures of non-oxidative leucine disposal). At the muscle level, BCAA infusion led to different amino acid kinetic responses based on arteriovenous (A-V) exchange measurements across the forearm depending on the amino acid tracer examined (i.e., L-[*ring*-2,6-^{3}H]-phenylalanine versus L-[1-^{14}C]-leucine). Specifically, the phenylalanine tracer showed no increase in indices of MPS (i.e. rate of disappearance (Rd) (nmol min^{-1} 100 ml^{-1})) in response to BCAA infusion, while the leucine tracer showed a marked increase. Based on the phenylalanine data, the authors concluded that BCAA do not stimulate MPS. A subsequent similar parallel group study by the same group[135] investigated the effects of a more prolonged (16 h) overnight intravenous infusion of BCAA in the overnight fasted state on whole-body and skeletal muscle amino acid kinetics in both men and women (18–34 years) using the same methodology. The results were compared against a group who received a 4 h systemic intravenous infusion of saline. Similar results were obtained at the whole-body and muscle level. Once again, infusion of BCAA did not stimulate rates of MPS based on A-V exchange measurements across the forearm using isotope-labelled phenylalanine, suggesting the lack of a stimulatory effect on MPS rates in their earlier study[135] was not due to an insufficient duration of BCAA infusion.

In a crossover study design, Liu and colleagues[136] evaluated the effects of isolated BCAA at rest, administered via intravenous infusion in the overnight fasted state, on whole-body phenylalanine flux (rate of appearance (Ra)), forearm phenylalanine kinetics and the phosphorylation of eIF-4E-BP1 ($\beta + \gamma/\alpha + \beta + \gamma$ ratio) and p70S6K ($\beta + \gamma/\alpha + \beta + \gamma$ ratio) with and without dexamethasone treatment. In the absence of dexamethasone treatment, infusion of BCAA improved forearm phenylalanine net balance when assessed at 6 h post infusion. However, while BCAA infusion increased the phosphorylation status of both eIF4E-BP1 and p70S6K, it did not increase indices of MPS (i.e. phenylalanine Rd (nmol min^{-1} 100 ml^{-1})). The authors concluded that BCAA act directly as nutrient signals in human skeletal muscle to activate mRNA translation and potentiate protein synthesis[136].

In 2016, Everman and colleagues[133] examined healthy young males and females in the overnight fasted state before and after insulin infusion to determine whether insulin stimulates MPS in relation to the availability of BCAA alone. No differences in MPS rates were found in response to BCAA versus saline infusion under both basal and insulin-stimulated conditions when MPS was assessed using the gold-standard precursor–product approach (i.e., L-[*ring*-^{2}H$_5$]-phenylalanine incorporation into muscle protein sampled via needle biopsy). Specifically, mean (SD) plasma concentrations of BCAA were 282 ± 40 and 310 ± 41 μmol/l during basal and insulin infusion periods in the saline condition, and 1059 ± 140 and 933 ± 91 μmol/l during basal and insulin infusion periods in the BCAA condition. The authors concluded that insulin does not stimulate MPS rates in the presence of increased circulating levels of plasma BCAA alone.

While the provision of BCAA via intravenous administration does provide valuable information on their effect on muscle protein turnover, outside of a controlled laboratory setting, this is an uncommon and highly impractical means of administration. Ferrando and colleagues[2] assessed the effects of orally ingested BCAA (5·2 g leucine, 2·6 g isoleucine, 3·2 g valine), co-ingested with carbohydrate (50 g) in the overnight fasted state, against an iso-nitrogenous and iso-caloric carbohydrate–EAA drink (4·0 g threonine, 3·8 g histidine, 3·2 g methionine) on leg A-V phenylalanine balance and postprandial MPS rates in young men using the precursor–product approach. Examination of leg phenylalanine kinetics via A-V balance revealed no stimulatory effect of BCAA on leg protein synthesis. While not statistically significant, BCAA stimulated MPS rates (i.e. fractional synthesis rate (FSR)) from 0·047 ± 0·002 during basal conditions to 0·093 ± 0·020 %/h in the postprandial period, while the EAA solution stimulated MPS rates from 0·059 ± 0·017 to 0·073 ± 0·015 %/h[2].

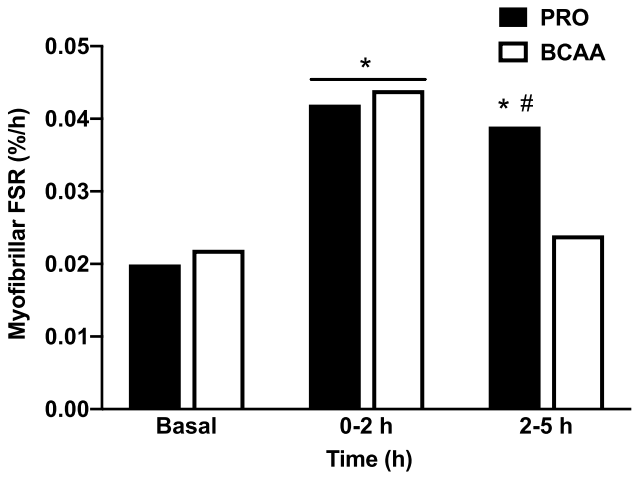

Fig. 1. Myofibrillar protein fractional synthesis rate (FSR; %/h) during the fasted (basal) state and over the early (0–2 h), and late (2–5 h) postprandial period following the ingestion of 30 g milk protein (PRO; complete source of protein containing ~6 g BCAA, of which 2·64 g was leucine) or 6 g branched-chain amino acids (BCAA; 3 g leucine, 1·5 g isoleucine, 1·5 g valine) in healthy older males. Values represent means. *Significantly different from basal; #significantly different from BCAA at the same timepoint. Adapted from Fuchs CJ, *et al.* (2019)[137].

Recently, Fuchs and colleagues[137] compared the impact of ingesting 6 g BCAA, 6 g branched-chain ketoacids and 30 g milk protein (containing 6 g BCAA) at rest in the overnight fasted state on postprandial myofibrillar protein synthesis (MyoPS) rates in older men (71 ± 1 years) using the precursor–product approach. It was reported that ingestion of BCAA stimulated (from 0·022 ± 0·002 %/h to 0·044 ± 0·004 %/h) postprandial MyoPS rates comparable to that elicited in response to ingestion of 30 g milk protein (from 0·020 ± 0·002 %/h to 0·042 ± 0·004 %/h) during the early (0–2 h) postprandial period. However, whereas the ingestion of 30 g of milk protein was able to sustain elevated MyoPS rates during the late (2–5 h) postprandial period (0·039 ± 0·004 %/h), the stimulation of MyoPS rates following isolated BCAA intake was short-lived, and not sustained during the late postprandial period (0·024 ± 0·005 %/h)[137] (Fig. 1). Interestingly, branched-chain ketoacid administration also elicited a transient stimulation of MyoPS rates that was similar to that achieved with BCAA. This work clearly demonstrates that while isolated BCAA (and branched-chain ketoacid) intake can stimulate postprandial MyoPS rates in older adults, the response

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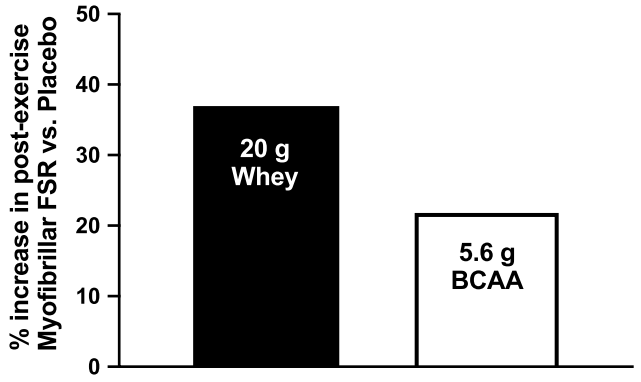

Fig. 2. Per cent increase in post-exercise myofibrillar protein fractional synthesis rate (FSR) versus placebo after ingestion of 20 g whey protein isolate (containing 10 g of EAA and 4·8 g of BCAA) or 5·6 g BCAA (containing 2·6 g leucine, 1·4 g isoleucine, 1·6 g valine). Post-exercise ingestion of 20 g whey protein isolate yields a 37% greater post-exercise myofibrillar protein FSR compared with 0 g whey protein isolate ingestion (placebo). Post-exercise ingestion of 5·6 g BCAA yields a 22% greater post-exercise myofibrillar protein FSR compared with 0 g BCAA ingestion (placebo). Adapted from Jackman *et al.* (2017)[138] and Witard *et al.* (2014)[46].

is transient, likely due to insufficient availability of the other amino acids required as substrate to yield a sustained stimulation of MyoPS rates[14].

Studies performed with resistance exercise

In 2017, Jackman and colleagues[138] reported that the oral intake of BCAA (BCAA: 5·6 g; 1·4 g isoleucine; 2·6 g leucine; 1·6 g valine) following an acute bout of resistance exercise performed 3 h following a standardized breakfast, stimulated 22% greater MyoPS rates in young men over 4 h post-exercise recovery, when compared with an energy-matched carbohydrate control. In this study, MyoPS rates were determined via the precursor–product approach. The greater rates of MyoPS following BCAA intake were accompanied by enhanced phosphorylation of Akt$^{(Ser473)}$, PRAS40$^{(Thr246)}$ and p70S6K1$^{(Thr389)}$ at 1 h after consumption versus baseline only in the BCAA trial[138]. These findings are in alignment with those of Liu and colleagues[136] who reported an increase in the phosphorylation p70S6K ($\beta + \gamma/\alpha + \beta + \gamma$ ratio) in response to BCAA infusion at rest. However, Jackman and colleagues[138] noted that the stimulation of MyoPS rates in response to BCAA intake following resistance exercise was ~50% less than the previously reported MyoPS response to a dose of whey protein containing similar amounts of BCAA[46,146]. These results suggest that, while isolated BCAA ingestion can result in greater MyoPS rates after an acute bout of resistance exercise as compared with an energy-matched carbohydrate control, ingestion of the full complement of EAA via high-quality dietary protein (e.g. whey protein) intake may be required to optimally stimulate MyoPS rates after resistance exercise (Fig. 2).

More recently, Jackman and colleagues[141] evaluated the effects of co-ingestion of BCAA (BCAA: 6·1 g; 1·4 g isoleucine; 2·8 g leucine; 1·9 g valine) with carbohydrate (30·6 g) on MyoPS rates after an acute bout of resistance exercise performed 3 h following a standardized breakfast in trained young men. BCAA co-ingestion with carbohydrate stimulated ~15% greater MyoPS rates over 4 h post-exercise recovery when compared with an energy-matched carbohydrate control. In qualitative terms, this 15% increase is similar to the 22% increase in post-exercise MyoPS rates with BCAA the authors reported previously[138], and again suggest that isolated BCAA intake may not result in an optimal muscle anabolic environment following resistance exercise.

In partial contrast to the studies from Jackman and colleagues[138,141], Moberg and colleagues[144] reported no differences between leucine (50 mg/kg), BCAA (110 mg/kg: 25% L-isoleucine, 45% L-leucine and 30% L-valine), EAA (290 mg/kg) and placebo (flavoured water) ingestion on postprandial MPS rates following acute resistance exercise. Although there were no differences between treatments on post-exercise MPS rates, p70S6K1 activity (pmol min^{-1} mg^{-1}) increased above resting values in all four trials, such that placebo < leucine < BCAA < EAA when assessed 90 min following exercise. Furthermore, p70S6K1 activity after 180 min of recovery remained ~60–95% higher in the BCAA and EAA trials versus the placebo and leucine trial[144]. Similar findings were also observed when evaluating the phosphorylation status of 4E-BP1$^{(Thr46)}$ and 4E-BP1$^{(Ser65)}$ at 90 and 180 min post-exercise. Taken together, these results suggest that a mixture of EAA promotes early signalling (90 min post-exercise) responses associated with translation initiation to a greater extent than BCAA; however, differences in signalling responses between BCAA and EAA become less apparent during the later stages (180 min) of post-exercise recovery.

A number of other studies have evaluated changes in molecular signalling implicated in the regulation of MPS in response to isolated BCAA intake in human muscle during recovery after exercise[76,139,140,142,143] (see Supplementary Table 1 for details). Collectively, these studies support the notion that BCAA intake may further enhance p70S6K1$^{(Thr389)}$[76,139,142] and rpS6$^{(Ser235/236)}$[139,142], but not eEF2$^{(Thr56)}$[139,144] phosphorylation during the early recovery period after resistance exercise. Alternatively, the effect of BCAA intake on mTOR$^{(Ser2448)}$ phosphorylation is less clear, with one study reporting an increase[144], and other studies reporting no difference[76,139,140] versus placebo ingestion during recovery after resistance exercise. Overall, BCAA intake appears to further enhance the phosphorylation status of some proteins within the mTORC1 pathway involved in the regulation of translation initiation of MPS in humans.

Fortification of dietary protein with a complete mixture of BCAA

Some research has evaluated the effects of supplementing various doses of protein with a complete mixture of BCAA on MyoPS rates both at rest and following resistance exercise in healthy adults[35,145,147]. For example, Churchward-Venne and colleagues[35] evaluated the effects of supplementing a 'sub-optimal' dose (6·25 g) of whey protein with different doses of leucine, as well as a mixture of BCAA (total BCAA intake: 7·73 g), compared with a more 'optimal' 25 g dose of whey protein. Ingestion of 6·25 g whey protein supplemented with BCAA in the overnight fasted state did not stimulate postprandial MyoPS rates, at rest or following acute resistance exercise, as effectively

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as 25 g whey protein. Similarly, Monteyne and colleagues[145] reported that supplementing a lower dose (18·7 g protein) of mycoprotein with BCAA (total: 2·5 g leucine, 1·5 g isoleucine and 1·9 g valine) failed to stimulate MPS rates, both at rest and following resistance exercise, as effectively as a larger dose (35·1 g protein) of mycoprotein matched for BCAA content. Finally, although MPS rates were not assessed, Engelen and colleagues[147] reported that adding BCAA to a soy protein meal did not alter whole-body protein synthesis or net balance when compared with a soy protein meal without BCAA fortification in a group of healthy older men studied under resting conditions. However, adding BCAA to a soy protein meal further stimulated whole-body protein synthesis in older men with COPD. While the addition of a mixture of BCAA to protein (i.e. to whey or mycoprotein) represents a different supplementation model/strategy than isolated ingestion of all three BCAA without other amino acids, these results further support the notion that ingestion of an ample amount of high-quality protein containing sufficient quantities of the full complement of amino acids appears necessary to robustly stimulate postprandial MPS rates.

Overall, some[137,138,141] but not all[2,133,144] studies applying the gold-standard precursor–product approach to assess MyoPS or MPS rates following oral (as opposed to intravenous) BCAA intake have demonstrated that BCAA can stimulate MPS rates in humans, both at rest[137] and following resistance exercise[138,141]. However, the stimulation of postprandial MPS rates following isolated BCAA intake is transient and sub-optimal when compared with the MPS response achieved following the ingestion of a complete protein (e.g. whey) matched for BCAA intake but providing the full complement of amino acids. Furthermore, the large majority of studies to date examining the effects of BCAA on MPS rates and associated molecular signalling responses have lacked an iso-caloric and/or iso-nitrogenous EAA control (Supplementary Table 1), making it difficult to determine whether the BCAA are more anabolic than other EAA in human muscle. Therefore, ingestion of a sufficient dose of high-quality dietary protein containing a complete mixture of amino acids represents a superior option to isolated BCAA intake for stimulating MPS rates in human muscle.

The effect of branched-chain amino acids on muscle protein breakdown and associated molecular signalling in humans

Studies examining the response of human skeletal muscle protein turnover to exercise and nutritional stimuli have focused predominantly on the response of MPS[96,148], with much less information available on the response of MPB. This is largely due to the technical challenges associated with obtaining dynamic measures of MPB[96,148]. As discussed, MPB is a determinant of NPB, and therefore can influence the overall anabolic response of muscle to nutrition and exercise interventions. The process of MPB also provides amino acid substrate for protein synthesis in other bodily organs and tissues, supports the repair, remodelling and synthesis of muscle proteins, and provides amino acids for hepatic gluconeogenesis[148]. A common claim pertaining to BCAA is that they are capable of suppressing rates of MPB during exercise and therefore are 'anti-catabolic'. In partial support of this notion, a number of early *in vitro* studies in rodent skeletal muscle tissue reported that BCAA reduced protein breakdown (for review see Ref.[149]). However, only a limited number of studies to date have examined the effects of a complete mixture of BCAA on indices of MPB *in vivo* in humans, and none has examined the effects of BCAA on MPB rates in response to resistance exercise.

The aforementioned studies by Louard and colleagues examining the effects of BCAA on whole-body and muscle protein metabolic responses[134,135] included measurements of whole-body and MPB (derived from A-V exchange measurements across the forearm using isotopically labelled amino acids). In response to a 3 h infusion of BCAA, phenylalanine flux (Ra) was reduced by 22%, indicative of a reduction in whole-body protein breakdown. Similar results were obtained from the phenylalanine tracer exchange data across the forearm; that is, muscle phenylalanine appearance (Ra) from protein breakdown was reduced in response to BCAA infusion[134]. This reduction in protein breakdown was unlikely due to a BCAA-mediated increase in insulin availability, as BCAA infusion did not alter insulin concentrations. A similar ~37% reduction in whole-body phenylalanine flux (Ra) and decrease in forearm protein breakdown (i.e. phenylalanine Ra) was observed by the same researchers in response to a prolonged (16 h) overnight intravenous infusion of BCAA[135]. Alternatively, the aforementioned studies by Ferrando and colleagues[2] and Liu and colleagues[136] failed to detect a decrease in indices of MPB in response to BCAA administration based on A-V exchange measurements across the leg and forearm respectively, using isotopically labelled phenylalanine. Nonetheless, BCAA reduced whole-body phenylalanine flux (Ra) in both studies[2,136], suggesting a suppression of whole-body protein breakdown. Other studies have also provided support for the notion that BCAA may attenuate whole-body protein breakdown, as BCAA have been reported to reduce whole-body phenylalanine flux (Ra) during the initial hours of post-exercise recovery[138,141]. While BCAA may attenuate both whole-body and MPB, the molecular mechanisms underpinning the anti-proteolytic effects of BCAA are unclear.

There is some evidence[76,143] that BCAA reduce the expression (mRNA and/or protein) of the ubiquitin ligases MuRF-1 and MAFbx in human skeletal muscle. For example, Borgenvik and colleagues[76] reported that BCAA (85 mg BCAA/kg body weight: 45% leucine, 30% valine and 25% isoleucine) reduced MAFbx mRNA expression in both resting and resistance exercised human muscle, and attenuated the increase in MuRF-1 total protein expression compared with a placebo in young adults. Similarly, Lysenko and colleagues[143] reported that BCAA supplementation (0·1 g BCAA/kg body weight: 50% leucine, 25% isoleucine and 25% valine) reduced the mRNA expression of MAFbx (Atrogin-1) and MuRF-1 at select timepoints during recovery from an acute bout of endurance exercise in young endurance-trained athletes[143]. Overall, a number of studies have demonstrated that a mixture of BCAA can attenuate both whole-body[2,134–136,138,141] as well as indices of muscle[134,135] protein breakdown, and reduce the mRNA expression of the

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ubiquitin ligases MAFbx and MuRF-1[76,143]. However, no studies to date have assessed whether BCAA intake can suppress the elevation in MPB rates that occurs during the post-exercise recovery period in response to resistance exercise[41,150]. Given that insulin, even at relatively low circulating concentrations (i.e. ~15–30 mU/l), can strongly suppress muscle proteolysis[87,88], mixed macronutrient intake (e.g. intake of both carbohydrate and protein) would also be expected to suppress MPB, and as discussed, better support the stimulation of MPS rates in human muscle.

Conclusion

The aim of this narrative review was to critically appraise the available research literature from studies performed in humans examining the effects of BCAA on MPS, MPB and associated molecular signalling pathway responses. Emphasis was placed on results from studies implementing a supplementation model involving isolated intake of all three BCAA (i.e. the provision of leucine, isoleucine and valine together as free amino acids, independent of other amino acids), rather than on the effects of leucine alone. Recent studies have demonstrated that isolated intake of BCAA can stimulate postprandial MyoPS rates above basal conditions at rest[137], and result in greater MyoPS rates as compared with energy-matched carbohydrate intake during recovery following resistance exercise[138,141]. However, the stimulatory effect of BCAA intake on postprandial MyoPS rates at rest and after resistance exercise is transient and/or reduced compared to the MyoPS response achieved following the ingestion of 25–30 g of a high-quality complete protein source containing all EAA[137,138]. Along these lines, isolated BCAA intake can enhance the activation/phosphorylation status of some signalling proteins within the mTORC1 pathway known to regulate translation initiation after exercise[76,136,138,139,142,144]. However, a complete mixture of all EAA (as would be contained within high-quality dietary protein) appears to stimulate translation initiation signalling in skeletal muscle more effectively than just the BCAA[144].

There is currently a paucity of data on the nutritional regulation of MPB rates following exercise. To our knowledge, no studies to date have examined the effects of isolated BCAA intake on MPB rates following resistance exercise. Nonetheless, BCAA appear capable of suppressing both whole-body[2,134,135] and muscle (limb)[134,135], protein breakdown during resting (i.e. non-exercised) conditions, and whole-body protein breakdown during recovery after resistance exercise[138,141]. The molecular mechanisms responsible for this effect are unclear but may partially relate to a BCAA-mediated reduction in the expression of the ubiquitin ligases MAFbx and MuRF-1[76,143]. While the suppression of MPB after exercise may contribute to a more anabolic environment, it is not clear that the suppression of whole-body and/or MPB after exercise is desirable for enhanced muscle hypertrophy, muscle remodelling and adaptation to training, and/or post-exercise exercise recovery processes[96,132]. Overall, based on the currently available evidence, orally ingested BCAA supplements activate molecular signalling involved in translation initiation, and can reduce the expression of the ubiquitin ligases MAFbx and MuRF-1. Along these lines, BCAA appear to reduce indices of MPB at rest, and whole-body protein breakdown both at rest and during recovery from resistance exercise; however, their effect on resistance exercise-induced MPB rates has not been evaluated. Although some studies have demonstrated that BCAA can transiently stimulate MyoPS rates, most studies to date have lacked an iso-caloric and/or iso-nitrogenous EAA control, making it unclear whether the BCAA are truly more anabolic than other EAA in human muscle. The ingestion of high-quality dietary protein providing the full complement of EAA (including the BCAA) more effectively supports the postprandial stimulation of MPS rates than intake of only the BCAA and is required to optimize the acute anabolic response to exercise and nutrition.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0954422423000197

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Competing interest

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Authorship

M.S.K. and T.A.C.-V. conceived the manuscript; M.S.K., S.J.H., Z.W.B. and T.A.C.-V. drafted the manuscript; S.J.H. prepared figures; S.J.H., Z.W.B. and T.A.C.-V. edited and revised manuscript; M.S.K., S.J.H., Z.W.B. and T.A.C.-V. take primary responsibility for the final content. All authors read and approved the final manuscript.

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