The effects of acute branched-chain amino acid supplementation on recovery from a single bout of hypertrophy exercise in resistance-trained athletes.

Journal: Applied Physiology, Nutrition, and Metabolism

Author names:

Mark Waldron[*1,2], Kieran Whelan[1], Owen Jeffries[1], Dean Burt[3], Louis Howe[1], Stephen David Patterson[1];

Institutions:
[1] School of Sport, Health and Applied Science, St Mary's University, London, UK
[2] School of Science and Technology, University of New England, NSW, Australia
[3] Sport and Exercise Science, Brindley Building, Staffordshire University, Leek Road, Stoke-on-Trent
* = corresponding author

Contact Details for the Corresponding Author:
Dr Mark Waldron
School of Sport, Health and Applied Science,
St Mary's University,
Waldegrave Road,
Twickenham,
London,
TW1 4SX
mark.waldron@stmarys.ac.uk

Running head: Effects of BCAA on muscle damage

Abstract

This study investigated the effects of acute branched-chain amino acid (BCAA) supplementation on recovery from exercise-induced muscle damage, among experienced resistance-trained athletes. In a double-blind matched-pairs design, 16 resistance-trained participants, routinely performing hypertrophy training, were randomly assigned to a BCAA (n = 8) or placebo (n = 8) group. The BCAAs were administered at a dosage of 0.087 g/kg body mass, with a 2:1:1 ratio of leucine, isoleucine and valine. The participants performed 6 sets of 10 full-squats at 70 % 1RM to induce muscle damage. All participants were diet-controlled across the study. Creatine kinase (CK), peak isometric knee-extensor force, perceived muscle soreness and counter-movement jump (CMJ) height were measured immediately before (baseline), 1-h, 24-h and 48-h post-exercise. There were *large* to *very large* time effects for all measurements between baseline and 24-48 h. Between-group comparisons, expressed as a percentage of baseline, revealed differences in isometric strength at 24-h (Placebo ~87% *c.f.* BCAA ~92 %; *moderate, likely*), CMJ at 24-h (Placebo ~93 % *c.f.* BCAA ~96 %; *small, likely*) and muscle soreness at both 24-h (Placebo ~685 % *c.f.* BCAA ~531 %; *small, likely*) and 48-h (Placebo ~468 % *c.f.* BCAA ~350 %; *small, likely*). Acute supplementation of BCAAs (0.087 g/kg) increased the rate of recovery in isometric strength, CMJ height and perceived muscle soreness compared to placebo after a hypertrophy-based training session among diet-controlled, resistance-trained athletes. These findings question the need for longer BCAA loading phases and highlight the importance of dietary control in studies of this type.

Key words: Amino Acids, muscle damage, hypertrophy, recovery, supplementation; exercise.

Introduction

Habitual resistance exercise can be used to increase muscle strength and compensatory growth known as 'hypertrophy' (Goldberg et al., 1975). Whilst the optimal training regime to increase muscle mass has not yet been established (Carpinelli et al., 2004; Philips, 2009; Mitchell et al., 2012), it is typical for athletes to perform between 3-5 repeated bouts of resistance exercise to fatigue per micro-cycle (ACSM, 2009). These sessions commonly comprise 2-6 sets of resistance exercise, targeting major muscle groups, at intensities ranging from 6-12 maximum repetitions (RM) to failure (ACSM, 2009). Performing resistance exercise in this manner is partly intended to create mechanical tension, thus disturbing the integrity of skeletal muscle (Schoenfeld, 2010; 2012). It has been postulated that the exercise–induced muscle damage (EIMD) caused by resistance exercise initiates a cascade of intracellular signalling processes that upregulate the synthesis of muscle proteins and increase cell size (Schoenfeld, 2012). In the days (24-72 hours) after performing this type of exercise, participants are likely to experience symptoms of EIMD, such as delayed-onset muscle soreness (DOMS), decreased force production and leakage of intramuscular proteins into circulation (i.e. creatine kinase; CK) (Sorichter et al., 1999).

Recovery from EIMD is an important part of the adaptive process, which can take up to 72 hours (Barnett, 2006; Howatson and van Someren, 2008; Nedelec et al., 2013). Given the demands of frequent resistance training, full and rapid recovery between bouts of exercise is desirable. Interventions that help to attenuate the effects of muscle damage would, therefore, be beneficial to the athlete by reducing the decline in physical function, thus permitting greater engagement with training in the days following exercise (Cheung et al., 2003; Proske and Morgan, 2001; Howatson and van Someren, 2008). Furthermore, interventions that lower

the perception of fatigue and DOMS or maintain the intracellular anabolic environment would also support the training or adaptation process. Branched-chain amino acids (BCAAs), in particular leucine, are commonly used as prophylactic interventions to attenuate symptoms of muscle damage (da Luz et al., 2011). Leucine supplementation has also been suggested to suppress muscle proteolysis (Zanchi et al., 2008) and reduce protein oxidation (Shimomura et al., 2009) after muscle-damaging exercise, thus maintaining the integrity of the sarcolemma. Indeed, the appearance of indirect markers of muscle damage, such as CK, have been reduced by both short- (Nosaka et al., 2006; Kirby et al., 2012; Shimomura et al., 2010) and long-term amino acid supplementation (loading) periods (Coombes and McNaughton, 2000; Howatson et al., 2012; Sharp and Pearson, 2010). The attenuation in CK efflux after BCAA supplementation has been linked to a reduction in secondary damage, caused by the inflammatory response (Howatson and van Someren, 2008). The dampening of acute inflammatory responses might also explain the commonly-reported reductions in DOMS following mixed amino acid (Nosaka et al., 2006), isolated leucine (Kirby et al., 2012) or mixed BCAA supplementation (Shimomura et al., 2010; Howatson et al., 2012). Reductions in DOMS and cell damage might, in turn, explain the accelerated recovery of muscle function after leucine or mixed BCAA supplementation (Kirby et al., 2012; Howatson et al., 2012).

There are numerous inconsistencies and limitations among previous studies that question the assumed recovery properties of BCAAs. Firstly, only one study has used participants with a recent resistance-training history (Howatson et al., 2012). Whilst it is suggested to omit resistance-trained participants from muscle-damage studies because of the repeated-bout effect (RBE) (McHugh, 2003), several studies have found that protective adaptation is absent amongst resistance-trained men and well-trained athletes (Bloomer et al., 2006; Falvo et al., 2007; Falvo et al., 2009). Indeed, if the RBE is absent amongst this population, it increases

the efficacy of BCAA supplementation among athletes who are training regularly and, most likely, require strategies to hasten recovery between training sessions. Furthermore, there has been no study to assess the efficacy of BCAA supplementation among resistance-trained athletes following a bout of hypertrophy-type exercise, opposed to eccentrically-biased exercise (i.e. 100 drop-jumps; Howatson et al., 2012). This is important, given that a recent study has suggested that that the efficacy of BCAA supplementation as a recovery agent is dependent on the degree of muscle damage, and it is likely to be more effective after 'moderate' muscle damage (Fouré et al., 2016), such as that induced by performing non-ballistic concentric/eccentric lifting patterns, realistic of hypertrophy training programmes. Additionally, the evidence to support the optimal timing of BCAA supplementation for recovery from EIMD is equivocal, with research showing positive effects after 30 min of pre-exercise supplementation (Howatson et al., 2012; Kirby et al., 2012), compared to others who have supplemented for up to three weeks (Sharp and Pearson, 2010). That 30 min of pre-exercise BCAA is sufficient to reduce markers of EIMD is consistent with its 1-2 hour peak bioavailability following ingestion (Dickinson et al., 2014; Fouré et al., 2016). This also indicates that longer supplementation periods might be unnecessary to promote recovery from muscle damage, whilst also incurring a greater financial cost for athletes. Lastly, only one study has monitored the dietary intake (Jackman et al., 2010) of participants, meaning that the amount of protein and other macronutrients ingested is often unaccounted for. This is important since the energy content and protein included in a diet might optimise the recovery of subjects, independent of the BCAA supplement.

Based on the above reasoning, the aim of this study was to investigate the effects of acute body-mass dependent BCAA supplementation on recovery from specific EIMD, among athletes who take part in long-term training programmes, designed to induce muscle

hypertrophy. It was hypothesized that the BCAA supplementation would attenuate the reductions in muscle function and increases in DOMS, as well as lowering the biochemical responses, compared to the placebo group.

Methods

Participants

Fourteen males (mean ± SD age 21.8 ± 1.6 years, stature 183.3 ± 6.2 cm, body mass 95.1 ± 11.8 kg, 1RM Squat 175 ± 32 kg) and two females (mean ± SD age 22 ± 1 years, stature 161.5 ± 1.5 cm, body mass 57 ± 3 kg, 1 RM squat 68.5 ± 2.5 kg) consented to take part in this study. All participants were experienced resistance-trained athletes, with a minimum of three years training history, who had consistently used a full-squat technique in their own hypertrophy programmes. To be included in this study, the participants had to be injury-free and train at least three times per week using resistance exercises that fall within the typical hypertrophy training sets and repetition ranges described in the literature (ACSM, 2009), with at least three years of training history. The BCAA group had 3.9 ± 0.7 years of resistance training experience, while the placebo group had 4.3 ± 1.6 years. Participants were initially screened for any recent injuries or movement compensations that may cause pain or discomfort when squatting or factors influencing their ability to perform the required moments. Ethical approval was granted for this study by the Institutional ethics committee (SMEC_2016-17_001).

Design

Two weeks prior to testing, participants were told to cease any use of nutritional supplements, additional to their normal diet, such as protein supplements, creatine and amino acids. The participants were advised to avoid any drugs with anti-inflammatory properties and not to use

compression garments or seek or therapeutic intervention, such as hydrotherapy treatments or forms of massage. They were also provided with a diet plan to follow from 48-h before the study until their final testing day. The intended energy content of their diet plans was based on published approaches (Alfonzo-Gonzales et al., 2004) and provided three options of breakfast, lunch, dinner and snacks. For consistency, the meal options comprised a macronutrient composition of 50% carbohydrate, 15% protein (of similar amino acid content) and 35% fat. Caloric intake was monitored throughout the study using 'My Fitness Pal' (MyFitnessPal Inc, Austin, TX). The reported caloric intake of the participants during the study (males and females) was 2486 ± 412 kcal/day and 2667 ± 449 kcal/day in the BCAA and placebo groups, respectively. The actual reported macronutrient compliance during the study was: 46 ± 9 % carbohydrates, 15 ± 3 % protein and 39 ± 8 % fat. The participants visited the laboratory at the same time of day (1000-1100) on four separate days, approximately 1-2-h after breakfast. During visit 1, the participants familiarized with the testing procedures, were tested for their 1 RM and weighed for subsequent calculation of the BCAA supplement. The participants were given specific instructions for how to perform a back squat, including tempo and joint positioning, as this would be the mode of muscle-damage during the study. Visit 1 was carried out 72-h before the next visit (visit 2) and no other exercise was performed in between. Familiarization was deemed to be sufficient after one visit as the participants were consistent in their performance on all tests and indicated that they were comfortable in performing them. After visit 1, the participants were assigned to one of the two conditions (BCAA supplement or placebo) in a double-blind, matched-pairs design. The participants were matched on body mass, sex and 1 RM strength. The supplements (placebo or BCAA) were consumed 30-min before and after the muscle damage protocol. Over the following 48-h, the supplements were provided 30-min before and after re-testing. The supplements were prepared by an independent laboratory technician, who was

also responsible for the random allocation of participants to each group. The randomisation was carried out by assigning each participant a number and using publicly available software to allocate their group (http://www.randomization.com/). On visit 2, the participants had capillary blood samples drawn from the finger for the measurement of baseline creatine kinase (CK) and then performed a battery of tests in the following order: perceived soreness, lower-limb isometric strength and countermovement jumping. After the baseline testing, the participants were supervised through the muscle-damage protocol. One hour after the damage protocol, the same measurements were taken. Visits 3 and 4 followed 24-h and 48-h, respectively, after the muscle damage protocol, where the same battery of tests were performed.

Procedure

Knee-extensor isometric strength

To test the maximal isometric strength of the knee-extensor muscles, each participant sat on a custom made, adaptable strength chair, with their back and knees fully supported. Their knee was firmly fixed at 100˚ and their hips at 110˚, which was verified using a goniometer. Their right leg was firmly strapped to the chair across the mid-thigh, whilst their ankle (immediately above malleoli) was fixed to a strain gauge (Interface SSM-AJ-500 Force Transducer; Interface, Scottsdale, AZ; 0.05% maximum error), sampling at 1000 Hz. The strain gauge recorded force as alteration in voltage. Calibration of the strain gauge with a known mass demonstrated the relationship between voltage and Newtons as linear, allowing determination of a regression formula to convert voltage to Newtons. A second calibration was performed with the same weights at the completion of testing, producing an ICC of 0.99. The strain gauge was attached to the participant using a high tension belt. The chair set-up was replicated for each participant in subsequent trials. Their upper-body was also tightly

fitted to the chair with two straps across each shoulder, which they were instructed to grip with their hands throughout the testing. A command of '3-2-1-GO' was given, after which the participants performed a maximal isometric knee extension for 5-s. Non-specific verbal encouragement was provided to the participants for motivation. Participants performed three maximal tests, separated by 2-min. A maximal voluntary contraction (MVC) was determined as the highest of three values. If the peak force (N) produced by participants systematically increased across the three tests, a fourth test was conducted. The reliability of this procedure was 2% (coefficient of variation; CV).

Counter-movement jumping (CMJ)

Participants performed a CMJ on a jump mat (Probiotics Inc, Huntsville, AL, USA) by standing with their feet at shoulder width, hands on hips and descending to ~90˚ before propelling themselves vertically to the highest possible height, keeping their legs fully extended. Standardised non-specific motivation and cues were provided to facilitate performance. The participants performed three jumps, separated by 2-min and the highest jump height (cm) was recorded. If the values systematically increased across the three tests, a fourth test was conducted. The test re-test reliability of this procedure was 1.2% (CV).

Blood sampling and analysis

The index fingertip of the subject was cleaned using a sterile alcohol swab and allowed to dry. Capillary blood was drawn from the finger and a sample of whole blood (30 μL) was collected into a heparinised capillary tube. The whole blood was centrifuged at 3000 rpm (4°C) for 5 min, and the resultant plasma was removed and stored at -80°C until subsequent analysis. Plasma CK was measured using a chemistry analyser (Rx Monza, Randox Laboratories Ltd., Crumlin, Antrim, UK). The intra-sample CV of the analyser is < 4% CV at

high and low concentrations and the expected baseline sample range is 37-2755 IU/L for CK, according to manufacturer's guidelines. To eliminate inter-assay variance, all samples were analysed in the same assay run

Perceived soreness

The participants were asked to rate their perceived muscle soreness in the lower-limbs from 0-10 on a 200 mm Visual Analogue Scale (VAS). The numbers were concealed from the participant on the reverse of the scale, whilst the verbal anchors of no muscle soreness (~0 on reverse), soreness upon movement (~5 reverse) and too sore to move (~10 reverse) were observed from the front of the scale. To do this, the participants performed a 5-s isometric squat, with their ankles, knees and hips at 90˚ and, after 5-s, moved a sliding scale to the number which they perceived to correspond to their level of soreness (Howatson et al., 2012).

Supplementation

Each participant was supplemented with either a placebo or a BCAA beverage, both of which contained 0.25 g/kg body mass of dextrose dissolved into 300 ml of water, thus ensuring drinks were indistinguishable in taste. The BCAA drinks were supplemented at a dosage of 0.087 g/kg body mass (Børsheim et al., 2002), consisting of leucine, isoleucine, and valine in a 2:1:1 ratio (Myprotein, Cheshire, UK). This dosage of AA has been shown to promote recovery from resistance exercise (Børsheim et al., 2002) and the ratio of leucine, isoleucine and valine was pre-determined by the manufacturer. Drinks were consumed 30-min before and immediately after the muscle damage protocol (Jackman et al., 2010). Over the following 48-h, the supplements were provided 30-min before and immediately after re-testing. On the final day, the supplement was taken with breakfast and 30-min before testing to provide two doses.

Muscle-damage protocol

A standardised warm-up was performed on the day, comprising walking, dynamic stretching and squatting up to 30% of the final load. The participants then performed back squats at an intensity of 70% of 1 RM for 10 repetitions across 6 sets (ACSM, 2009). Two minutes rest was given between sets, where participants remained standing and were free to walk around a 10 m x 10 m area. To replicate an optimal hypertrophy session, the participants were told to perform all repetitions as per their familiarisation, with an eccentric phase of 3-s, followed by a moderate 2-3 s concentric phase (Schoenfeld, 2010). If a set dropped below 8 repetitions, or the investigator judged the quality of technique to regress, then the weight was decreased by 10% until the desired repetition range or lifting form was achieved.

Statistical analyses

Based on best-practice recommendations for research in sports nutrition (See Burke, 2008), effect sizes (ES) and magnitude-based inferences (MBIs) were used to identify mechanistic differences in the dependent variables between the two experimental conditions (placebo or BCAA). All of the dependent variables were expressed as change relative to baseline. Effect sizes were defined as; *trivial* = 0.2; *small* = 0.21–0.6; *moderate* = 0.61–1.2; *large* = 1.21–1.99; *very large* > 2.0 (Batterham and Hopkins, 2006). Raw data were log-transformed to account for non-uniformity of effects. Threshold probabilities for substantial effects based on the 90% confidence limits were: <0.5% most unlikely, 0.5–5% very unlikely, 5–25% unlikely, 25–75% possibly, 75–95% likely, 95–99.5% very likely, 99.5% most likely. Thresholds for the magnitude of the observed change in the dependent variables were determined as the within-participant standard deviation × 0.2 (small) 0.6 (moderate) and 1.2 (large). Effects with confidence limits across a likely small positive or negative change were

classified as unclear (Hopkins et al., 2009). The uncertainty of effects was based on 90% confidence limits for all variables. A custom spreadsheet was used to perform all of the calculations (http://www.sportsci.org/.).

Results

There were *trivial* differences between BCAA and placebo groups for the total energy intake (2486 ± 412 kcal/day *cf.* 2667 ± 449 kcal/day; *trivial, unlikely*), carbohydrate (1157 ± 354 kcal/day *cf.* 1230 ± 300 kcal/day; *trivial, unlikely*), fat (980 ± 283 kcal/day *cf.* 1049 ± 275 kcal/day; *trivial, unlikely*) and protein energy intake (378 ± 79 kcal/day *cf.* 394 ± 101 kcal/day; *trivial, unlikely*).

Changes in isometric strength (% baseline) are presented in Figure 1 (mean ± SD). There were *large* reductions in isometric strength between baseline and post-exercise for the BCAA group (1031 ± 273 N *cf.* 976 ± 238 N, respectively *most likely* ↓), whilst the placebo group showed *very large* changes (899 ± 248 N *cf.* 734 ± 186 N, respectively; *most likely* ↓). By 24-h post-testing, the reduction in strength remained *very large* for the placebo group (*most likely* ↓) and *large* for the BCAA group (*most likely* ↓), which were subsequently reduced to *moderate, unclear* changes among the placebo and BCAA conditions at 48-h. Both groups did not return to baseline strength levels during the study. Pairwise between-group tests revealed a greater reduction in isometric strength at 24-h in the placebo group compared to the BCAA group (*moderate, likely* ↓), indicating a delayed recovery of muscle function.

*********Figure 1 near here*********

Changes in CK concentration (% baseline) are presented in Figure 2 (mean ± SD). There were *moderate* increases in CK between baseline and post-exercise for the BCAA group (339 ± 77 IU/L *cf.* 783 ± 407 IU/L, respectively; *very likely* ↑), whilst there were *small, unclear* increases in the placebo group (357 ± 121 IU/L *cf.* 538 ± 235 IU/L, respectively). By 24-h post-testing, the increase in CK was *large* and *most likely* for both groups, which remained *large,* and *most likely* between baseline and 48-h. Both groups did not return to baseline CK levels during the study. Pairwise between-group tests revealed *large, possible* increases in CK concentration at 24-h and 48-h in the BCAA group compared to the placebo group.

*********Figure 2 near here*********

Changes in CMJ height (% baseline) are presented in Figure 3 (mean ± SD). There were *moderate, very likely* reductions in CMJ height between baseline and post-exercise for the both groups (BCAA = 56.3 ± 6.9 cm *cf.* 54.6 ± 8.2 cm; Placebo = 55.4 ± 5.4 cm *cf.* 52.8 ± 6.2 cm). By 24-h post-testing, the reduction in CMJ height was *very large* and *most likely* for both groups, which improved to a *trivial,* and *unclear* for both groups between baseline and 48-h, indicating a return to baseline. Pairwise between-group tests revealed *small, likely* reductions in CMJ height at post-exercise and 24-h in the placebo group compared to the BCAA group.

*********Figure 3 near here*********

Changes in perceived muscle soreness (% baseline) are presented in Figure 4 (mean ± SD). There were *large, most likely* increases in perceived muscle soreness between baseline and post-exercise for the both groups (BCAA = 1.6 ± 0.9 AI *cf.* 8.6 ± 3.4 AI; Placebo = 1.8 ± 8.6

AI *cf.* 8.6 ± 4.4 AI). By 24-h post-testing, the increase in perceived muscle soreness remained *large* and *most likely* for both groups, which improved to a *trivial,* and *unclear* between baseline and 48-h, indicating a return to baseline. Pairwise between-group tests revealed *small, likely* increases in perceived muscle soreness at 24-h and 48-h in the placebo group compared to the BCAA group.

*********Figure 4 near here**********

Discussion

We investigated the effects of BCAA supplementation on recovery from muscle damage after a hypertrophy-based protocol, among participants regularly taking part in this form of exercise. All of the participants in this study exhibited indirect signs of exercise-induced muscle damage, with both the placebo and BCAA groups declining in strength and CMJ height and increasing DOMS and CK concentration across the 48-h recovery period. However, as hypothesised, the primary finding of this study was that a BCAA supplement of 0.087 g/kg body mass was sufficient to reduce the effects of a hypertrophy-based training session on isometric strength, CMJ height and DOMS compared to placebo. The differences between groups were predominantly noted at the 24-48-h period, whereby the BCAA group showed differences (*small* to *large*) in strength, CMJ and DOMS compared to placebo (Figures 1-4), indicating faster recovery towards baseline values. That baseline values of strength or CMJ height were not re-established after 48-h was not unexpected as other studies have shown that muscle function and other performance measures do not return to baseline 72-h to 96-h after muscle damage when BCAAs are orally administered (Jackman et al.,

2010; Howatson et al., 2012; Kirby et al., 2012). Most importantly, our findings support the suggestion that BCAA supplementation can increase the rate of recovery in muscle function among well-trained habitual weight-lifters, following a muscle damage protocol that mimicked a typical hypertrophy training session.

Similar findings have been reported previously, where a comparable dosage of a BCAA supplement, administered across a 7-day loading period, accelerated the recovery of muscle function and DOMS (Howatson et al., 2012). Kirby et al. (2012) also reported an improvements in recovery of isometric strength, but not squat jump height, after muscle damage using a short-term (beginning 30-min prior to exercise) leucine supplementation regime, similar to the current study. On this basis, Kirby and co-authors questioned the usefulness of leucine as a recovery agent if its effects do not transfer to ballistic tasks. Indeed, others have reported similar findings, with no change in vertical jump height between placebo and BCAA groups after muscle damage (Howatson et al., 2012). In disagreement with these studies, we found that CMJ height was recovered faster in the BCAA group compared to placebo at 24-h post-damage. The use of a standard CMJ test, opposed to a static jump (Kirby et al., 2012), might partly explain this difference as it is known that movements involving the stretch-shortening cycle (SSC) can be effected by muscle damage, partly owing to structural changes in non-contractile elements and an associated loss of muscle-tendon stiffness (Komi, 2000). *In vitro* studies have shown that leucine administration can promote the restoration of damaged connective tissue in rat skeletal muscle (Perieira et al., 2014), which is responsible for the transfer of energy between the muscle and tendon structures (Turrina et al., 2013). Therefore, it is feasible that the repair of damaged connective tissue was facilitated by BCAA supplementation, thus supporting energy transfer during SSC movements. There are other mechanisms, such as impairment of reflex-sensitivity (Komi, 2000) that might explain the

poorer CMJ performance post-muscle damage. The lowered DOMS after BCAA supplementation is likely to have caused less neural inhibition, thus enabling improved reflex sensitivity and performance (Nicol et al., 2003). Based on the above findings, we suggest that long-term BCAA supplementation may not be necessary for the recovery of isometric muscle function after muscle damage and that this occurs in parallel to the recovery of CMJ performance. Of course, these differences could also be attributed to the application of an ostensibly more appropriate statistical technique (Batterham and Hopkins, 2006; Burke, 2008) compared to previous studies, particularly when the difference between groups is *small*.

Heavy resistance exercise has been shown to induce the release of muscle proteins, such as CK, into the blood stream (Kraemer et al., 1993). As anticipated, CK concentration increased in the 24-h after muscle damage in both conditions, reflecting disruption of the sarcolemmal membrane. However, BCAA supplementation appeared to increase CK compared to placebo in the current study, which was not expected. Our findings add to the equivocality of current research, with some reporting no change in the CK response after muscle damage (Jackman et al., 2010) and others showing an attenuation of the CK response after mixed amino acid or BCAA supplementation (Nosaka et al., 2006; Howatson et al., 2012; Kirby et al., 2012). The current findings could be attributed to the well-described intra-individual CK response to muscle damage, resulting in large standard deviations and random variations in CK values across the subsequent days (Clarkson and Ebbeling, 1988). Irrespective of the reasons for these findings, the current data question the supposition that CK efflux after muscle damage is blunted by BCAA availability. Furthermore, given that soreness was also lower in the BCAA group, despite higher CK values, these findings do not support the involvement of BCAAs in a proposed mechanism that relies on parallel changes in these measures to explain

a reduction in secondary muscle damage (Howatson et al., 2012). These findings support the suggestion that CK might not be a useful single marker of muscle damage (Chrismas et al., 2013).

This was the first study to test the effect of BCAA supplementation on muscle damage in a sample of experienced resistance-trained athletes, who perform habitual hypertrophy training, using a mode of exercise that reflects their day-to-day activities. Our findings show that muscle damage is caused among athletes of this type and that acute BCAA supplementation, prior to and during the recovery period, is capable of accelerating recovery in the 24-48-h after a muscle damaging bout of exercise. This is particularly relevant to athletes taking part in regular hypertrophy training as programmes of this type necessitate a maximum of 48-h between training days. In addition, this is the first study to prescribe and monitor the daily diet of resistance-trained participants, thus controlling the total energy and macronutrient intake during the BCAA supplementation period. This is an extremely important aspect of the current study because, without such measures being taken, the available energy, as well as the amount and quality of amino acids being ingested alongside the supplement is unknown. Indeed, lack of suitable dietary control has been suggested as a major limitation among studies that have investigated the effects of BCAA or protein supplementation on recovery from muscle damage (Pasiakos et al., 2015). Our findings, therefore, support studies that have reported positive effects of acute BCAA (or isolated leucine or amino acid) supplementation on recovery from muscle damage (Kirby et al., 2012) and extend this to diet-controlled, experienced resistance-trained athletes.

Whilst the diet of the current participants was under control, it should be stated that, given the design of this study which did not include a separate mixed amino acid group, it is unclear

whether the accelerated recovery was due to the BCAA alone mixture or the addition of amino acids to the diet. Future studies should extend the current work by including amino acid and isolated leucine groups to the experimental design. The isolated leucine group is a particularly important part of this suggestion as leucine has the most potent effects on muscle protein synthesis via mTor pathways (Philips, 2009), which is not the case for the remaining BCAAs, isoleucine and valine (Churchward-Venne et al., 2014). BCAAs, collectively, have long-held presumed stimulatory effects on protein synthesis (Blomstrand et al., 2006), which might explain their inclusion in recovery drinks. Indeed, this was part of the rationale for the current study. However, given the reported competition between leucine, isoleucine and valine for cellular transport and subsequent metabolism (Cynober, 2002), it is possible that valine and isoleucine inhibited the effects of leucine on muscle protein synthesis and that the combination of all three BCAAs is unnecessary or detrimental to muscle recovery (De Bandt and Cynober, 2006).

Administration of leucine-rich amino acids has also been shown to reduce the appearance of inflammatory cytokines, whilst increasing muscle protein synthesis after eccentric exercise in rodents (Kato et al., 2016) and after endurance exercise in athletes (Rowlands et al., 2016). Muscle soreness is partly related to local inflammation, which is a necessary part of the recovery process that follows acute mechanical damage of the myofibres (Howatson and van Someren, 2008). The proposed anti-inflammatory effects of leucine post-exercise might explain its capacity to lower muscle soreness. However, there are putative roles for all BCAA during the acute inflammatory phase of muscle damage. This is because of the known transamination of all BCAA into glutamate and, thus, contribution to the glutamate-glutamine pool, which is a known substrate for inflammatory cells (Nicastro et al., 2012). It is, therefore, important that future research is designed to examine the effects of isolated BCAA,

most notably leucine, on the inflammatory responses to resistance exercise and associated muscle soreness.

Given the unanticipated CK response after the muscle-damaging exercise in the current study, it would have been useful to measure other blood markers of muscle damage, such as myoglobin, which is known to increase after strenuous exercise (Brancaccio et al., 2010) and can be reduced after protein supplementation (Cockburn et al., 2008). This would have helped to comprehend the unexpected CK response and provided a more comprehensive understanding of the mechanisms by which a BCAA supplement is able to accelerate recovery muscle damage, without appearing to alter the level of blood proteins. Indeed, replication of this study using different combinations of BCAA dosage would be beneficial, given the reports that question the necessity of valine and isoleucine as part of a BCAA supplement (De Bandt and Cynober, 2006; Churchward-Venne et al., 2014). Furthermore, future studies should investigate the chronic effects of BCAA supplementation on recovery from muscle damage as athletes undertaking hypertrophy training will require long term recovery strategies and the findings of this study were limited to a 48-h recovery period. However, the diet control and supervised supplementation regime that were imposed herein to isolate the acute effects of BCAA supplementation would be methodologically challenging for future researchers, yet not impossible to achieve.

Conclusion

Acute oral supplementation of BCAAs at a concentration of 0.087 g/kg body mass was sufficient to increase the rate of recovery in isometric strength, CMJ height and perceived soreness compared to placebo after a hypertrophy-based training session among resistance-trained athletes. This means that, based on a 100 kg athlete supplementing twice daily, as

little as 17.2 g/day of BCAAs is necessary to accelerate recovery from hypertrophy training sessions. However, further studies are required to understand whether the provision of BCAAs, the amino acid content alone or isolated BCAAs are mediating this response. This study also highlights the importance of controlling the energy and macronutrient intake of participants during research of this type, owing to the potential confounding influence of unaccounted dietary food sources on recovery from muscle damage.

Disclaimer: The authors report no conflicts of interest associated with this manuscript.

References

ACSM. American College of Sports Medicine position stand. 2009. Progression models in resistance training for healthy adults. Med. Sci. Sports Exerc, 41: 687–708: doi: 10.1249/MSS.0b013e3181915670.

Alfonzo-González, G., Doucet, E., Alméras, N., Bouchard, C. and Tremblay, A. 2004. Estimation of daily energy needs with the FAO/WHO/UNU 1985 procedures in adults: comparison to whole-body indirect calorimetry measurements. Eur. J. Clin. Nutr, 58(8):1125-31: doi: 10.1038/sj.ejcn.1601940.

Barnett, A. 2006. Using recovery modalities between training sessions in elite athletes. Sport Med, 36(9): 781-796: PMID: 16937953.

Batterham, A. M. and Hopkins, W. G. 2006 Making meaningful inferences about magnitudes. Int. J. Sports Physiol. Perform, 1(1): 50-57. PMID: 19114737.

Blomstrand, E., Eliasson, J., Karlsson, H. K. R. and Kohnke, R. 2006. Branched-Chain Amino Acids Activate Key Enzymes in Protein Synthesis after Physical Exercise J. Nutr, 136: 269–273, 2006: PMID: 16365096.

Bloomer, R. J., Falvo, M. J., Fry, A. C., Schilling, B. K., Smith, W. A. and Moore, C. A. 2006. Oxidative stress response in trained men following repeated squats or sprints. Med. Sci. Sports Exerc, 38:1436-1442: doi: 10.1249/01.mss.0000227408.91474.77.

Børsheim, E., Tipton, K. D., Wolf, S. E. and Wolfe, R. R. 2002. Essential amino acids and muscle protein recovery from resistance exercise. Am. J. Phys. Endocr. Metabol, 283(4): 648-657: PMID: 12217881.

Brancaccio, P. Lippi, G. and Maffulli, N. 2010. Biochemical markers of muscular damage. Clin. Chem. Lab. Med, 48(6):757-767: PMID: 20518645.

Burke, L. M. 2008. Caffeine and sports performance. Appl. Physiol. Nutr. Metab, 33(6):1319-1334: PMID: 19088794.

Carpinelli, R. N., Otto, R. M. and Winett, R. A. 2004. A critical analysis of the ACSM position stand on resistance training: Insufficient evidence to support recommended training protocols. J. Exerc. Physiol. Online, 7(3): 1-60.

Cheung, K., Hume, P. and Maxwell, L. 2003. Delayed onset muscle soreness: treatment strategies and performance factors. Sports Med, 33(2):145-64: PMID: 12617692.

Clarkson, P. M. and Ebbeling, C. 1988. Investigation of serum creatine kinase variability after muscle-damaging exercise. Clin. Sci. (Lond), 75(3): 257–261: PMID: 3416560.

Cockburn, E., Hayes, P.R., French, D. N., Stevenson, E. and St Clair Gibson, A. 2008. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. Appl. Physiol. Nutr. Metab. 33:775–783: doi: 10.1139/H08-057.

Coombes, J. S. and McNaughton, L. R. 2000. Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. J. Sports Med. Phys. Fitness, 40(3): 240-246: PMID: 11125767.

Chrismas, B. C. R., Taylor, L., Carrol, S., Smith, A. Pemberton, P., Seigler, J. C. et al. 2009. Reproducibility of creatine kinase: how useful is this measurement tool? 11th ISEI Symposioum, Newcastle, Australia, 9-12 Sept. 2013. *Exercise Immunology: Prescriptions for Health.*

Churchward-Venne, T. A., Breen, L., Di Donato, D. M., Hector, A. J. Mitchell, C. J., Moore, D. R., et al. 2014. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. Am. J. Clin. Nutr. 99:276–286. doi: 10.3945/ajcn.113.068775.

Cynober, L. A. 2002. Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance. Nutr, 18:761–766: PMID: 12297216

da Luz, C. R., Nicastro, H., Zanchi, N. E., Chaves, D. F. S. and Lancha, A. H. 2011. Potential therapeutic effects of branched-chain amino acids supplementation on resistance exercise-based muscle damage in humans. J. Int. Soc. Sports Nutr, 8:23: doi: 10.1186/1550-2783-8-23

De Bandt, J. and Cynober, L. J. 2006. Therapeutic Use of Branched-Chain Amino Acids in Burn, Trauma and Sepsis. J. Nutr, 136: 308–313: PMID: 16365104.

Dickinson, J. M., Gundermann, D. M., Walker, D. K., Reidy, P. T., Borack, M. S., Drummond, M. J., et al. 2014. Leucine-enriched amino acid ingestion after resistance exercise prolongs myofibrillar protein synthesis and amino acid transporter expression in older men. J. Nutr, 144:1694-16702: doi: 10.3945/jn.114.198671.

Falvo, M. J., Schilling, B. K., Bloomer, R. J., Smith, W. A. and Creasy, A. C. 2007. Efficacy of prior eccentric exercise in attenuating impaired exercise performance after muscle injury in resistance trained men. J. Strength Cond. Res, 21:1053-1060. doi: 10.1519/R-21406.1

Falvo, M. J., Schilling, B. K., Bloomer, R. J. and Smith, W. A. J. 2009. Repeated bout effect is absent in resistance trained men: an electromyographic analysis. Electromyogr. Kinesiol, 19: 529-535: doi: 10.1016/j.jelekin.2008.10.010.

Fouré, A., Nosaka, K., Gastaldi, M., Mattei, J. P., Boudinet, H., Guye, M., et al. 2016. Effects of branched-chain amino acids supplementation on both plasma amino acids concentration and muscle energetics changes resulting from muscle damage: A randomized placebo controlled trial. Clin. Nutr, 35: 83-94: PMID: 25886707.

Goldberg, A. L., Etlinger, J. D., Goldspink, D. F. and Jablecki, C. 1975. Mechanism of work-induced hypertrophy of skeletal muscle. Med. Sci. Sports, 7: 185-198: PMID: 128681

Hopkins, W. G., Marshall, S. W., Batterham, A. M. and Hanin, J. 2009. Progressive statistics for studies in sports medicine and exercise science. Med. Sci. Sports Exerc, 41(1): 3-13: doi: 10.1249/MSS.0b013e31818cb278.

Howatson, G. and van Someren, K. A. 2008. The prevention and treatment of exercise-induced muscle damage. Sport Med, 38: 483–503: PMID: 18489195.

Howatson, G., Hoad, M. Goodall, S., Tallent, J., Bell, P. G. and French, D. N. 2012. Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study. Amino Acids, 9(20): doi: 10.1186/1550-2783-9-20.

Jackman, S. R., Witard, O. C., Jeukendrup, A. E. and Tipton, K. D. 2010. Branched-chain amino acid ingestion can ameliorate soreness from eccentric exercise. Med. Sci. Sports Exerc, 42(5): 962-970: doi: 10.1249/MSS.0b013e3181c1b798.

Kato, H., Miura, K., Nakano, S., Suzuki, K., Bannai, M., and Inoue, Y. 2016. Leucine-enriched essential amino acids attenuate inflammation in rat muscle and enhance muscle repair after eccentric contraction. Amino Acids, 48: 2145–2155: doi: 10.1007/s00726-016-2240-1.

Kirby, T. J. Triplett, T. N., Haines, T. L., Skinner, J. W., Fairbrother, K. R. and McBride, J. M. 2012. Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. Amino Acids, 42:1987–1996: doi: 10.1007/s00726-011-0928-9.

Komi, P. V. 2000. Stretch-shortening cycle: a powerful model to study normal and fatigued muscle. J. Biomech, 33(10): 1197-1206: PMID: 10899328.

Kraemer, W. J., Dziados, J. E., Marchitelli, L. J., Gordon, S. E., Harman, E. A., Mello, R.,et al. 1993. Effects of different heavy-resistance exercise protocols on plasma beta-endorphin concentrations. J. Appl. Physiol, 74(1): 450-459: PMID: 8444727.

McHugh, M. P. 2003. Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. Scand. J. Med. Sci. Sports, 13: 88-97: PMID: 12641640.

Mitchell, C. J., Churchward-Venne, T. A., West, D. W., Burd, N. A., Breen, L., Baker, S. K. et al. 2012. Resistance exercise load does not determine training-mediated hypertrophic gains in young men. J. Appl. Physiol, 113: 71–77: doi: 10.1152/japplphysiol.00307.

Nedelec, M., McCall, A., Carling, C. Legall, F. Berthoin, S. and Dupont, G. 2013. Recovery in Soccer: Part II—Recovery Strategies. Sport Med, 43(1): 9-22: doi: 10.1007/s40279-012-0002-0.

Nicastro, H., Ribeiro da Luz, C., Chaves, D., Bechara, L. R. G., Voltarelli, V. A. Rogero, M. et al. 2012. Does Branched-Chain Amino Acids Supplementation Modulate Skeletal Muscle Remodeling through Inflammation Modulation? Possible Mechanisms of Action. J. Nutr, Metab, doi:10.1155/2012/136937

Nicol, C., Kuitunen, S., Kyröläinen, H., Avela, J. Komi, P. V. 2003. Effects of long- and short-term fatiguing stretch-shortening cycle exercises on reflex EMG and force of the tendon-muscle complex. Eur. J. Appl. Physiol, 90(5-6): 470-9: doi: 10.1007/s00421-003-0862-y

Nosaka, K., Sacco, P. and Mawatari, K. 2006. Effects of amino acid supplementation on muscle soreness and damage. Int. J. Sport Nutr. Exerc. Metab, 16(6): 620–635: PMID: 17342883.

Pasiakos, S.M., Lieberman, H.R. & McLellan, T.M. 2015. The effects of protein supplements on muscle mass, strength, and aerobic and anaerobic power in healthy adults: a systematic review. Sports Med, 45(1):111-131: doi: 10.1007/s40279-014-0242-2.

Pereira, M. G., Silva, M. T., Carlassara, E. O., Gonçalves, D. A., Abrahamsohn, P. A., Kettelhut, I. C., et al. 2014. Leucine supplementation accelerates connective tissue repair of injured tibialis anterior muscle. Nutrients, 6(10): 3981-4001: doi: 10.3390/nu6103981.

Philips, S. 2009. Physiologic and molecular bases of muscle hypertrophy and atrophy: impact of resistance exercise on human skeletal muscle (protein and exercise dose effects). Appl. Physiol. Nutr. Metab, 34: 403–410: doi: 10.1139/H09-042.

Proske, U. and Morgan, D. L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J. Physiol, 537(2): 333-345: PMID: 11731568

Rowlands, D. S., Nelson, A. R., Raymond, F., Metairon, S., Mansourian, R., Clarke, J., et al. 2016. Protein-leucine ingestion activates a regenerative inflammo-myogenic transcriptome in skeletal muscle following intense endurance exercise. Physiol. Genomics, 48: 21-32: doi: 10.1152/physiolgenomics.00068.2015.

Schoenfeld, B. J. 2010. The mechanisms of muscle hypertrophy and their application to resistance training. J. Strength Cond. Res, 24(10): 2857-2872: doi: 10.1519/JSC.0b013e3181e840f3.

Schoenfeld, B. J. 2012. Does exercise-induced muscle damage play a role in skeletal muscle hypertrophy? J. Strength Cond. Res, 26(5):1441-1453: PMID: 22344059.

Sharp, C. P. and Pearson, D. R. 2010. Amino acid supplements and recovery from high-intensity resistance training. J. Strength Cond. Res, 24(4): 1125-1130: doi: 10.1519/JSC.0b013e3181c7c655.

Shimomura, Y., Kobayashi, H., Mawatari, K., Akita, K., Inaguma, A., Watanabe, S., et al. 2009. Effects of squat exercise and branched-chain amino acid supplementation on plasma free amino acid concentrations in young women. J. Nutr. Sci. Vitaminol, 55(3): 288-291: PMID: 19602839.

Shimomura, Y., Inaguma, A., Watanabe, S., Yamamoto, Y., Muramatsu, Y., Bajotto, G., et al. 2010. Branched-chain amino acid supplementation before squat exercise and delayed-onset muscle soreness. Int. J. Sport Nutr. Exerc. Metab, 20: 236–244: PMID: 20601741

Sorichter, S., Puschendorf, B. and Mair J. 1999. Skeletal muscle injury induced by eccentric muscle action: muscle proteins as markers of muscle fiber injury. Exerc. Immunol. Rev, 5:5-21: PMID: 10519060

Turrina, A., Martínez-González, M. A., Stecco, C. 2013. The muscular force transmission system: role of the intramuscular connective tissue. J. Bodyw. Mov. Ther, 17(1): 95-102: PMID: 23294690.

Zanchi, N. E., Nicastro, H. and Lancha, A. H. 2008. Potential antiproteolytic effects of Lleucine: observations of in vitro and in vivo studies. Nutr. Metab. (Lond), 5:20: doi: 10.1186/1743-7075-5-20.

Figure 1. Changes in isometric strength (% baseline) after muscle damage among the placebo and BCAA groups. † = moderate difference between conditions at that time point.

Figure 2. Changes creatine kinase concentration (% baseline) after muscle damage among the placebo and BCAA groups. * = large difference between conditions at that time point.

Figure 3. Changes counter-movement jump (CMJ) height (% baseline) after muscle damage among the placebo and BCAA groups. ¥ = small difference between conditions at that time point.

Figure 4. Changes perceived soreness (% baseline) after muscle damage among the placebo and BCAA groups. ¥ = small difference between conditions at that time point.