Branched Chain Amino Acid Plus Glucose Supplement Reduces Exercise-Induced Delayed Onset Muscle Soreness in College-Aged Females

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Title:
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Abstract:

The three branched chain amino acids (BCAAs), leucine, isoleucine and valine, are the amino acids that are metabolized in the greatest quantity by skeletal muscle during exercise. Supplementation of BCAAs has been used to stimulate muscle protein synthesis following exercise. The purpose of this study was to determine if supplementation of BCAAs in combination with glucose would reduce exercise-induced delayed onset muscle soreness (DOMS). Using a double-blind crossover design, subjects (n=20) were randomly assigned to either BCAA (n=10) or placebo (n=10) groups. Subjects performed a squatting exercise to elicit DOMS and were required to rate their muscle soreness every 24 hours for four days following exercise while continuing to consume the assigned supplement. After a three-week recovery period subjects returned for a second visit to perform the same procedure only this time they were administered whichever treatment they did not receive during the first visit. An overall trend of lower reported muscle soreness was observed for BCAA beginning 24 hours after exercise, although these results were not statistically significant (p=0.1057). Reported muscle soreness for mean muscle soreness for all participants during BCAA consumption was 33% lower than placebo at the 24-hour rating (p=0.1423). However, there was a statistically significant difference between BCAA and placebo for females at the 24-hour rating (p=0.0182).

Introduction:

The branched chain amino acids (BCAAs) leucine, isoleucine, and valine are three of the nine amino acids that are essential for human protein synthesis. The BCAAs are similar in structure and are catabolized via the same metabolic pathway, resulting in breakdown products that feed directly into the Krebs cycle to resynthesize adenosine triphosphate (ATP) (1). The BCAAs account for 35% of the essential amino acids found in skeletal muscle protein (2). During exercise, skeletal muscle mitochondria will metabolize BCAA through a two-step process. The first involves the transamination of the BCAA to an alpha-keto acid by the enzyme branched-chain amino transferase (BCAT). The BCAA in the alpha-keto acid form remain in the tissue amino acid pool. From there, these alpha-keto acids can either be further catabolized by the enzyme branched chain alpha-keto acid dehydrogenase (BCKD) to form products which feed into the Krebs cycle or are used to resynthesize muscle protein (3). The BCKD complex is thought to be the rate-limiting step in BCAA catabolism and is activated through dephosphorylation (1). Studies have shown that there is relatively low activity of BCKD in skeletal muscle as compared to liver, due to increased levels of a deactivating enzyme BCKD-kinase that is present in skeletal muscle. Therefore, the BCAA that are catabolized to alpha-keto acids during exercise are more likely be used to resynthesize skeletal muscle protein after exercise, rather than be used to resynthesize ATP through the Krebs cycle (4).

Skeletal muscle oxidizes a greater proportion of BCAA than any of the other amino acids during exercise (5). Studies have shown that eccentric muscle contractions elicit the greatest magnitude of delayed onset muscle soreness (DOMS) which is the pain

felt in skeletal muscles upon palpation or movement following exercise, generally peaking within 24-48 hours (6). By performing a controlled squatting exercise, the skeletal muscles of the legs undergo eccentric contractions, breaking down muscle protein and creating small tears in the muscle fibers.

Recent studies have attempted to use BCAA supplementation before exercise to increase the amount of free BCAA in the amino acid pool, in an effort to increase muscle protein synthesis after exercise (7,8). The results from one single-blind study showed significant reduction in post-exercise DOMS in females after supplementation with 5g BCAA prior to eccentric exercise (5). In this study, glucose was used to substitute for the BCAA in the placebo, but was not used in the test supplement. While this placebo may have been formulated in an attempt to make a direct correlation with BCAA supplementation and a reduction in DOMS, it should be noted that consumption of carbohydrates, mainly glucose, stimulates insulin production in the body. Through mechanisms of cell signaling, insulin stimulates muscle protein synthesis by inductively increasing the production of ribosomes in the cell, as well as controlling the initiation and elongation of mRNA (9,10). In a study designed to test the synergistic effects of supplementation with BCAA and carbohydrates, it was determined that muscle protein synthesis was significantly increased in subjects receiving the combination of BCAA and carbohydrate, compared to those receiving only BCAA (8). The extent of DOMS reduction was not measured in this study. It was however noted that further experimentation on the effects of BCAA with carbohydrate supplementation on DOMS would be a valuable avenue to pursue.

Many studies that have tested the effects of BCAA supplementation on DOMS have used a non-nutritive sweetener in their placebo instead of glucose (7,11,12). As previously stated, insulin is needed to induce muscle protein synthesis and this cannot occur without the ingestion of carbohydrates such as glucose. Thus the purpose of this study was to evaluate the efficacy of a BCAA plus glucose supplement versus a glucose-containing placebo on exercise-induced DOMS.

METHODS:

Participants:

Men (n=9) and women (n=11) aged 18-25 who engaged in no more than one hour of light to moderately intense physical activity per week were recruited from the Greater Burlington, Vermont area. Subjects were recruited using flyers, in-class announcements at the University of Vermont, and an ad placed on Craigslist. Exclusion criteria included individuals that engaged in more than one hour per week of light to moderate physical activity, had been involved in strict athletic competition or weight training in the past six months, were pregnant or nursing, had a known muscular disease, diabetes mellitus, cardiovascular disease, respiratory disease, and/or were currently taking a protein-based dietary supplement. Subjects were tested in conformity with a protocol approved by the University of Vermont Institutional Review Board and all participants were required to sign an informed consent form prior to participation.

General Study Design:

The study was a randomized, controlled, double-blind crossover design. Each participant was tested with the BCAA supplement and the placebo separated by a recovery period of 3-7 weeks depending on subject availability. During visit 1, participants' height and weight were measured using a sliding balance scale and body fat percentage was determined using bioelectrical impedance (model TBF-305; Tanita Corp. of America Inc., Skokie, IL, USA). Subjects were asked to rate their muscle soreness using a numerical rating scale (NRS) prior to consumption of either the BCAA supplement or placebo. Subjects then performed a squatting exercise consisting of 3 sets of 12 squats with a one minute rest between sets, followed by another muscle soreness rating immediately after the exercise. Participants were asked to consume another pre-measured dosage of either the test supplement or placebo at 24-hour intervals for the next four days. Immediately following consumption of the BCAA supplement or placebo subjects again rated their muscle soreness. Following a minimum of at least three weeks from completion of the first round of supplement administration, participants returned for visit 2 to perform the same procedure as outlined in visit 1, but were administered whichever treatment they did not receive during the first visit.

BCAA Supplement:

This study used a commercially available BCAA mixture supplement, Epic® (Epic H2O, LLC, Williston, VT, USA), as the test supplement. The composition of the test supplement was as follows: 1.22g of a mixture of the BCAA: L-leucine, L-isoleucine, and L-valine, 5.6g dextrose, 136mg chloride, 93mg sodium, 22mg potassium, 90 IU

Vitamin A (as beta-carotene), 15mg Vitamin C (as ascorbic acid), 7.5IU Vitamin E (as dl-aplha-tocopheryl acetate), 0.19mg Thiamin (as thiamine hydrochloride), 0.21mg Riboflavin, 2.5mg Niacin (as niacinamide), and 0.25mg Vitamin B6 (as pyridoxine hydrochloride).

The placebo was formulated to match both the taste and color of the test supplement. Crystal Light® Lemonade powder (Kraft Foods, Northfield, IL, USA) was mixed with 5.6g of powdered dextrose (Now Foods, Bloomingdale, IL, USA) to match the amount of dextrose present in the test supplement. Ingredients found in the Crystal Light® Lemonade powder include the following: citric acid, potassium and sodium citrate, aspartame, magnesium oxide, contains less than 2% of natural flavor, lemon juice solids, acesulfame potassium, soy lecithin, artificial color, yellow 5, and BHA.

Both the test supplement and placebo were delivered in a powdered form. The supplement and/or placebo were pre-measured into five separate unlabeled test tubes that were placed in a paper bag to be given to the subject at each visit. Subjects self-administered the supplement and/or placebo by mixing one tube with approximately 8oz of water in a colored water bottle supplied to each participant. All participants verbally indicated complete consumption of both supplement and placebo to the principal investigator.

Exercise:

During each visit subjects performed 3 sets of 12 squats (total of 36 squats) with a one minute resting period between sets to induce DOMS. In an effort to reduce injury, subjects were shown how to correctly perform a squat prior to beginning the exercise.

During each set, the subject's exercise form was critiqued and necessary changes were made for the remaining squats. The exercise was performed once during visit 1 and again during visit 2. Participants were asked to not alter their exercising habits for the duration of the study.

Muscle Soreness:

Subjects rated general muscle soreness using a discrete Numerical Rating Scale (NRS) numbered 0-10 with verbal anchors of 0 being "no pain" and 10 being "the worst pain you have ever felt". It has been proposed that subjects will show greater compliance and accuracy of rating when using a NRS as compared to a Verbal or Visual scale (13). Subjects rated their muscle soreness twice during each visit (pre-exercise and post-exercise) in addition to one rating every 24 hours for the next four days, approximately five minutes after BCAA or placebo consumption.

Statistical Analysis:

A power analysis indicated that a sample size of 20 participants would result in a power of 0.88 to detect a change in means of 1.5 on the scale of muscle soreness rated by participant. The data were analyzed using the SAS System for Windows, version 9.3 (SAS Institute, Inc., Cary, NC). An F-test was used to compare the mean values of muscle soreness between sexes, treatments and time periods at a significance level set at $p< 0.05$.

RESULTS:

Participants:

The characteristics of the study participants are shown in Table 1. All 20 participants completed all parts of the study.

	Age (yrs)	Height (cm)	Weight (kg)	% Body Fat	BMI
Males (n=9)	22.1 ± 2.3	177.9 ±5.8	73.9 ±10.9	15.1 ±5.7	23.1±2.7
Females (n=11)	21.0 ±1.7	161.8 ±5.7	62.5 ±12.5	33.5 ± 8.7	23.8 ±4.3

TABLE 1. Participants' characteristics. Values are expressed as means ± SD. % Body fat calculated using bioelectrical impedance.

Muscle Soreness:

The mean muscle soreness scores for all participants in response to the squatting exercise are illustrated in Figure 1. The greatest muscle soreness during BCAA supplementation was seen directly following the squatting exercise (2.00±0.22), at time point labeled "post" in Figure 1. Ratings for BCAA gradually decreased after the post-exercise rating. During placebo supplementation peak muscle soreness was observed 24 hours post-exercise (2.30±0.36) with a decrease in muscle soreness following the 24-hour rating. To analyze the difference between BCAA and placebo, the datum point directly following exercise (labeled "post" in Figures 1, 2A, and 2B) was eliminated to allow for a 24-hour time interval between all muscle soreness ratings. This time point was not reflective of DOMS as it measured the soreness directly following the exercise, whereas DOMS does not appears until approximately 8-24 hours post-exercise (6). Also, the BCAA supplement would not be expected to have an effect on DOMS at the point directly following exercise because absorption and utilization of the supplement by the body would not have occurred in such a short amount of time. However, it was noted

that the degree of reported muscle soreness immediately following exercise for both groups were almost identical (BCAA=2.00±0.22, placebo=1.95±0.26), indicating that the measurement scale was quite accurate and reliable between tests for visit 1 and visit 2. An overall trend of lower reported muscle soreness in the BCAA supplement group was observed beginning 24 hours after exercise along with a 33% lower reported muscle soreness for BCAA at the 24-hour rating. However, these results were not significantly different from the placebo group (p=.1057).

Muscle soreness in female subjects (Fig. 2A) peaked 24 hours post-exercise (2.45±0.58) during placebo supplementation. DOMS occurring in the BCAA trials was greatest directly following exercise (1.81±0.26) and gradually decreased during the following four days. For females, there was a significant difference in DOMS between BCAA and placebo at 24 hours post-exercise (p=0.0182). Muscle soreness for male subjects (Fig. 2B) was greatest directly following exercise for both BCAA and placebo trials (2.22±0.36 and 2.33±0.42). Unlike the female curves, the male curves demonstrated no effect of BCAA supplementation since both BCAA and placebo peaked immediately following exercise and then gradually decreased over the next four days. In addition, mean muscle soreness for females in BCAA trials was slightly less than males directly following exercise and throughout the 72 hours post-exercise period.

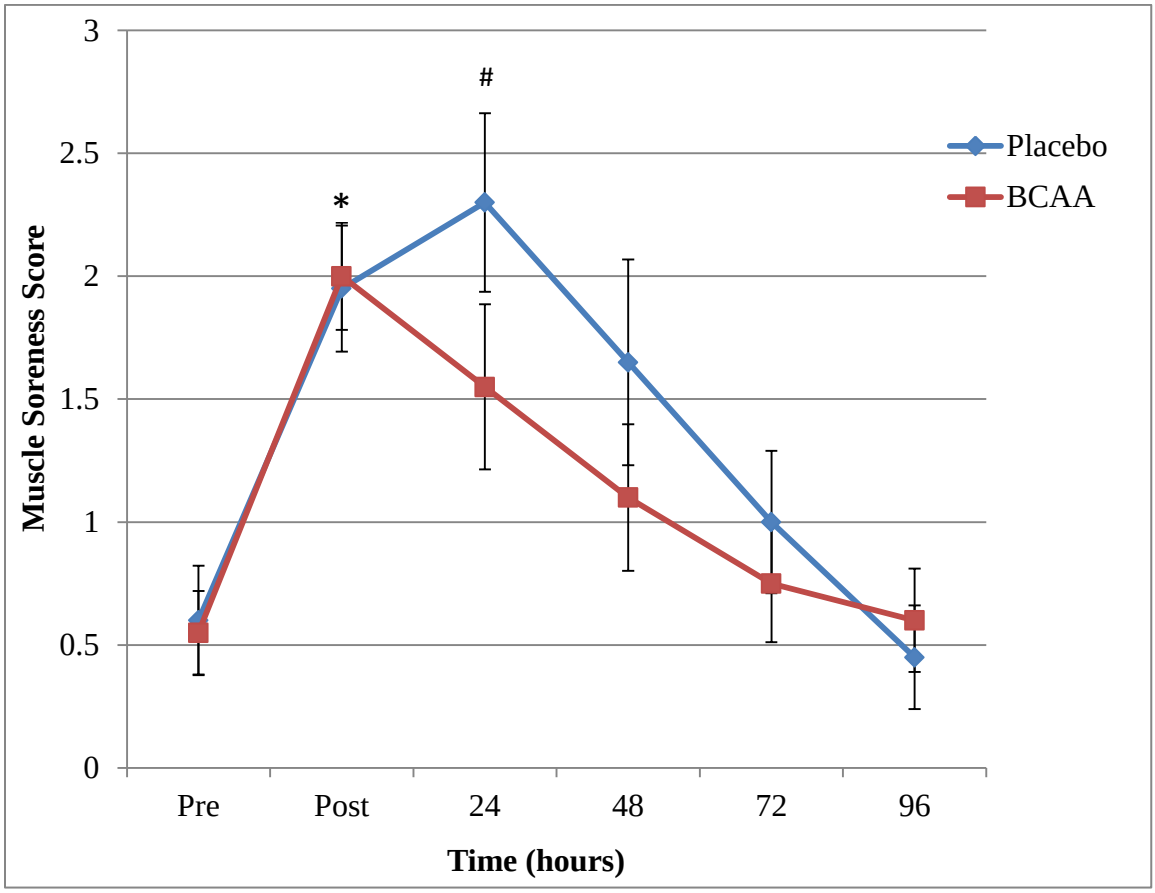

FIGURE 1. Effect of branched chain amino acid (BCAA) supplementation on delayed onset muscle soreness (DOMS) before (pre) and immediately following (post) squat exercise, measured in 24-hour intervals. Values are means ± SEM for all participants (n=20). No significant difference between overall mean scores for BCAA and placebo (p=0.1057). #No significant difference between BCAA and placebo (p= 0.1423). *Datum point not used in statistical analysis.

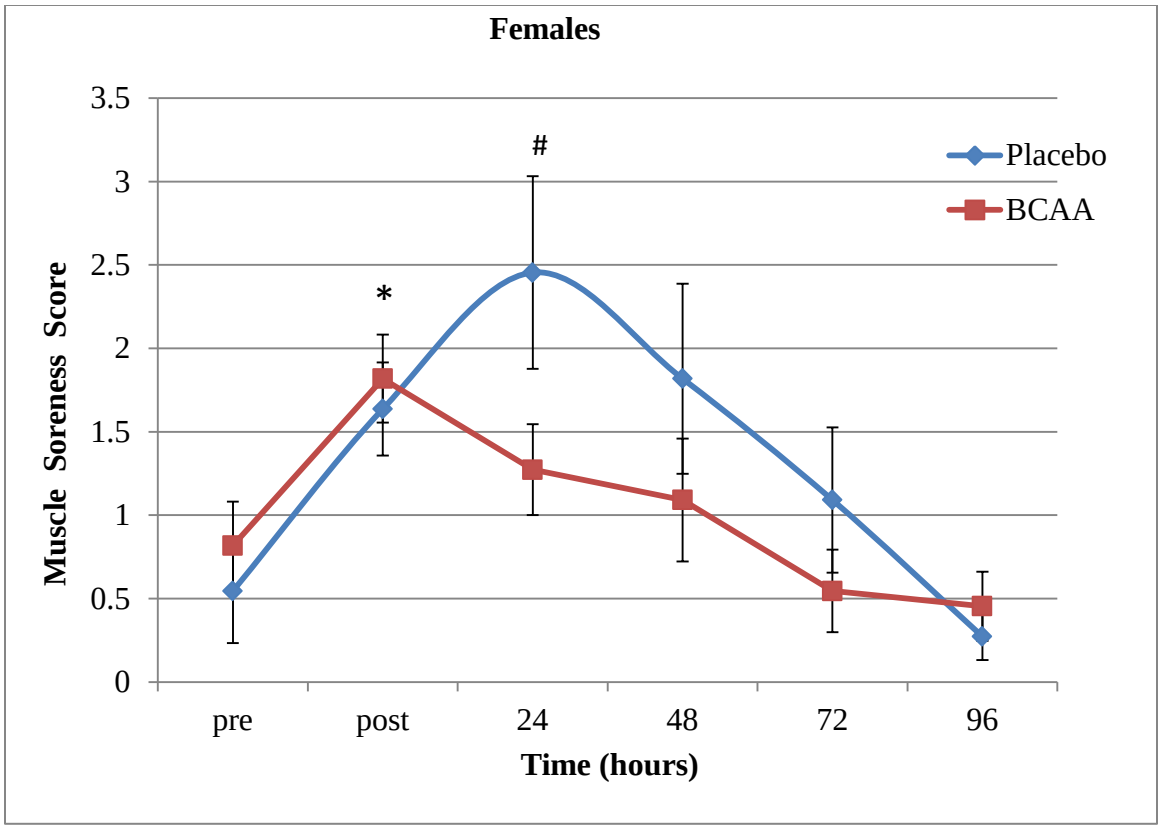

FIGURE 2A. Effect of BCAA supplementation on DOMS in females (n=11) before (pre) and after (post) squat exercise, measured in 24-hour intervals. Values are means ± SEM. #Significant difference between BCAA and placebo (p=0.0182) *Datum point not used in statistical analysis.

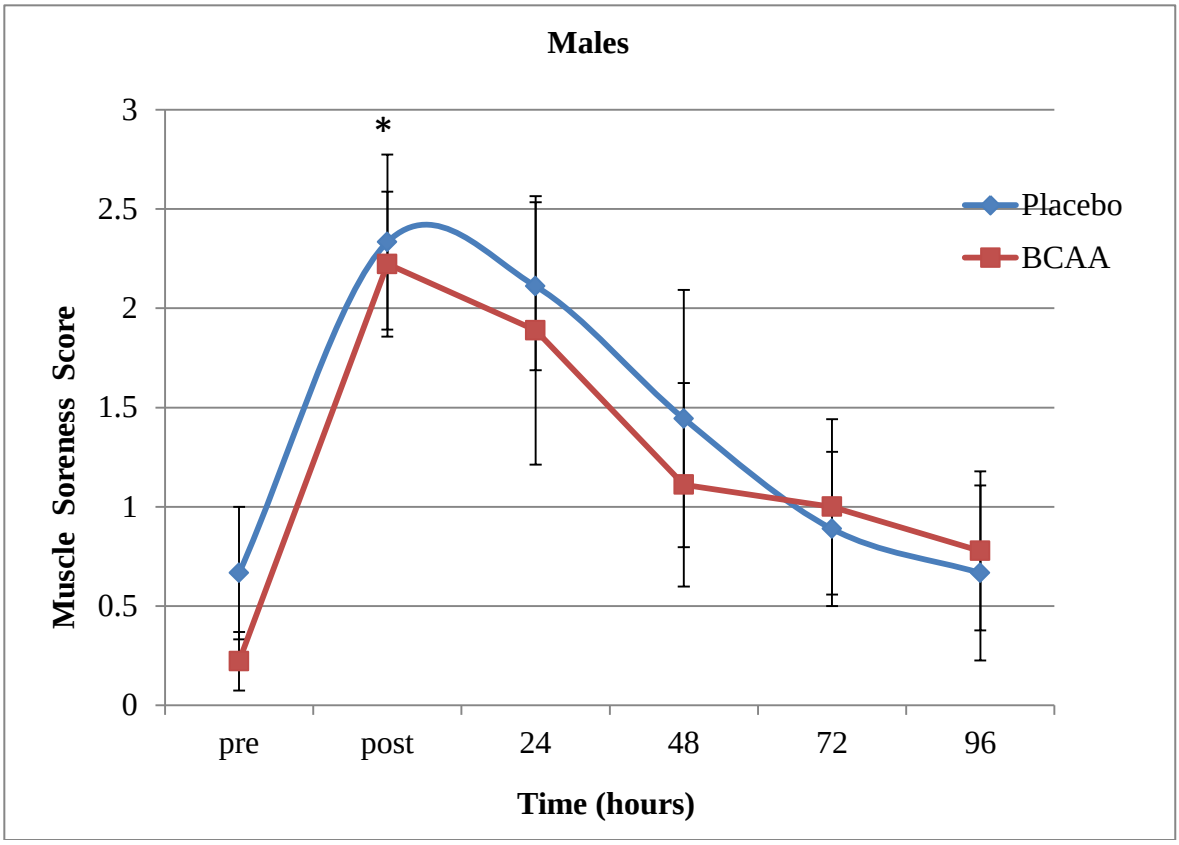

FIGURE 2B. Effect of BCAA supplementation on DOMS in males (n=9) before (pre) and after (post) squat exercise, measured in 24-hour intervals. Values are means ± SEM. No significant difference between mean scores for BCAA and placebo at any time point. *Datum point not used in statistical analysis.

DISCUSSION:

Previous studies have reported that consumption of BCAA prior to exercise and in the days following exercise can attenuate DOMS (12,14). In our study there was a significant difference in DOMS for females during BCAA supplementation at the 24-hour rating ($p=0.0182$). Although, there was no significant difference in mean muscle soreness between BCAA and placebo trials for all participants ($p=0.1057$), the overall trend in mean muscle soreness (Fig. 1) did show that BCAA supplementation decreased both the intensity and duration of DOMS with females having a greater difference than males (Fig. 2A and 2B).

In our study we wanted to test the effect that supplementation of BCAA mixed with glucose had on DOMS. We recognized that insulin activation is required to begin the process of cell signaling for muscle protein synthesis. Without insulin, muscle protein synthesis would not occur, hence the purpose of having glucose in the BCAA supplement. To ensure that the results obtained from this study were an implication of the effect of the BCAA, the same quantity of glucose found in the test supplement (5.6g) was added to the placebo. With glucose being present in the placebo, muscle protein synthesis would be stimulated, but limited by the lack of BCAA in the placebo.

In looking at the results of our study, we were unable to determine the reason for the difference in muscle soreness seen between sexes. However, one possible explanation may be attributed to a difference in muscle mass between male and female subjects. Males tend to have a greater total body weight and percentage of lean muscle mass as compared to females. Both sexes received the same quantity of BCAA (1220 mg/dose). However, females would have ingested more BCAA per kilogram of body weight

(19.5±3.3 mg/kg) as compared to males (16.5±1.9 mg/kg). This may have resulted in increased muscle protein synthesis and recovery from DOMS for females, which could have contributed to the statistically significant difference seen at the 24-hour muscle soreness rating ($p=0.0182$).

In using a crossover design a smaller sample size can be used since each participant acts as his or her own control. In this study, the sample size was calculated by a power analysis using data from a study in which a similar exercise was performed (5). It was determined that with 88% power a sample size of 20 subjects would allow for a detectable change in mean muscle soreness of 1.5 on the rating scale at $p<0.05$. Analysis of the overall mean muscle soreness scores for all participants during BCAA versus placebo showed a p-value of 0.1057, meaning the difference in perceived muscle soreness for both treatments was not statistically significant. One reason for not being able to detect a significant difference may have been attributed to the numerical rating scale (NRS) used in this study. The NRS was discretely numbered from 0 to 10. Participants were therefore unable to rate their soreness at any number between those set on the scale even if they felt their soreness would have been more appropriately reflected using a non-discrete number. One participant made mention of this during the study, stating their pain felt like a "2.5". However, since this was not a given value on the NRS, the subject reported a lower score of "2". Additionally, the verbal anchor representing number 10, the most extreme muscle soreness rating, was "worst pain you have ever felt". This statement may have been too extreme for the magnitude of muscle soreness that participants would have felt after performing a squatting exercise. A verbal anchor of "extremely sore" at the number 10 may have been more appropriate for this study. For

these reasons it is possible that using a more sensitive scale such as a continuous line scale, like those used in similar studies (7,15), may have yielded a more statistically significant difference between BCAA and placebo.

It has been observed that 30-35% of subjects will exhibit no delayed onset muscle soreness following eccentric exercise (16). While the cause for this remains unknown, subjects in our study showed a similar trend. Three male and three female subjects reported having no DOMS during at least one of the trials at the 24-96 hour ratings as indicated by a score of 0 on the NRS. Two of the subjects had no DOMS following placebo supplementation whereas three subjects reported no DOMS during BCAA supplementation. However, only one of the six subjects reported having no DOMS during either BCAA or placebo supplementation. Subjects may have also experienced a protective effect against subsequent muscle damage following the first bout of eccentric exercise (17). By using a crossover-design, subjects were exposed to two separate bouts of eccentric squatting exercise, separated by at least a three-week resting period. This was done to give time for full muscle recovery while controlling for potential short-term protective effects. One study suggested a two-week resting period would be an adequate amount of time between bouts of exercise (18), but another study found the protective effect might last up to six months (19). In our study eleven subjects had a lower muscle soreness rating after the second bout of eccentric exercise as compared the first. However, only three of these subjects reported the decreased muscle soreness while consuming the placebo, meaning the other seven subjects were consuming the BCAA supplement during the second bout of exercise. While there is evidence in support of the

protective effect, the disagreement seen in the literature regarding the amount of time needed to control for this effect suggests additional research is required.

In summary, although we observed a trend of decreased DOMS following the supplementation of BCAA plus glucose, the difference was not significant ($p=0.1432$). There was however a significant difference between BCAA and placebo for female subjects at the 24-hour rating ($p=0.0182$). Perhaps, with a modified NRS and a subject-specific dosage of BCAA, subsequent studies may show a significant reduction in DOMS for all participants. The practical implications of BCAA supplementation and its relationship to DOMS should be recognized as both athletes and individuals looking to increase their level of physical activity may benefit from further research in this field.

Works Cited:

1) Harper, A. E, Miller, R. H., & Block, K. P. (1984) Branched-chain amino acid metabolism. *Annual Review of Nutrition, 4, 409–454.*

2) Shimomura, Y., Murakami, T., Nakai, N., Nagasaki, M., & Harris, R. A. (2004). Exercise promotes BCAA catabolism: effects of BCAA supplementation on skeletal muscle during exercise. *Journal of Nutrition*, 134(6), 1583S-87S.

3) Harris, R. A., Popov, K. M., Zhao, Y., & Shimomura, Y. (1994). Regulation of branched chain amino acid catabolism. *Journal of Nutrition,* 124(8), 1499S-1502S.

4) Suryawan, A., Hawes, J. W., Harris, R. A., Jenkins, A. E., & Hutson, S. M. (1998). A molecular model of human branched-chain amino acid metabolism. *American Journal of Clinical Nutrition*, 68(1), 72-81.

5) Shimomura, Y., Yamamoto, Y., Bajotto, G., Sato, J., Murakami, T., Shimomura, N., & Mawatari, K. (2006). Nutraceutical effects of branched-chain amino acids on skeletal muscle. *Journal of Nutrition*, 136(2), 529S-32S.

6) Connolly, D. A., Sayers, S. P., & McHugh, M. P. (2003) Treatment and prevention of delayed onset muscle soreness. *Journal of Strength and Conditioning Research*, 17(1), 197–208.

7) Jackman, S. R., Witard, O. C., Jeukendrup, A. E., & Tipton, K. D. (2009). Branched-chain amino acid ingestion can ameliorate soreness from eccentric exercise. *Medicine & Science in Sports & Exercise,* 42(5), 962-70.

8) Rasmussen, B. B., Tipton, K. D., Miller, S. L., Wolf, S. E., & Wolfe, R. R. (2000). An oral essential amino acid-carbohydrate supplement enhances protein anabolism after resistance exercise. *Journal of Applied Physiology*, 88, 386-92.

9) Gelfand, R. A., & Barrett, E. J. (1987). Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *Journal of Clinical Investigation,* 80, 1-6.

10) Proud, C. G. (2006). Regulation of protein synthesis by insulin. *Biochemical Society Transactions,* 34(2), 213-16.

11) Shimomura, Y., Kobayashi, H., Mawatari, K., Akita, K., Inaguma, A., Watanabe, S., Bajotto, G., & Sato, J. (2009) Effects of squat exercise and branched-chain amino acid supplementation on plasma free amino acid concentration in young women. *Journal of Nutritional Science and Vitaminology*, 55, 288-91.

12) Shimomura, Y., Inaguma, A., Watanabe, S., Yamamoto, Y., Muramatsu, Y., Bajotto, G., Sato, J., Shimomura, N., Kobayashi, H., & Mawatari, K. (2010) Branched-chain amino acid supplementation before squat exercise and delayed-onset muscle soreness. *International Journal of Sport Nutrition and Exercise Metabolism,* 20, 236-44.

13) Hjermstad, M. J., Fayers, P. M., Haugen, D. F., Caraceni, A., Hanks, G. W., Loge, J. H., Fainsinger, R., Aass, N., & Kaasa, S. (2011) Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for assessment of pain intensity in adults: a systematic literature review. *Journal of Pain and Symptom Management,* 41(60), 1073-85.

14) Nosaka, K., Sacco, P., & Mawatari, K. (2006) Effects of amino acid supplementation on muscle soreness and damage. *International Journal of Sports Nutrition and Exercise*, 16, 620-35.

15) Flackoll, P.J., Judy, T., Flinn, K., Carr, C., Flinn, S. (2003) Postexercise protein supplementation improves health and muscle soreness during basic military training in marine recruits. *Journal of Applied Physiology*, 96, 951-6.

16) Sayers, S. P., Knight, C. A., Clarkson, P. M., Van Wegen, E. H., & Kamen, G. (2001) Effect of ketoprofen on muscle function and sEMG activiy after eccentric exercise. *Medicine & Science in Sport & Exercise*, 33(5), 702-10.

17) 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. *Scandinavian Journal of Medicine & Science in Sports*, 13, 88-97.

18) Chen, H.L., Nosaka, K., Chen, T.C. (2012) Muscle damage protection by low intensity eccentric contractions remains for 2 weeks but not 3 weeks. *European Journal of Applied Physiology,* 112(12), 555-65.

19) Nosaka, K., Sakamoto, K., Newton, M., & Sacco, P. (2001) How long does the protective effect on eccentric exercise-induced muscle damage last? *Medicine & Science in Sport & Exercise*, 33(9), 1490-5.