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Beau K. Greer

Sacred Heart University, greerb@sacredheart.edu

John L. Woodard

Florida State University

Jim P. White

Florida State University

Eric M. Arguello

Florida State University

Emily M. Haymes

Florida State University

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Branched-Chain Amino Acid Supplementation and Indicators of Muscle Damage After Endurance Exercise

**Beau Kjerulf Greer, John L. Woodard, Jim P. White,
Eric M. Arguello, and Emily M. Haymes**

The purpose of this study was to determine whether branched-chain amino acid (BCAA) supplementation attenuates indirect indicators of muscle damage during endurance exercise as compared with an isocaloric, carbohydrate (CHO) beverage or a noncaloric placebo (PLAC) beverage. Nine untrained men performed three 90-min cycling bouts at 55% $\dot{V}O_{2peak}$. Subjects, blinded to beverage selection, ingested a total of 200 kcal of energy via the CHO or BCAA beverage before and at 60 min of exercise, or they drank the PLAC beverage. Creatine kinase (CK), lactate dehydrogenase (LDH), isokinetic leg-extension and -flexion torque, and muscle soreness were assessed before and immediately, 4 h, 24 h, and 48 h postexercise. The trials were separated by 8 wk. CK activities were significantly lower after the BCAA trial than in the PLAC trial at 4, 24, and 48 h postexercise, as well as lower than the CHO beverage at 24 h postexercise. CK was lower in the CHO trial at the 24- and 48-h time points than in the PLAC trial. LDH activities were lower in the BCAA trial at 4 h than in the PLAC trial. As compared with the CHO and PLAC trials, ratings of perceived soreness were lower at 24 h postexercise, and leg-flexion torque was higher at the 48-h time point after the BCAA trial. The present data suggest that BCAA supplementation attenuates muscle damage during prolonged endurance exercise in untrained college-age men. CHO ingestion attenuates CK activities at 24 and 48 h postexercise as compared with a placebo beverage.

Key Words: ergogenic aids, rhabdomyolysis, sport drinks

Exercise-induced muscle damage is characterized by delayed-onset muscle soreness, Z-line streaming, general myofibril disorganization, impaired maximal force production, and the appearance of muscle proteins in the blood (4). In terms of aerobic performance, supplementation is usually focused on whether it will improve competition performance. It has become evident that the focus must also be directed on training. Considering that athletes might exhibit significant signs of muscle damage immediately prerace (18), attenuating the damage during the

Greer is with the Dept. of Physical Therapy and Human Movement Sciences, Sacred Heart University, Fairfield, CT 06825. Woodard, White, Arguello, and Haymes are with the Dept. of Food, Nutrition, and Exercise Sciences, Florida State University, Tallahassee, FL 32306.

general training cycle through supplementation might encourage a better chance for optimal performance during competition.

Unfortunately, strategies to reduce the amount of damage a muscle incurs during exercise remain scarce and are often not supported by peer-reviewed literature. Ingesting branched-chain amino acids (BCAA) for 7 d before endurance exercise, as well as immediately pre- and postexercise, decreases postexercise creatine kinase (CK) and lactate dehydrogenase (LDH) activities (5). Saunders et al. (26) have also reported that adding protein calories to a carbohydrate (CHO) beverage attenuates the postexercise CK response more than a beverage containing only CHO. Because neither of those studies used isocaloric beverages to compare the treatments, the specific aim of this study was to determine whether BCAA supplementation would attenuate indirect markers of muscle damage after endurance exercise as compared with both an isocaloric CHO beverage and a noncaloric placebo (PLAC) beverage. Recent investigations reported that a carbohydrate-protein-antioxidant beverage lowers CK, LDH, and muscle soreness postexercise in comparison with an isocaloric CHO beverage (21, 25). In both those studies, however, the 2 beverages had markedly different dietary antioxidant levels that might have influenced the degree of injury to the muscle (14). The current study is unique in that it provides an isocaloric comparison between BCAA and CHO beverages, as well as controlling for antioxidant intake before and during exercise.

Supplementation with BCAA might reduce the extent of muscle damage via the release of anabolic hormones (3) or by inhibiting proteolysis (2, 29, 30). Glucose feeding may provide a similar effect because it might reduce protein catabolism (15, 23, 31). We hypothesized that the BCAA-containing beverage would attenuate indirect indicators of muscle damage compared with the CHO and PLAC beverages. We also theorized that the CHO beverage would provide a reduction in muscle damage compared with the PLAC beverage.

Methods

Subjects

Nine healthy, untrained men participated in this study. Based on a pilot-data effect size of 1.5, this subject number exceeded the amount necessary to achieve a power of 0.80. Individuals who had engaged in regular aerobic or anaerobic training within the past year, and those who were taking or had taken any dietary supplement (with the exception of multivitamins or minerals) within the preceding 6 months were excluded from the study. Each volunteer completed a medical-history form that revealed contraindications to exercise. Provided there were no contraindications, subjects signed an informed consent approved by the Florida State University Institutional Review Board for Research Involving Human Subjects after being informed of the nature of the study and the risks involved. At this time, subjects were given instructions regarding the dietary and physical activity restrictions of the study. Although it was assumed that untrained participants would not engage in any exercise outside of the experimental trials, any strenuous physical activity was discouraged throughout the course of the experiment.

Baseline Testing and Dietary Measures

Body weight was measured before all exercise trials on a Seca scale (Model 707, Seca Corp., Columbia, MD) to the nearest 0.5 kg. Subjects were weighed in their workout clothes but no shoes. A continuous, graded exercise test (Åstrand maximal cycle protocol) (1) on a Monark cycle ergometer (Monark Exercise AB, Vansbro, Sweden) was used to assess peak oxygen consumption via open-circuit indirect spirometry (Truemax 2400 Metabolic Measurement System, Consentius Technologies, Sandy, UT). This exercise mode was chosen because previous studies investigating BCAA or protein use during endurance exercise had used cycling as the method for inducing muscle damage (5, 25, 26), even though there are other modes that create a higher degree of damage (4).

All volunteers received written and verbal instructions from the primary investigator concerning the dietary requirements for this study. Subjects kept detailed records of dietary intake for 3 d before and the day of each exercise session and were asked to avoid eating for 4 h before each exercise trial. They were encouraged to maintain the same dietary patterns for the 3 d before each trial (dietary choices on the day of exercise were not permitted to change). Dietary information was analyzed using a commercially available dietary-analysis software package (Nutritionist Five, version 2.0, First DataBank, Inc., San Bruno, CA). Mean energy and macronutrient intake, as well as intake of vitamins C and E, were computed to verify that they did not significantly differ between trials. These nutritional variables were chosen for their potential effects on muscle damage (14). No specific action was taken if a subject did not follow the same dietary patterns as he did before a previous exercise bout with the exception of the day of exercise (in which the action would be to not allow the exercise trial to take place). If, however, statistical analyses revealed a significant difference between dietary patterns, the dietary variable would have been used as a covariate in all analyses.

The 3 experimental trials differed only in the content of beverage consumed. Subjects were assigned to 1 of 3 treatment orders to ensure that no trial had an advantage in regard to the repeated-bout effect. Beverages were administered 5 min before the initiation of exercise, as well as at the 60-min mark. The PLAC beverage was noncaloric and contained only water, lemon flavor, salts, and artificial sweetener. A CHO-containing beverage (Gatorade, Inc., Chicago, IL) was used, as well as an isocaloric BCAA-containing beverage ("Ni," Musashi, Notting Hill, Australia). One serving (2.5 g) of the BCAA beverage contained 480 mg isoleucine, 1.22 g leucine, and 730 mg valine (10 kcal). The beverages were indistinguishable in taste (with the addition of artificial sweetener, salts, and lemon flavor), and their appearance was hidden. Subjects were not told which beverage they were consuming in order to prevent any potential placebo effects on ratings of perceived soreness. The total amount of energy given to subjects over the 2 time points for the BCAA and CHO trials was 200 kcal. This amount was chosen because it is consistent with what prior research has shown to be an effective amino acid dose to reduce muscle damage (5, 26).

Experimental Procedures

There were 3 treatment phases for this study, which began no sooner than 2 wk after initial $\text{VO}_{2\text{peak}}$ testing. Muscle protein (CK and LDH) activities in blood, maximal-voluntary-contraction (MVC) torque of the knee extensors and flexors, and ratings of perceived soreness were used as indirect markers of muscle damage. The phases were separated by 8 wk to allow for full muscle recovery from damage and to reduce the influence of the repeated-bout effect that is most evident in regard to CK and muscle-soreness measurements (24). Values for trials 1, 2, and 3 were compared with ANOVA to test for order effects.

After participants had 15 min of seated rest, a resting blood sample was drawn aseptically from an antecubital vein. After this procedure and approximately 15 min before an exercise trial commenced, MVC leg-flexion and -extension contraction torque were assessed through isokinetic work on a Biodex dynamometer (Biodex, Shirley, NY). Subjects performed 3 sets of 3 repetitions of leg flexion and extension at 180°/s with their right leg. One minute of rest was given between sets, and the peak values for flexion and extension were recorded.

Subjects engaged in a 5-min warm-up on a Monark cycle ergometer at 50 revolutions per minute and a resistance of 1 kp. After the warm-up, the resistance determined during the preliminary procedure was increased to a workload eliciting an intensity of 55% $\text{VO}_{2\text{peak}}$. Steady-state exercise continued for 90 min, and VO_2 was monitored every 15 min to ensure that the 3 trials had equivalent metabolic workloads in case ergometer calibration was significantly affected during the trial.

Postexercise MVC leg-flexion and -extension torque were measured 10 min after the completion of the time trial, as well as 4, 24, and 48 h postexercise. In addition to the preexercise sample, a resting blood sample was taken immediately postexercise, as well as 4, 24, and 48 h postexercise; blood sampling was not carried out for a longer period because it was expected that LDH would peak approximately 4 h postexercise and CK would peak approximately 24 h postexercise after noncentrally biased aerobic work (20). CK and LDH activities were determined by manual assays (Pointe Scientific, Inc., Detroit, MI) (13, 19). Both variables were adjusted for plasma volume shifts using the formula devised by Dill and Costill (8). Muscle soreness of the quadriceps (measured on a scale of 1 to 10) was self-rated on the same time course as the blood sampling (33).

Statistical Analyses

The study followed a 3×5 , trial \times time, within-subjects design with repeated measures in regard to indicators of muscle damage, and a 3×7 design was employed for VO_2 measurements. Within-group differences were also examined in regard to these variables. Analyses of variance (ANOVA) with repeated measures were used to analyze the variance of experimental treatments, and dietary information and order effects were assessed using a 1-way ANOVA. Statistical significance was set at $P < 0.05$. A Tukey honestly significant difference post hoc test was used to determine significant differences between means, and Mauchly's test of sphericity was used to prove homogeneity of repeated-measures variability. SPSS was used for all statistical calculations (SPSS Inc., Chicago, IL).

Results

Nine subjects completed all the testing procedures for the study. Subject characteristics are presented in Table 1. ANOVA showed that dietary intakes did not differ between trials ($P > 0.05$), so no dietary factors were used as a covariate. In addition, measures of VO_2 did not differ between trials at any time point, indicating that each trial provided similar metabolic stress.

Blood Markers

A log-10 transformation was used to reduce the variability within the trials for CK data because Mauchly's test was violated. The coefficient of variation for CK assays was approximately 4.5%. ANOVA with repeated measures revealed a significant main effect for trial and time and a trial \times time interaction ($P < 0.05$). Significantly lower serum CK activities were found in the BCAA trial at the 4-, 24-, and 48-h time points than in the PLAC trial ($P < 0.05$); the CK levels in the BCAA trial were also lower than in the CHO trial at 24 h postexercise ($P < 0.05$; Figure 1). The CK response was attenuated in the CHO trial as compared with the

Table 1 Subject Characteristics

Variable	Mean \pm standard deviation
Age (y)	21.6 \pm 3.2
Weight (kg)	84.2 \pm 17.0
Body-mass index	26.3 \pm 4.3
Maximal oxygen uptake ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	36.3 \pm 2.2
Maximal oxygen uptake (L/min)	3.0 \pm 0.5

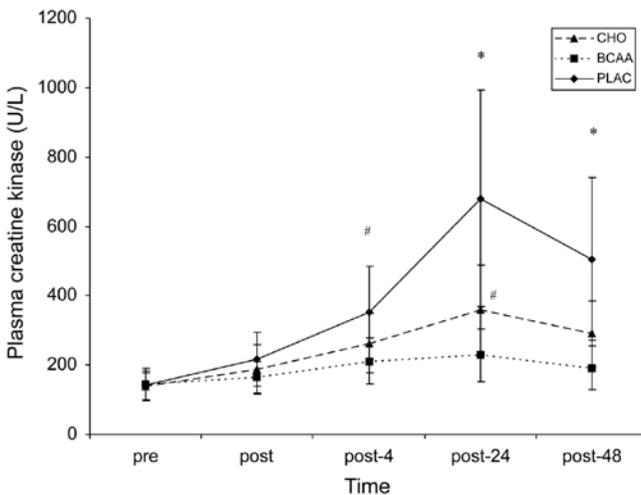


Figure 1 — Mean (\pm standard deviation) creatine kinase levels. CHO indicates carbohydrate; BCAA, branched-chain amino acids; PLAC, placebo. *Significantly different ($P < 0.05$) from the BCAA or CHO trial. #Significantly different ($P < 0.05$) from the BCAA trial.

PLAC trial at 24 and 48 h ($P < 0.05$; Figure 1). In all 3 trials, CK activities did not return to preexercise levels by 48 h ($P < 0.05$) and peaked at 24 h postexercise. The PLAC trial was the only trial to show a significantly elevated CK response immediately postexercise ($P < 0.05$).

Repeated-measures ANOVA revealed a significant main effect for trial and time and a trial \times time interaction for LDH ($P < 0.05$). A significant difference was found between the BCAA and PLAC trials at 4 h postexercise ($P < 0.05$; Figure 2). All trials had returned to preexercise levels by 48 h ($P > 0.05$) and peaked at 4 h postexercise. The coefficient of variation for LDH assays was approximately 2.5%.

MVC Torque

There were no significant main effects for trial or trial \times time interaction in regard to MVC leg-extension torque ($P > 0.05$). There was, however, a significant main effect for time ($P < 0.05$). In all trials, MVC torque significantly decreased from pre- to postexercise and did not return to preexercise values by 48 h postexercise ($P < 0.05$; Figure 3).

There was a significant main effect for time and a significant trial \times time interaction in regard to leg flexion ($P < 0.05$). Leg-flexion torque in the BCAA trial was significantly greater ($P < 0.05$) than both the CHO and PLAC trials at 48 h postexercise. Torque was reduced postexercise in all trials ($P < 0.05$) and remained below preexercise values for at least 24 h; only in the BCAA trial did it return to preexercise values by 48 h postexercise ($P > 0.05$; Figure 4).

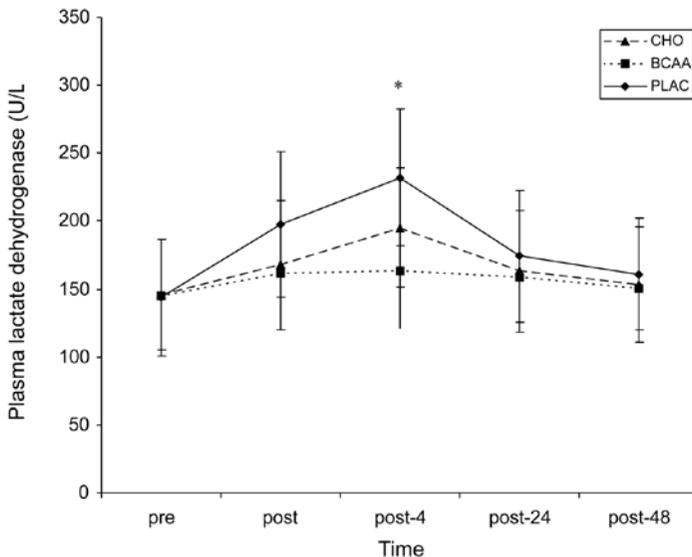


Figure 2 — Mean (\pm standard deviation) lactate dehydrogenase levels. CHO indicates carbohydrate; BCAA, branched-chain amino acids; PLAC, placebo. *Significantly different ($P < 0.05$) from the BCAA trial.

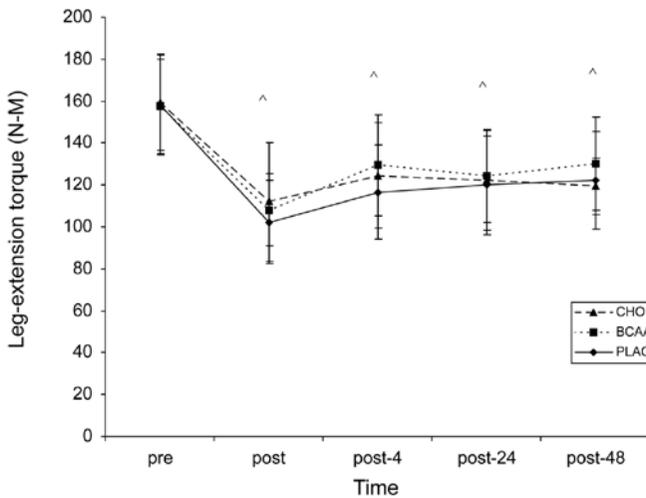


Figure 3 — Mean (\pm standard deviation) maximal leg-extension torque. CHO indicates carbohydrate; BCAA, branched-chain amino acids; PLAC, placebo. ^All points significantly different ($P < 0.05$) from the preexercise value.

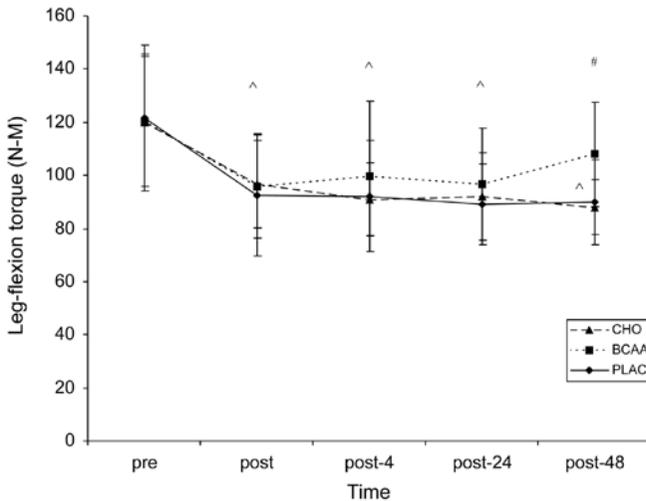


Figure 4 — Mean (\pm standard deviation) maximal leg-flexion torque. CHO indicates carbohydrate; BCAA, branched-chain amino acids; PLAC, placebo. ^All points significantly different ($P < 0.05$) from the preexercise value. #Significantly different ($P < 0.05$) from the CHO and PLAC trials.

Soreness

Repeated-measures ANOVA showed significant main effects for trial and time and a trial \times time interaction ($P < 0.05$) in regard to ratings of perceived muscle soreness. The rating of perceived soreness at 24 h postexercise was lower in the BCAA trial than in the CHO and PLAC trials ($P < 0.05$). This time point also represented the maximal soreness rating in each trial. The CHO and PLAC trials' ratings of perceived soreness at 24 h postexercise were greater than preexercise values ($P < 0.05$) but had returned to preexercise values by 48 h postexercise ($P > 0.05$; Figure 5).

To determine whether any adaptations occurred between the trials, we tested the order effect (trial 1 vs. trial 2 vs. trial 3). Analyses of variance did not reveal any significant main effects in regard to CK, LDH, leg-flexion and -extension torque, or ratings of perceived soreness ($P > 0.05$).

Discussion

The current data suggest that BCAA supplementation attenuates muscle damage during prolonged endurance exercise in untrained college-age men. BCAA ingestion resulted in decreased CK activities compared with the PLAC trial at 4, 24, and 48 h postexercise, as well as compared with the CHO trial at 24 h. It is interesting that the CHO also reduced CK activities more than the PLAC trial at 24 and 48 h. Although these results indicate that BCAA intake might be a superior choice, it is possible that energy intake during exercise, regardless of the macronutrient

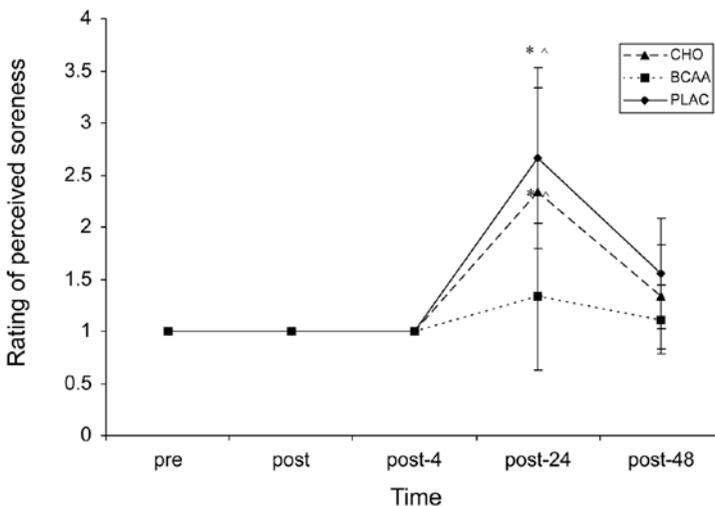


Figure 5 — Mean (\pm standard deviation) rating of perceived soreness. CHO indicates carbohydrate; BCAA, branched-chain amino acids; PLAC, placebo. *Significantly different ($P < 0.05$) from the BCAA trial. ^Significantly different ($P < 0.05$) from preexercise values.

composition, will result in an attenuated CK response after endurance exercise because it might decrease the need for muscle protein oxidation (16).

Although significant differences were found in regard to CK, the average effect size for the maximum change in the current study between the BCAA and PLAC trial (effect size = 1.45 at 24 h postexercise) appears to be much lower than in similar studies (26). In the Saunders et al. study, the CHO+protein group received 139 extra calories of energy during the exercise trial than the CHO group, which alone might account for the larger effect size. It is also feasible that feeding whole protein is more effective than just BCAA or that amino acids and CHO ingestion have a synergistic effect in reducing muscle damage during endurance exercise. Because CHO intake during exercise produces a small but significant insulin response that would aid BCAA entry into the muscle cell (7), amino acid ingestion might attenuate muscle damage more effectively with coingestion of CHO. Leucine ingestion alone has also been shown to stimulate an insulin response (10), and an approximately 10-fold increase in sensitivity of muscle protein synthesis to insulin is reported in rats when CHO is coinfused with BCAA compared with CHO alone (12). Consequently, the small increases in insulin from CHO feeding during exercise might have a more dramatic effect on BCAA uptake in leg muscles if the CHO is coingested with BCAA. Both insulin and amino acids, most notably leucine, decrease rates of proteolysis in humans (11), and it also appears that insulin's suppression of proteolysis is greatly attenuated when plasma amino acid concentrations are low (9).

The current study found that CHO ingestion decreases CK levels at 24 and 48 h after endurance exercise compared with a noncaloric placebo. There is additional evidence to suggest that glucose ingestion during endurance exercise decreases the rate of muscle protein breakdown. In a recent study, van Hamont et al. (31) reported that protein catabolism, as determined indirectly by sweat and urine urea excretion, is significantly reduced during endurance cycling when glucose is ingested. CHO supplementation during treadmill exercise decreased plasma myoglobin concentrations in well-trained runners (23). In addition, glucose infusion with amino acids significantly lowers ureagenesis compared with amino acid infusion alone in dogs (15). It has also been hypothesized that reductions in tricarboxylic-acid-cycle intermediates are responsible for increased protein catabolism (32). Therefore, glucose ingestion during exercise might reduce protein catabolism because it has been shown to increase tricarboxylic-acid-cycle intermediates (28). Conversely, Couture et al. (6) reported no difference in protein oxidation with or without glucose feeding during endurance exercise. Because the CHO-induced potential attenuation of damage was only seen in the CK data, formal conclusions should not be drawn regarding this issue until it is investigated further.

In regard particularly to the CK data, avoiding the bias an outlier might have on a single group response was the initial reason for the repeated-bout design in the present study (22). Because the repeated-bout effect can last for several weeks, a repeated-bout design could also create a large variability within each trial but provides a more powerful design (4). A test of the trial-order effect showed no significant differences for any variable in this study. At the CK peak (24 h postexercise), the mean difference between the first and third trials was 85.96 U/L. Because this difference, and the differences in all other time points, might have occurred by chance ($P > 0.05$), we suggest that 8 wk might be a long enough

duration between trials to avoid the CK repeated-bout effect in future studies involving non-eccentrically biased exercise.

The LDH results support the hypothesis that BCAA supplementation would alleviate some degree of damage, because the level at 4 h postexercise was significantly lower than in the PLAC trial. In partial contrast to the present results, Coombes and McNaughton (5) reported lower LDH concentrations in a BCAA-supplemented group for 5 d postexercise than in a placebo group. Subjects in the present study, however, exercised for a shorter duration (15 min less) at a lower intensity (55% $\text{VO}_{2\text{peak}}$ vs. 70% $\text{VO}_{2\text{max}}$) than in the Coombes and McNaughton study. Because LDH does not increase postexercise to the magnitude that CK does, a higher exercise intensity or longer duration might have been needed to lead to greater differences between trials.

Although subjects in the BCAA trial produced higher torque levels than in the other 2 trials at 4-, 24-, and 48-h time points in both extension and flexion, only the 48-h flexion torque was significantly different. While the biceps femoris functions as a hip extensor, the medial hamstring is primarily a knee flexor, and both work eccentrically to stabilize the pelvis during cycling. Decreased muscle damage or improved recovery might be responsible for the attenuation of knee-flexion torque loss at 48 h in the BCAA trial.

In regard to isokinetic torque, one possible explanation for the lack of significant differences between trials and the lack of supplement efficacy is that maximal torque is primarily determined by the activity of high-threshold motor units (the alpha motor neuron and faster twitch muscle fibers), even though all thresholds of motor unit are activated during an MVC (17). It is assumed that during moderate-intensity endurance cycling, muscle injury would be confined primarily to lower threshold motor units (the alpha motor neuron and slower twitch muscle fibers). Consequently, using a test that does not specifically target the muscle fiber type that incurred the most damage might not have been the most appropriate method. There were still, however, significant decreases in torque in all trials because lower threshold motor units, those assumed to have incurred the most damage, contribute to maximal force production (17).

It has been reported that BCAA supplementation before resistance training significantly reduces delayed-onset muscle soreness (27). Likewise, subjects' ratings of perceived soreness support the hypothesis that BCAA supplementation did reduce muscle damage in the thigh to a greater extent than the CHO and PLAC beverages. The hypothesis that energy intake during exercise (regardless of macronutrient composition) might help prevent muscle damage is not supported by these results because there were no differences between the CHO and PLAC trials.

Although we did not investigate mechanisms, there are several theories as to why BCAA supplementation would reduce muscle damage. When ingested before aerobic exercise, exogenous BCAA increases concentrations of human growth hormone and helps attenuate a drop in testosterone, resulting in a more anabolic environment (3). The administration of either BCAA or alpha-ketoisocaproate, the keto analogue of leucine, inhibits protein catabolism *in vitro* (2, 30). In addition, it has been suggested that a decrease of amino acid in the free muscle pool, as would occur during prolonged exercise, might act as a signal to promote muscle protein degradation, thereby replenishing the pool (29). Therefore, keeping the pool high in BCAA through supplementation might suppress the signal for muscle protein breakdown or speed recovery.

In conclusion, BCAA supplementation before and during endurance exercise attenuates some indirect markers of muscle damage compared with an isocaloric or noncaloric nonprotein beverage in untrained college-age men. Serum activities of CK and LDH, as well as ratings of perceived soreness, support this conclusion. Glucose feeding before and during exercise also reduced postexercise CK activities at 24 and 48 h postexercise compared with a noncaloric beverage. The current study is the first to show that BCAA ingestion before and during exercise might help prevent muscle damage in comparison with an isocaloric beverage in untrained individuals. It is recommended that formal conclusions regarding the efficacy of BCAA or protein ingestion to reduce muscle damage should not be drawn until direct measures of damage are employed. In addition, the synergistic effect between amino acids and glucose should be further investigated in exercising humans because many BCAA or protein supplements are marketed for use with CHO-electrolyte beverages. In addition, if isokinetic muscle actions are used to evaluate the degree of muscle damage after endurance exercise, it might be advisable to also include a short-term local muscle-endurance test that will better target the muscle fibers used in the exercise session than an MVC would.

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