
THE EFFECTS OF ADDING LEUCINE TO PRE AND POSTEXERCISE CARBOHYDRATE BEVERAGES ON ACUTE MUSCLE RECOVERY FROM RESISTANCE TRAINING

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ABSTRACT

Stock, MS, Young, JC, Golding, LA, Kruskall, LJ, Tandy, RD, Conway-Klaassen, JM, and Beck, TW. The effects of adding leucine to pre and postexercise carbohydrate beverages on acute muscle recovery from resistance training. *J Strength Cond Res* 24(8): 2211–2219, 2010—The present study examined the effects of adding leucine to pre and postexercise carbohydrate beverages on selected markers of muscle damage, delayed-onset muscle soreness (DOMS), and squat performance for up to 72 hours after lower-body resistance training. Seventeen resistance trained men (mean \pm SD age 22.9 ± 2.9 years) and 3 resistance trained women (mean \pm SD age 21.6 ± 2.6 years) performed 6 sets of squats to fatigue using 75% of the 1 repetition maximum. Each subject consumed a carbohydrate beverage 30 minutes before and immediately after exercise with or without the addition of $22.5 \text{ mg}\cdot\text{kg}^{-1}$ ($45 \text{ mg}\cdot\text{kg}^{-1}$ total) of leucine in a randomized, double-blind fashion. Serum creatine kinase (CK), lactate dehydrogenase (LDH), and DOMS were analyzed immediately before (TIME1), 24 (TIME2), 48 (TIME3), and 72 (TIME4) hours after exercise. The subjects repeated the squat protocol at TIME4 to test recovery. No differences were observed between groups for squat performance, defined as the total number of repetitions performed during 6 sets of squats, for both TIME1 and TIME4. The addition of leucine did not significantly decrease CK and LDH activity or DOMS. These results suggested that adding leucine to carbohydrate beverages did not affect acute muscle recovery and squat performance during

both initial testing and during a subsequent exercise bout 72 hours later in resistance trained subjects.

KEY WORDS amino acids, eccentric, delayed-onset muscle soreness

INTRODUCTION

Leucine, isoleucine, and valine, the branched-chain amino acids (BCAAs), make up approximately one-third of muscle protein (4), and are essential to the diet. Consumption of these amino acids before or during exercise may attenuate skeletal muscle proteolysis (19) and improve ratings of perceived exertion and mental fatigue during exercise (5). Investigators have recently examined the effects of essential amino acid (EAA) consumption on changes in human skeletal muscle protein synthesis (10,11), as well as satellite cell activation (26). Studies have demonstrated that leucine plays an important role in cell signaling and muscle growth, and the mechanisms by which these processes occur are independent of plasma insulin levels (1,2).

Very few studies have examined the effects of oral leucine supplementation alone (i.e., not in combination with isoleucine and valine or as a whole protein such as whey or casein) on exercise performance or body composition. In a study by Pitkänen et al. (24), power athletes consumed beverages before strength training and maximal anaerobic running with the addition of 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$ leucine, respectively. The authors reported no acute improvements in exercise performance between leucine supplementation and a placebo, but consumption of the leucine-containing beverages resulted in positive changes in blood amino acid concentrations. Using a dose of $50 \text{ mg}\cdot\text{kg}^{-1}$, Mero et al. (21) reported that leucine supplementation prevented a decrease in serum leucine during intensive training. Crowe et al. (8) recently demonstrated that when men and women competitive outrigger canoeists supplemented their diet with 45

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mg·kg⁻¹·d⁻¹ of leucine for 6 weeks, endurance performance and upper-body power were enhanced more so than training alone. Given that the ratio of f-TRP to BCAA did not change with leucine supplementation, the authors speculated that “the ergogenic effects of leucine were more likely related to a reduction in skeletal muscle damage with training or an increase in skeletal muscle protein synthesis” (p 671). However, no study has examined muscle damage and soreness with acute leucine supplementation.

Exercise-induced muscle damage and delayed-onset muscle soreness (DOMS) frequently occur when an individual performs a task for the first time. When the task is repeated in the days after, the muscles involved in the task adapt to the stimulus and future exercise bouts result in lower levels of muscle damage, a phenomenon known as the repeated bout effect (20). Other than the repeated bout effect, strategies to prevent or reduce exercise-induced muscle damage during exercise are poorly understood. The effects of pre or postexercise nutrition, a concept termed nutrient timing (15), have recently been examined. The theory behind many of these studies is that by supplementing the diet with additional protein and amino acids before, during, and/or after training, individuals may recover faster from workouts via increased muscle protein synthesis, thereby improving performance over time. Baty et al. (3) demonstrated that ingestion of a carbohydrate-protein beverage before, during, and after resistance training decreased blood markers of muscle damage and favorably altered cortisol and insulin levels compared to a placebo. Similar carbohydrate-protein studies using trained cyclists have found significantly lower blood creatine kinase (CK) levels compared with an isocarbohydrate treatment (28) and an isocaloric carbohydrate treatment (27). Coombes and McNaughton (7) demonstrated decreased blood CK and lactate dehydrogenase (LDH) in cyclists supplementing their diets with BCAA. Contrary to studies finding positive effects associated with protein or amino acid supplementation, Green et al. (12) found no differences in isometric quadriceps strength, blood CK, or lower extremity muscle soreness in subjects that consumed carbohydrate, carbohydrate-protein, or placebo beverages after downhill treadmill running. Millard-Stafford et al. (22) also failed to find a significant difference in CK between carbohydrate-protein and carbohydrate ingestion but did report improvement in soreness with ingestion of a carbohydrate-protein beverage.

Although the previously mentioned studies have compared the effects of carbohydrate vs. carbohydrate-protein beverages on muscle damage and DOMS after exercise, no study has isolated the effects of the leucine content. Because leucine has been shown to activate the mTOR signaling pathway, which turns on the translational machinery necessary for muscle protein synthesis (1,2), and non-EAAs do not enhance muscle protein synthesis (6), it is presently unknown as to what extent leucine alone contributes to studies finding positive results associated with carbohydrate-protein supplementation. Therefore, using the dose Crowe et al. (8)

found to be sufficient (45 mg·kg⁻¹), the purpose of the present investigation was to examine the effects of adding dietary leucine to pre and postexercise carbohydrate beverages on selected blood markers of muscle damage and DOMS in resistance trained subjects following their normal ad libitum diet. A secondary purpose was to examine exercise performance, defined as the total number of repetitions performed during 6 sets of squats to fatigue, both acutely, and 72 hours after the initial exercise bout. We hypothesized that leucine’s ability to augment muscle protein synthesis would lead to a reduction in damage to myofibrillar and cytoskeleton proteins, thereby preventing an increase in serum CK and LDH in the subject consuming leucine-carbohydrate beverages. We further speculated that if the subjects consuming leucine-carbohydrate beverages displayed reduced levels of these muscle proteins, less DOMS would be experienced, giving these subjects the ability to maintain or improve squat performance during a subsequent resistance training bout.

METHODS

Experimental Approach to the Problem

To address the primary hypothesis of the present investigation, a randomized, double-blind, between-subjects design was used. The subjects reported to the laboratory on 5 separate occasions. During the initial visit, the subjects performed 1 repetition maximum (1RM) testing for the barbell squat exercise. The subjects were then asked to refrain from physical activity for the next week in an effort to ensure low baseline levels of muscle damage and recovery before initial testing. One week after 1RM testing, the subjects returned to the laboratory for their second visit, and 5 mL of blood was drawn from the antecubital vein, which served as the preexercise sample (TIME1). Each subject then consumed a carbohydrate beverage with (LCHO) or without (CHO) the addition of leucine (Musashi, Notting Hill, United Kingdom). Thirty minutes after ingestion, each subject performed 6 sets of squats to fatigue with 75% of their 1RM and a 3-minute rest between sets. Immediately upon completion of the workout, the subjects consumed a beverage identical to their respective preexercise beverage. After the pre and postexercise treatments, no further nutritional intervention was provided. At 24 hours postexercise (TIME2), 48 hours postexercise (TIME3), and 72 hours postexercise (TIME4), the subjects returned to the laboratory for blood sampling, reporting of DOMS, and returned food logs that were completed at each feeding. After their blood draw at TIME4, the subjects again performed 6 sets of squats to fatigue with 75% of their 1RM and a 3-minute rest between sets, this time with no nutritional intervention. All blood samples were subsequently analyzed for CK and LDH content as indirect markers of skeletal muscle damage at each time point (TIME1-TIME4). Food logs were analyzed for total calories, protein, CHO, leucine, isoleucine, and valine content for each 24-hour period postexercise.

TABLE 1. Subject responses to 24-hour postexercise (TIME2) questions concerning the workout.*

	LCHO		CHO	
	Number of scores	Percentage	Number of scores	Percentage
How did you feel upon completing the workout?				
1. Strong	0	0.0	0	0.0
2. Strong, but tired	3	30.0	1	10.0
3. Tired	1	10.0	1	10.0
4. Weak	4	40.0	2	20.0
5. Very weak	1	10.0	4	40.0
6. Very weak, sick, nauseous	1	10.0	2	20.0
How did the drink affect your performance?				
1. Improved performance	5	50.0	5	50.0
2. No change in performance	5	50.0	5	50.0
3. Decreased performance	0	0.0	0	0.0

*CHO = carbohydrate; LCHO = leucine-carbohydrate.

Subjects

Seventeen men (mean ± SD age 22.9 ± 2.9 years, height 177.5 ± 6.5 cm, body mass 82.7 ± 13.3 kg, 1RM squat 151.9 ± 27.5 kg) and 3 women (mean ± SD age 21.6 ± 2.6 years, height 161.7 ± 6.7 cm, body mass 61.4 ± 8.7 kg, 1RM squat 67.4 ± 1.3 kg) volunteered to participate in the study. All subjects were resistance trained (≥1 year) with similar resistance training backgrounds (mean ± SD 4.5 ± 2.5 years) and performed the squat exercise in their current training programs. Individuals with a history of heart disease, hypertension, diabetes, thyroid disease, hypoglycemia, or musculoskeletal injury were not permitted to participate. Individuals were also unable to participate if they had consumed nutritional supplements that may affect muscle mass (i.e., creatine, beta alanine, beta-hydroxy-beta-methylbutyrate [HMB]) or anabolic hormones (i.e., androstenedione) within 3 months before the study. All subjects completed a health history questionnaire, were aware of the risks involved with participation, and signed a written informed consent document before initiating the study. The study was approved by the University of Nevada Las Vegas' Institutional Review Board for Human Subjects.

One Repetition Maximum Strength Testing

The 1RM squat test was performed according to standard guidelines (16) to assess maximal lower-body strength 1 week before TIME1. Each subject performed a warm-up set using a resistance that was approximately 40–60% of his or her perceived maximum and then performed 3–4 subsequent trials to determine the 1RM. A minimum of 3 minutes of rest was given between each trial. Proper squat technique required the subject to place an Olympic barbell across the trapezius muscle. Each subject squatted to the parallel position, which was attained when the greater trochanter of the femur reached the same position as the patella. All

attempts were performed in a rack to ensure safety. The subjects were given verbal feedback with each repetition to ensure adequate squat depth. Seventy-five percent of the 1RM was then calculated and used as the constant load during TIME1 and TIME4.

Testing and Beverage Protocol

The subjects arrived at the laboratory 1 week after 1RM strength testing (TIME1) and were provided a 0.25 g·kg⁻¹ carbohydrate solution mixed in 16 oz of water 30 minutes before and immediately after exercise (0.50 g·kg⁻¹ total) in a

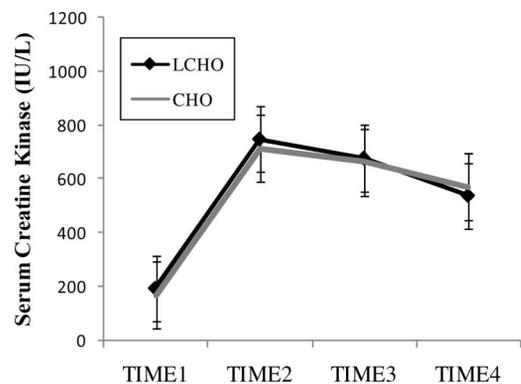


Figure 1. Data presented are mean ± SEM creatine kinase (CK) responses in the leucine-carbohydrate (LCHO) and carbohydrate (CHO) groups measured at baseline (TIME1), 24 (TIME2), 48 (TIME3), and 72 (TIME4) hours postexercise. There were no between-group (CHO and LCHO) differences, but CK activity (marginal means, collapsed across group) at TIME2 and TIME3 were significantly greater ($p < 0.05$) than TIME1.

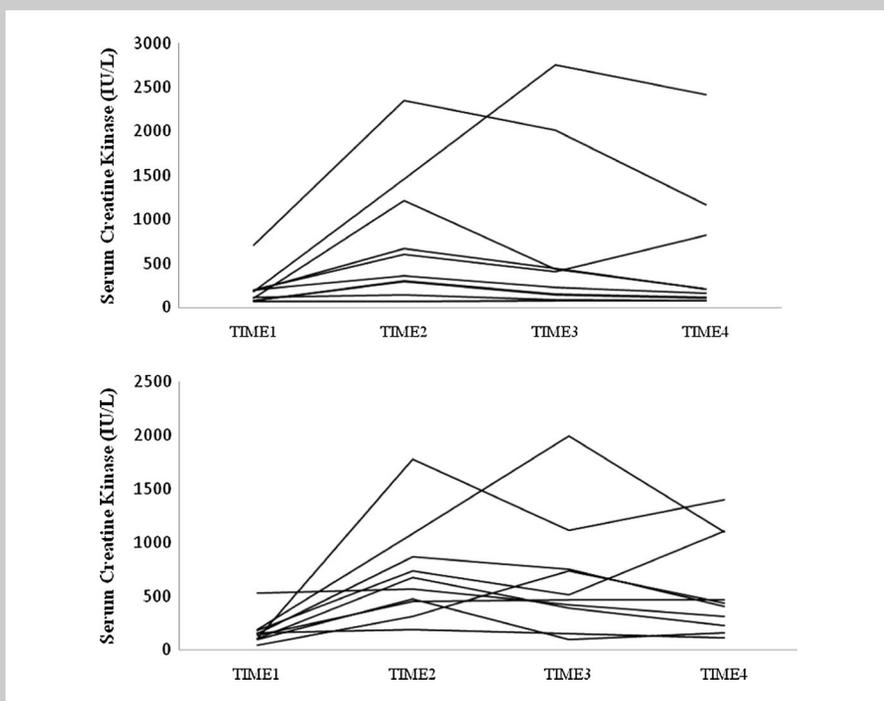


Figure 2. Data presented are individual creatine kinase (CK) responses for each of the 10 subjects in both the leucine-carbohydrate (top figure) and carbohydrate (bottom figure) groups measured at baseline (TIME1), 24 (TIME2), 48 (TIME3), and 72 (TIME4) hours postexercise.

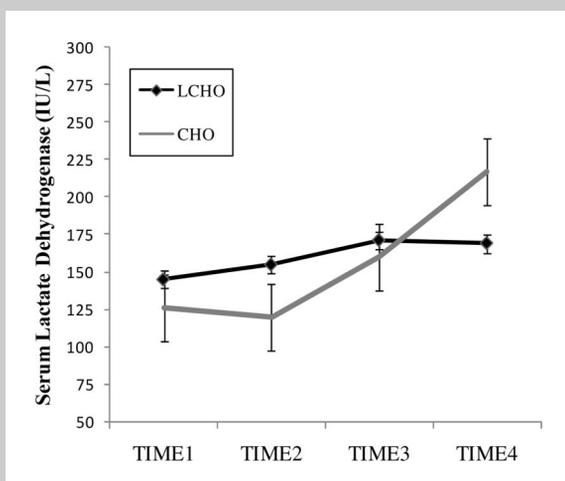


Figure 3. Data presented are mean \pm SEM lactate dehydrogenase (LDH) responses in the leucine-carbohydrate (LCHO) and carbohydrate (CHO) groups measured at baseline (TIME1), 24 (TIME2), 48 (TIME3), and 72 (TIME4) hours postexercise. There were no between-group (CHO and LCHO) differences and no significant increases in LDH (marginal means, collapsed across groups) from TIME1.

covered plastic container in the presence of the study investigator. The independent variable was the addition of 22.5 mg·kg⁻¹ of leucine powder to both the pre and postexercise test beverages (45 mg·kg⁻¹ total). Blinded taste

tests determined that a carbohydrate solution mixed in 16 oz of water was sufficient to dilute the taste of leucine, and the tastes of the beverages were indistinguishable. Assembly of the beverages was performed by a research assistant not familiar with the study.

Thirty minutes after preexercise beverage ingestion, the subjects performed a brief standardized warm-up of the lower body. Each subject then performed 6 sets of squats to fatigue using a load equivalent to 75% of the predetermined 1RM with 3 minutes of rest between sets, and the subjects were verbally encouraged to perform as many repetitions as possible. Subjects repeated this exercise protocol at TIME4 using identical procedures. Attempts not using the acceptable range of motion were discarded. Other studies have used similar resistance training protocols to assess nutritional

interventions on lower-body performance (14,17), and pilot data determined that this protocol significantly elevated CK, LDH, and DOMS in 6 resistance trained subjects.

Serum Creatine Kinase and Lactate Dehydrogenase Activity

Each subject provided a venous blood sample immediately before supplement ingestion (TIME1), and TIME2-TIME4. Venous samples were collected from a superficial forearm vein while subjects were in a supine position. The blood was allowed to clot at room temperature for 15 minutes. Samples were then centrifuged and frozen at -80°C until analysis. At the conclusion of the study, all samples were thawed at room temperature and processed on a Sirus Clinical Chemistry Analyser (Stanbio Laboratory, Boerne, TX, USA) for CK and LDH analysis. Results indicated that the intraclass correlation coefficients (model 3,1) for the CK and LDH values were $R = 0.96$ and $R = 0.98$, respectively (34).

Delayed-Onset Muscle Soreness

Immediately before supplement ingestion (TIME1), and TIME2-TIME4, the subjects were asked to quantify their level of DOMS over the past 24 hours using the following anchors on a 7-point Likert scale used in previous studies (33): 1 = a complete absence of muscle soreness; 2 = a light pain felt when only when touched/a vague ache; 3 = a moderate pain felt only when touched/a slight persistent pain; 4 = a light pain when walking up or down the stairs; 5 = a

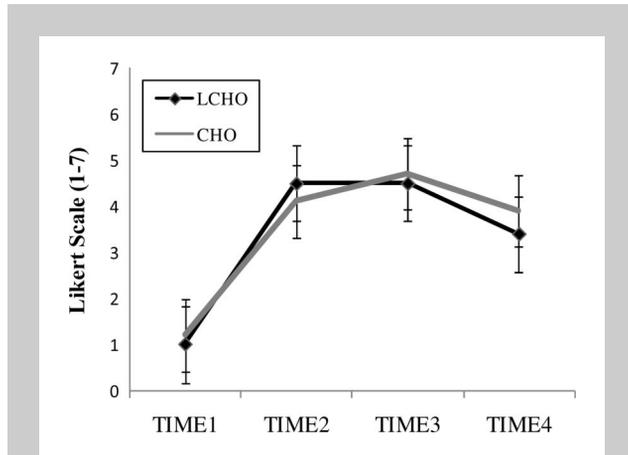


Figure 4. Data presented are mean \pm SEM Likert scale ratings for delayed-onset muscle soreness (DOMS) in the leucine-carbohydrate (LCHO) and carbohydrate (CHO) groups measured at baseline (TIME1), 24 (TIME2), 48 (TIME3), and 72 (TIME4) hours postexercise using the following anchors: 1 = a complete absence of muscle soreness; 2 = a light pain felt only when touched/a vague ache; 3 = a moderate pain felt only when touched/a slight persistent pain; 4 = a light pain when walking up or down stairs; 5 = a light pain when walking on a flat surface/painful; 6 = a moderate pain, stiffness, or weakness when walking/very painful; 7 = a severe pain that limits my ability to move. There were no between-group (CHO and LCHO) differences, but DOMS (marginal means, collapsed across groups) at TIME2-TIME4 were significantly greater ($p < 0.05$) than TIME1.

light pain when walking on a flat surface/painful; 6 = a moderate pain, stiffness, or weakness when walking/very painful; 7 = a severe pain that limits my ability to move. Using questions designed by Baty et al. (3), the subjects were asked how they felt upon completing the workout 24 hours postexercise using the following choices: 1 = strong; 2 = strong, but tired; 3 = tired; 4 = weak; 5 = very weak; 6 = very weak,

sick, nauseous. Furthermore, the subjects were asked how the drinks affected their performance using the following choices: 1 = improved performance, 2 = no change in performance, 3 = decreased performance. The responses to 24-hour postexercise questions concerning the workout can be found in Table 1.

Diet Analysis

Dietary intake was monitored throughout the study via food logs collected at each of the postexercise visits to the laboratory (TIME2-TIME4). Each subject was instructed to follow their normal ad libitum diet but avoid protein powders, drinks, and bars. Food logs were analyzed for total calories, protein, CHO, leucine, isoleucine, and valine content with the use of The Food Processor SQL (ESHA Research, Salem, OR, USA). In the event that the nutrient content of a specific food was not available, USDA standard references were used as an estimate.

Statistical Analyses

Three separate 2 (beverage) \times 4 (time) analyses of variance (ANOVAs) with repeated measures were used to analyze the CK, LDH, and DOMS data. A 2 (beverage) \times 2 (time) ANOVA with repeated measures was used to analyze the total squat repetitions to fatigue data. Independent samples *t*-tests were used to compare responses from questions in Table 1, and diet analysis for each of the 24 hour postexercise periods in which food logs were kept (i.e., TIME1-TIME2). Linear regression was used to calculate the coefficient of determination (r^2) and significance level for the dietary protein intake vs. CK relationship, and the dietary leucine intake vs. CK relationship. Follow-up analyses included 1-way repeated measures ANOVAs, paired-samples *t*-tests, and Bonferroni post hoc comparisons. The alpha level was set at $p \leq 0.05$. All data are presented as mean \pm SEM.

TABLE 2. Dietary intake.*†‡

	TIME1-TIME2		TIME2-TIME3		TIME3-TIME4	
	LCHO	CHO	LCHO	CHO	LCHO	CHO
kcal	3,128 \pm 470	2,411 \pm 171	2,770 \pm 421	2,471 \pm 367	2,697 \pm 395	2,015 \pm 272
Protein (g)	155 \pm 22	137 \pm 13	123 \pm 27	129 \pm 14	132 \pm 24	105 \pm 11
Total protein (g·kg ⁻¹)	1.85 \pm 0.24	1.83 \pm 0.14	1.42 \pm 0.30	1.73 \pm 0.17	1.57 \pm 0.28	1.39 \pm 0.13
CHO (g)	353 \pm 57	284 \pm 34	318 \pm 28	287 \pm 44	305 \pm 32	266 \pm 38
Leucine (g)	10.6 \pm 1.7	8.0 \pm 0.8	8.2 \pm 2.2	9.2 \pm 0.8	8.4 \pm 2.0	6.6 \pm 0.7
Isoleucine (g)	9.1 \pm 1.3	9.7 \pm 1.1	7.2 \pm 1.3	8.1 \pm 0.9	8.0 \pm 1.1	6.7 \pm 0.8
Valine (g)	8.5 \pm 1.0	8.0 \pm 0.8	6.6 \pm 1.2	7.4 \pm 0.7	7.0 \pm 1.1	5.9 \pm 0.7
BCAA (g)	28.2 \pm 3.7	25.7 \pm 2.6	21.9 \pm 4.6	24.6 \pm 2.2	23.4 \pm 4.1	19.2 \pm 2.1

*CHO = carbohydrate; LCHO = leucine-carbohydrate; BCAA = branched-chain amino acids.

†Values are mean \pm SEM.

‡These data do not include the contents of the pre and postexercise LCHO or CHO study beverages during TIME1. There were no between-group (CHO and LCHO) differences for any of the 3 days postexercise.

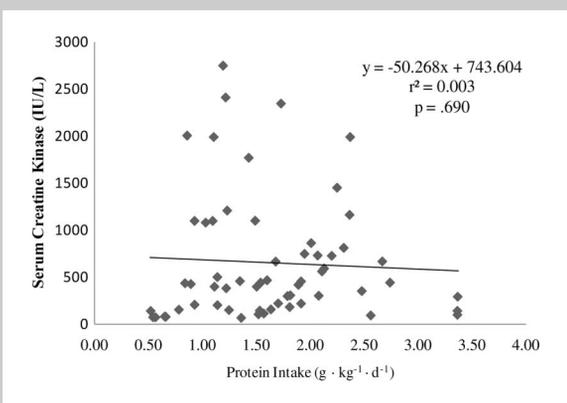


Figure 5. Data represent the relationship between the 24-hour protein content of the diet and each individual subject's corresponding postexercise serum creatine kinase (CK) level. Data are presented for all subjects independent of group.

RESULTS

Serum Creatine Kinase Activity

Serum CK levels were not different among treatment groups ($F = 0.031$, $p = 0.972$; Figure 1). Levels peaked in both groups at TIME2 and declined thereafter. This elevation was significant at TIME2 ($p = 0.001$) and TIME3 ($p = 0.032$) but declined at TIME4 ($p = 0.063$) to levels that were not significantly different from TIME1. Figure 2 displays the responses of individual subjects for both the LCHO and CHO groups.

Serum Lactate Dehydrogenase Activity

Serum LDH levels were not different among treatment groups ($F = 1.754$, $p = 0.191$; Figure 3). Qualitatively, LDH activity in the CHO group appeared to display a more robust

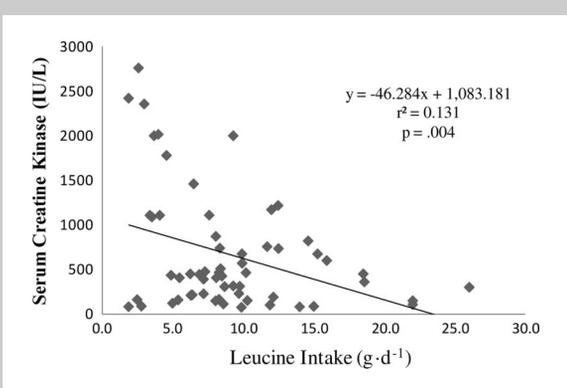


Figure 6. Data represent the relationship between the 24-hour leucine content of the diet and each individual subject's corresponding postexercise serum creatine kinase (CK) level. Data are presented for all subjects independent of group. These data do not include the leucine content of the pre and postexercise beverages for the subjects in the leucine-carbohydrate group.

rise than the LCHO group at TIME4. However, an independent samples t -test for this time point indicated that these differences were not significant ($p = 0.088$). Although CK activity peaked at TIME2 for both groups, little relative change was observed in LDH at TIME2 ($p = 1.00$).

Delayed-Onset Muscle Soreness

Delayed-onset muscle soreness was not different among treatment groups ($F = 0.948$, $p = 0.424$; Figure 4). All subjects reported DOMS at TIME2 ($p = 0.001$), TIME3 ($p = 0.001$), and TIME4 ($p = 0.001$) that was significantly different from TIME1. The responses to the questions regarding the workout and how the drinks affected performance can be viewed in Table 1. Subjects in the LCHO tended to have a more positive response when asked how they felt after the initial workout than those in the CHO group, but the responses were not statistically significant ($p = 0.085$).

Diet Analysis

Variables for daily dietary intake are shown in Table 2. No significant differences in total calories, protein, protein relative to body mass, CHO, leucine, isoleucine, valine, or BCAA were seen between the groups for each of the 24-hour postexercise periods in which food logs were kept (i.e., TIME1-TIME2). The relationship between the protein content of the diet over a 24 hour period and the corresponding CK level for each subject was not statistically significant ($r^2 = 0.003$, $p = 0.690$; Figure 5). In contrast, the relationship between the leucine content of the diet over a 24-hour period and the corresponding CK level for each subject was statistically significant ($r^2 = 0.131$, $p = 0.004$; Figure 6).

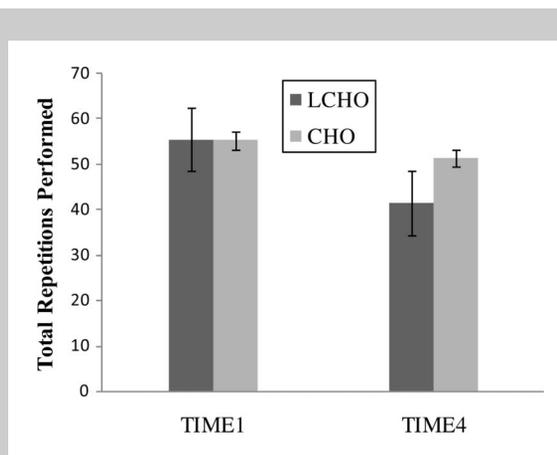


Figure 7. Data presented are mean \pm SEM total number of repetitions performed during 6 sets of squats to fatigue using 75% of each subject's 1 repetition maximum in the leucine-carbohydrate (LCHO) and carbohydrate (CHO) groups measured after preexercise beverage consumption (TIME1) and during an identical workout 72 hours later (TIME4). There were no between-group (CHO and LCHO) differences, but the mean \pm SEM number of repetitions performed (marginal means, collapsed across groups) during TIME1 was significantly different ($p < 0.05$) from TIME4.

Squat Repetitions to Fatigue

Squat repetitions to fatigue were not different between groups ($F = 2.765$, $p = 0.114$; Figure 7). An independent samples t -test indicated that there was not a significant difference between groups for total repetitions performed ($p = 0.979$) during the 6 sets performed during TIME1, suggesting that there was no acute effect in consuming either preexercise beverage. Furthermore, this suggested that there was also no difference in exercise volume, defined as sets \times repetitions, in initiating muscle damage. The subjects in the CHO group performed more repetitions to fatigue during TIME4 than the subjects in the LCHO group, but the difference was not significant ($p = 0.210$).

DISCUSSION

The results from the present study indicated that there were no differences in CK, LDH, DOMS, and squat repetitions to fatigue in subjects consuming pre and postexercise carbohydrate beverages with or without the addition of leucine. This is the first study that has attempted to isolate the effects of adding leucine to a carbohydrate beverage on recovery from lower-body resistance exercise. This experiment is also unique in that we monitored dietary intake for up to 72 hours postexercise. Within the context of our study design, the addition of $45 \text{ mg}\cdot\text{kg}^{-1}$ leucine split into pre and postexercise carbohydrate beverages did not reduce blood markers of exercise-induced muscle damage or reduce DOMS.

When consumed before or after exercise, supplementation with amino acids has been shown to enhance training adaptations by stimulating muscle protein synthesis, reducing muscle protein breakdown, or both (6). Essential amino acids are primarily responsible for this enhancement, whereas non-EAAs do not provide any additional stimulation of muscle anabolism (6). Among the EAAs, the most outstanding effects have been observed with BCAA, and particularly leucine. Crowe et al. (8) recently reported that 6 weeks of leucine supplementation improved endurance performance and upper-body power in outrigger canoeists. These improvements occurred without a significant change in the plasma ratio of tryptophan to BCAA, leading the authors to speculate that chronic leucine supplementation allowed the subjects to train with reduced exercise-induced muscle damage. Although the present investigation examined acute exercise-induced muscle damage after only 2 carbohydrate-leucine beverages (pre and postexercise), our results are in contrast to the theory put forth by Crowe et al. (8).

In the present investigation, the sole intervention was the addition of leucine to pre and postexercise carbohydrate beverages. It is interesting to note that of the nutrient intervention studies that have found improvements in performance or selected markers of muscle damage with amino acid supplementation, positive results have been found when the supplementation period lasts several days before or after exercise (7,8,18,23). For example, Nosaka et al. (23) demonstrated that when subjects consumed a supplement containing

12 amino acids or placebo 30 minutes before and immediately after exercise, no significant differences were found between groups. However, when supplementation continued over a 4-day period, CK, aldolase, myoglobin, and muscle soreness were significantly lower for the amino acid condition. Furthermore, the optimal dose of leucine needed to improve exercise performance or recovery in athletes is unclear. In the present investigation, a dose of $45 \text{ mg}\cdot\text{kg}^{-1}$ was used and split into pre and postexercise feedings based upon the positive findings reported by Crowe et al. (8). However, other leucine studies have used doses ranging from $50 \text{ mg}\cdot\text{kg}^{-1}$ (21) to $200 \text{ mg}\cdot\text{kg}^{-1}$ (24).

In addition to studies investigating the ability of amino acids to stimulate muscle protein synthesis when combined with exercise, several studies have investigated the synergistic effects of combined protein and carbohydrate ingestion. Studies have found reduced serum CK activity after resistance (3) or endurance training (27,28) with consumption of a 4:1 ratio of carbohydrate to protein. Studies by Green et al. (12) and Wojcik et al. (35) both found no differences in CK for up to 3 days when subjects consumed carbohydrate-protein, carbohydrate, or placebo beverages after exercise. Additionally, Green et al. (12) reported that although not statistically significant, subjects in the placebo group displayed reduced CK at all time points compared to subjects ingesting a carbohydrate-protein or carbohydrate only beverage. Methodological differences such as exercise type (resistance training, endurance exercise, eccentric contractions, etc.), the number of supplement treatments, and the dose of the supplement make comparing results between such studies difficult. Furthermore, many different methods of measuring the rate of recovery from a bout of muscle damaging exercise have been used. Among the blood markers, CK has been the most frequently used and studied, perhaps because of its large degree of change from baseline compared to other markers. In the present investigation, CK peaked at TIME2 in both groups and declined thereafter. In contrast, little change was found in LDH levels at TIME2 and TIME3 in both groups. These results indicated that LDH may be less responsive marker to muscle damage after resistance training.

A secondary purpose of the present investigation was to determine the effects of adding leucine to pre and postexercise carbohydrate beverages on squat repetitions to fatigue both acutely and 72 hours later. As hypothesized, there was no difference between groups during the initial workout when subjects consumed their respective beverage 30 minutes before exercise, and to our knowledge, no study has demonstrated acute performance improvements from BCAA or leucine supplementation. Kraemer et al. (18) reported that subjects consuming amino acids during an overreaching program were able to maintain strength and displayed reduced CK, indicating a potential relationship between muscle damage and performance in resistance trained subjects. Based on the potential relationship between reduced CK and force production, it was our hypothesis that the

subjects consuming a leucine-carbohydrate beverage before and after resistance training would display reduced serum levels of CK and LDH compared to subjects consuming a carbohydrate beverage, which would allow for subjects to perform more repetitions of the squat exercise during a subsequent training bout. Our results indicated that the ability to perform repeated repetitions of the squat exercise declined independent of the beverages consumed. Additionally, before performing the second bout of squats to fatigue, both groups reported significant DOMS, suggesting that 72 hours may not be a sufficient time period to recover from 6 sets of squats to fatigue even in resistance trained individuals.

In the present investigation, the subjects were instructed to maintain their normal ad libitum diet but avoid protein powders, drinks, and bars. Interestingly, the results from the present study indicated that the leucine content of the diet accounted for approximately 13% of the variance in CK (Figure 6), whereas the protein intake was not significantly associated with CK levels postexercise (Figure 5). It is important to note that although there were no significant between-group differences for any of the nutrients analyzed, the average daily protein intakes for the LCHO and CHO groups were 1.62 and 1.65 g·kg⁻¹, respectively, which evidence has shown is sufficient to preserve positive nitrogen balance in strength athletes (32). Differing from our study design, Coombes and McNaughton (7) tested the hypothesis that BCAA supplementation would improve muscle recovery even in conjunction with a high protein diet. To do so, the authors performed daily food recalls. In the event that a subject's protein and BCAA intake for that specific day were too low, the subject was instructed to drink additional milk. The authors reported that BCAA supplementation decreased serum CK and LDH after endurance exercise even when the subjects consumed a diet rich in BCAA. With the contrasting results of the present investigation and those of Coombes and McNaughton (7), future studies should investigate the amounts of leucine and BCAA in the diet, and meal timing and frequency, needed to optimize recovery from resistance training.

As with all research studies, these findings are context specific. It is important to acknowledge the potential limitations of the present investigation. Although CK is frequently used as an indirect measure of muscle damage after exercise, a large amount of variability existed between subjects postexercise in the present study. As recently noted by Green et al. (12), a significant factor in comparing the results of exercise-induced muscle damage studies with an intervention as the independent variable is the authors' decision to use a crossover design or a between-subjects design. Using a crossover design has the advantage of allowing subjects to serve as their own controls and, therefore, significantly reduces variability. However, the use of a crossover design allows subjects to perform an activity multiple times, and it is therefore unknown to what extent the results are because of a true treatment effect vs. a consequence of the repeated bout

effect. Although the subjects in the present investigation were resistance trained, the high volume and intensity of the squat protocol resulted in significantly increased CK levels and DOMS in both groups. Additionally, it should be noted that of the 3 women recruited for the present investigation, 2 were in the LCHO group and 1 was in the CHO group, and exclusion of these subjects from data analysis does not significantly change the results of any of the dependent variables. In regards to changes in CK after exercise, 2 studies have reported no sex differences (9,30), whereas 2 other studies (29,31) have reported that women demonstrate lower CK responses before and after eccentric exercise. Studies investigating DOMS after exercise have not found sex differences (9,25,29). Thus, when designing the study, it was our decision to allow both men and women to volunteer for the present investigation under the contention that all subjects were healthy, resistance trained, and familiar with the squat exercise. However, the high degree of variability in CK found in the present study suggests that these results must be interpreted with caution.

In summary, these results do not provide support for adding leucine to carbohydrate beverages before and after resistance training within the context of this study. Although leucine has been shown to play an important role in augmenting muscle protein synthesis after exercise (1,2), the addition of 45 mg·kg⁻¹ leucine divided into pre and postexercise carbohydrate beverages was insufficient at reducing acute exercise-induced muscle damage and DOMS in the current study. When combined with other studies (7,8,18,23), the results of the present investigation suggested that future studies should investigate higher doses of leucine on muscle recovery after exercise, or do so using a longer supplementation period (i.e., days or weeks).

PRACTICAL APPLICATIONS

Allowing proper recovery between workouts is an important consideration when designing resistance training programs. It is in the best interest of coaches to consider methods of enhancing muscle recovery after resistance training. Although past studies have shown improvement in performance (8) and muscle recovery (3,6,7) after exercise with amino acid supplementation, the results of the present study suggested that blood CK and LDH, DOMS, and squat performance were not improved after resistance training by adding leucine to pre and postexercise carbohydrate beverages. It should be noted, however, that these findings are context specific, and leucine's effects on muscle protein synthesis and cell signaling (1,2) suggest further studies are needed to investigate the optimal dose of leucine, and meal timing and frequency, needed to optimize recovery from resistance training.

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