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ORIGINAL ARTICLE

Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise

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Abstract The purpose of this study was to determine the effect of leucine supplementation on indices of muscle damage following eccentric-based resistance exercise. In vitro, the amino acid leucine has been shown to reduce proteolysis and stimulate protein synthesis. Twenty-seven untrained males (height 178.6 ± 5.5 cm; body mass 77.7 ± 13.5 kg; age 21.3 ± 1.6 years) were randomly divided into three groups; leucine (L) ($n = 10$), placebo (P) ($n = 9$) and control (C) ($n = 8$). The two experimental groups (L and P) performed 100 depth jumps from 60 cm and six sets of ten repetitions of eccentric-only leg presses. Either leucine (250 mg/kg bm) or placebo was ingested 30 min before, during and immediately post-exercise and the morning of each recovery day following exercise. Muscle function was determined by peak force during an isometric squat and by jump height during a static jump at pre-exercise (PRE) and 24, 48, 72, and 96 h post-exercise (24, 48, 72, 96 h). Additionally, at these time points each group's serum levels of creatine kinase (CK) and myoglobin (Mb) along with perceived feelings of muscle soreness were determined. None of the C group dependent variables was altered by the recurring testing procedures. Peak force was significantly decreased across all time points for both experimental groups. The L group experienced an attenuated drop in mean peak force across all post-exercise time points compared to the P group. Jump height significantly decreased from PRE for both the L and P group at 24 h and 48 h. CK and Mb was significantly elevated from PRE for both experimental groups at 24 h. Muscle soreness increased across all time points for the both the L and P group, and the L group experienced a significantly higher increase in mean muscle soreness post-exercise. Following exercise-induced muscle damage, high-dose leucine supplementation may help maintain force output during isometric contractions, however, not force output required for complex physical tasks thereby possibly limiting its ergogenic effectiveness.

Keywords Creatine kinase · Myoglobin · Muscle soreness · Force output

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Introduction

Resistance exercise involves subjecting the musculature to mechanical overload thereby resulting in microtrauma to the muscle (Baty et al. 2007; Ebbeling and Clarkson 1989; Gibala et al. 1995). The eccentric phase of the muscle contraction produces the highest level of exercise-induced muscle damage (Gibala et al. 1995). Exercise-induced muscle damage is succeeded by biochemical changes within the circulation, including increases in creatine kinase (CK) activity (Brancaccio et al. 2007) and

myoglobin (Mb) concentrations (Yamamoto et al. 2008). Physical performance can also become altered as a result of damage to the muscle. Following exercise-induced muscle damage there is a reduction in the ability of the muscle to contract with maximal force (Pearce et al. 1998), which is observed in all three types of muscle actions; eccentric, concentric, and isometric (Turner et al. 2008). Explosive muscle actions, such as those used during a vertical jump, can also become impaired as a result of muscle damage. Harrison and Gaffney (2004) found that eccentric exercise significantly reduced take-off velocity and subsequent performance for both static jumps and countermovement jumps. Similarly, Byrne and Eston (2002) showed a decrease in vertical jump performance following ten sets of ten barbell squats, with performance remaining diminished for 72 h post-exercise.

Various supplemental protocols have been investigated as a method to attenuate levels of muscle damage, and subsequent reductions in functionality of the muscle, following a bout of resistance exercise (Baty et al. 2007; Cooke et al. 2009; Jackman et al. 2010; Sharp and Pearson 2010). Many of these investigations have utilized supplements containing amino acids, though the composition of specific amino acids has been highly variable. Of particular interest have been the branched-chain amino acids (BCAAs), due to their relative abundance in skeletal muscle (Coombes and McNaughton 2000; Greer et al. 2007; Jackman et al. 2010). Coombes and McNaughton (2000) had subjects supplement with 12 g of BCAAs or placebo per day for 14 days and on the 7th day of supplementation a 120 min cycling bout at 70% VO_{2max} was performed. Following the cycling bout, CK levels were significantly lower at 2, 3, 4, 24, 72, and 120 h post-exercise in the group that consumed BCAAs (Coombes and McNaughton 2000). Acute ingestion of 50 g of BCAAs also resulted in significantly lower CK levels at 4, 24, and 48 h following a cycling protocol of 90 min at 55% of VO_2 peak (Greer et al. 2007). BCAA supplementation has also been shown to reduce the level of delayed-onset muscle soreness (DOMS) experienced 24 h post-exercise and attenuated the decrease in leg-flexion torque 48 h post-exercise (Greer et al. 2007). Similarly, Shimomura et al. (2010) recently showed that a single high dose of BCAAs (100 mg/kg) immediately before seven sets of 20 squats significantly attenuated DOMS and decreases in isometric muscle force in the days following exercise. Conversely, Jackman et al. (2010) recently reported no effect of BCAA supplementation on CK, Mb, or force production following 120 eccentric contractions of the knee extensors. These discrepancies in findings may be due to the differences in the quantity of BCAA ingested, as Jackman et al. (2010) only consumed 14.6 g of BCAAs in close proximity to the exercise protocol when compared to the 50 g consumed during exercise in Greer et al. (2007). Additionally, the magnitude of muscle damage may account for some of the inconsistencies in these findings, as the investigations showing positive effects of supplementation occurred with modest levels of muscle damage (Coombes and McNaughton 2000; Greer et al. 2007; Shimomura et al. 2010).

Leucine, one of the BCAAs, has been shown to be a potent stimulus for protein synthesis (Anthony et al. 2000), as well as an anti-proteolytic amino acid (Nakashima et al. 2005). When leucine is given concurrently with the other BCAAs, isoleucine and valine, protein synthesis becomes stimulated (Blomstrand et al. 2006; Buse and Reid 1975). However, when leucine is removed from the mixture, protein synthesis is abated (Buse and Reid 1975). Therefore, it can be argued that leucine is the rate-limiting BCAA controlling protein synthesis during the recovery period following exercise. Much of the research examining the effect of leucine during resistance exercise has focused on the leucine metabolite, β-Hydroxy β-methylbutyrate (HMB). The effect of HMB supplementation on muscle damage following eccentric-based exercise has shown conflicting results. Supplementation with 3 g/day of HMB and 0.3 g/day of α-isoketocaproic acid for 14 days prior to performing an elbow flexion damage protocol resulted in an attenuated CK response and percent decrement in one repetition maximum (1RM) strength in the 72 h following the exercise (van Someren et al. 2005). However, when an identical supplement protocol was applied to a downhill running model, there was no effect on CK response, isometric and isokinetic torque, or muscle soreness (Nunan et al. 2010). These results cannot be generalized to leucine as HMB accounts for only 5–10% of the metabolites produced from the degradation of leucine (Van Koevering and Nissen 1992). Consequently, there may be additional metabolites produced from the oxidation of leucine that possess ergogenic effects. To date only one investigation has examined the effect of oral supplementation with leucine, in conjugation with carbohydrates, on markers of muscle damage, exercise performance and DOMS following intensive resistance exercise. Stock et al. (2010) had subjects perform six sets of squats to fatigue at 75% of the subject's 1RM while consuming either 22.5 mg/kg of leucine or placebo with 0.25 g/kg of carbohydrate solution. They reported no effect of leucine on CK, lactate dehydrogenase or muscle soreness in the 72 h following exercise, as well as no differences in performance when the same exercise protocol was repeated 72 h post-exercise (Stock et al. 2010). However, this investigation used a relatively low dose of leucine (22.5 mg/kg) with no supplementation in the days following the exercise protocol. Low doses may be beneficial when administered chronically over a period; however, higher doses (>150 mg/kg)

may be required to have any effect on a single bout of intense exercise (Greer et al. 2007). With the amount of literature that shows that leucine supplementation may help preserve skeletal muscle during various catabolic states, as well as stimulate protein synthesis, it remains unclear whether high-dose leucine supplementation is a viable mean for attenuating the muscle damage response brought about by a single bout of eccentric-based resistance exercise.

Therefore, the purpose of this investigation was to determine the effect of supplementation with high doses of leucine on markers of muscle damage, both biomechanical and biochemical, as well as muscle soreness following eccentric-based resistance exercise. It was hypothesized that leucine supplementation would attenuate the levels of indirect muscle damage markers and muscle soreness following exercise-induced muscle damage, and help to facilitate the restoration of muscle function.

Methods

Subjects

A total of 27 healthy, college-aged male subjects were recruited for this investigation (height 178.6 ± 5.5 cm; body mass 77.7 ± 13.5 kg; age 21.3 ± 1.6 years). Subjects were excluded based on (a) participation in a structured lower-body resistance exercise program in the 6 months prior to the investigation, (b) use of ergogenic aids in the previous 3 months, (c) or if currently taking any prescription drugs for chronic inflammation. Prior to initial testing, subjects were required to read and sign an informed consent, which was approved by the Institutional Review Board at Appalachian State University.

Study design

The study was a double-blind, placebo controlled design consisting of two supplement groups, leucine ($n = 10$) and placebo ($n = 9$), and a control group ($n = 8$). Sample size was determined by running a power analysis (power = 0.8) on previously published data examining high-dose BCAA supplementation on markers of muscle damage following strenuous exercise (Greer et al. 2007). Subjects were randomly assigned to their respective groups. Subjects were asked to refrain from performing any type of upper-body resistance exercise or strenuous activity 48 h prior to each testing session and in the 96 h following each testing session. Anthropometric measurements (height, weight, age) and each subject's 1RM in the leg press exercise were all obtained in the first testing session (Table 1). Additionally, during the first testing session baseline values were obtained for peak force during an isometric squat and static jump height. During the second testing session, the subjects performed an eccentric-based resistance exercise protocol in which three doses of the supplement [leucine (250 mg/kg) or placebo] was consumed 30 min before, during, and immediately after the exercise protocol. Subjects also consumed one dose of the supplement prior to each post-testing session. The control group did not perform the resistance exercise protocol nor ingest any form of supplement. Subjects then reported for post-testing evaluation for muscle function at 24, 48, 72, and 96 h (24, 48, 72, 96 h) following the eccentric-based resistance exercise protocol. Subjects were instructed to refrain from taking any type of non-steroidal anti-inflammatory drugs (e.g., ibuprofen, Advil^{TM}) during the duration of the study. Subjects reported to the laboratory in a fasted state for the resistance exercise session and for all of the post-testing sessions. Blood samples were taken at PRE, 24, 48, 72, and 96 h and analyzed for CK and Mb. Measures of muscle function and perceived muscle soreness were also obtained at PRE, 24, 48, 72, and 96 h. Perceived muscle soreness was assessed via a visual analogue scale (VAS). Subjects were instructed to keep dietary records for the 48 h prior to the resistance exercise session and in the 72 h following the resistance exercise session. Dietary records were analyzed for total caloric intake, as well for quantity of each macronutrient (carbohydrates, protein, and fat).

Muscle function assessment

During the first testing session, subjects performed three maximal isometric squats, as well as three static jumps. Subjects performed the same tests again 24, 48, 72, and 96 h, however, during these sessions, only two trials for each test were performed in order to minimize accumulating fatigue and muscle damage. Subjects were

Table 1 Anthropometric and strength values for each group (mean ± SD)

Group	Height (cm)	Body mass (kg)	Age (years)	Leg press 1RM (kg)
Leucine	178.45 ± 6.60	76.79 ± 12.47	21.00 ± 1.05	206.58 ± 53.79
Placebo	178.71 ± 5.99	79.59 ± 14.65	21.67 ± 1.94	218.37 ± 70.06
Control	178.74 ± 4.08	76.76 ± 14.84	21.13 ± 1.73	209.47 ± 95.62

encouraged to give a maximal effort during the isometric squat and each of the static jumps, and 3 min of rest was given between each trial. Each isometric squat was performed with the subject standing on a force plate (BP6001200, AMTI, Watertown, MA) with a fixed-position barbell placed across their upper back. Subjects were instructed to exert maximal force against the bar for 3 s. All isometric squats were performed with a knee angle of 100° as determined by a goniometer.

All vertical jump testing was performed with the subject standing on a force plate while holding a weightless (plastic) bar across their upper back. The right side of the barbell was attached to two linear position transducers (LPTs) (PT5A-150, Celesco Transducer Products, Chatsworth, CA). The weightless bar acted to counterweight the pull of the two LPTs resulting in zero load. The LPTs were located above-anterior and above-posterior to the subject and, when attached to the bar, resulted in the formation of a triangle. This allowed for the calculation of vertical and horizontal displacements through trigonometry involving constants and displacement measurements. This method of collecting kinematic variables has previously been validated (Cormie et al. 2007). The combined retraction tension of the LPTs was 16.4 N; this was accounted for in all calculations. Analogue signals from the force plate and LPTs were collected for every trial at 1,000 Hz using a BNC-2010 interface box with an analogue-to-digital card (NI PCI-6014, National Instruments, Austin, TX). Custom programs designed using LabVIEW (Version 8.2, National Instruments) were used for recording and analyzing the data.

Signals from the two LPTs and the force plate underwent rectangular smoothing with a moving average half-width of 12. A force–time curve was calculated for each isometric squat. Isometric squat peak force was measured as the highest force output achieved during the 3 s isometric contraction. Displacement–time curves were calculated for each static jump. Jump height was measured during the concentric phase of the vertical jump. Jump height was determined as the difference between maximum displacement reached during the jump and initial displacement while in a standing position. Within each time point, the trial that resulted in the greatest isometric peak and jump height was used for statistical analysis.

Leg press 1RM testing

A warm-up protocol consisted of loads equal to 30% (8–10 repetitions), 50% (4–6 repetitions), 70% (2–4 repetitions), and 90% (1 repetition) of an estimated one repetition maximum (1RM). Subjects were given up to four maximal attempts to achieve their 1RM. This protocol has previously been used to determine a subject's 1RM in the squat exercise (McBride et al. 2009). During all leg press attempts, subjects were required to lower the weight to a point where an 80° knee angle was attained as determined by a goniometer. Rest periods of 5 min were utilized between trials. Any necessary modifications during testing were determined by a Certified Strength and Conditioning Specialist.

Eccentric-based resistance exercise protocol

The resistance exercise protocol emphasized the eccentric component of each exercise as eccentric muscle contractions have been shown to induce the highest levels of muscle damage (Gibala et al. 1995). The resistance exercise protocol was modified from damage protocols previously utilized by Miyama and Nosaka (2004) and Cooke et al. (2009). Both protocols were shown to induce significant levels of muscle damage as shown through increases in plasma CK. Subjects were instructed to perform a 5 min warm-up on a cycle ergometer. The exercise protocol consisted of five sets of 20 maximal drop jumps from a height of 60 cm with a 10 s interval between jumps and a 2 min rest between sets. Subjects then performed six sets of 10 eccentric contractions on the leg press with a weight equal to 120% of their previously determine 1RM, with a 3 min rest period given between sets. During all repetitions, the eccentric portion of the movement was performed with a 3 s tempo until the subjects reached a knee flexion of greater than 90°. If the desired tempo could not be maintained due to fatigue, subjects were allowed a 10-15 s rest before continuing with the set.

Supplement protocol

Supplement groups included a leucine (L) group ($n = 10$) and a placebo (P) group ($n = 9$). The control (C) group ($n = 8$) did not receive any form of supplement. Leucine supplementation consisted of 250 mg/kg body weight of L-leucine per dose. This dosage was established based on previous dosages of BCAAs shown to reduce muscle damage (Greer et al. 2007). Additionally, 3 g of non-caloric sweetener (Splenda®) was added to the supplement and acted as the supplement during the placebo condition. Doses were provided 30 min prior to resistance exercise, immediately pre-exercise, immediately post-exercise, and immediately before each of the post-testing sessions (24, 48, 72 and 96 h). This dosing protocol was utilized as previous literature has demonstrated that acute amino acid supplementation around the exercise bout is insufficient at attenuating muscle damage unless supplementation continued during the recovery period (Nosaka et al. 2006). Plasma leucine concentrations have been show to elevate

15 min following ingestion and peak at 30 min with 100 mg/kg doses of BCAAs (Shimomura et al. 2010). The placebo consisted of Splenda® alone at all time points. Each dose for both the L and P groups were mixed with a liquid solution containing 2 g of a low-calorie flavoring mixture (Crystal Light®) to increase palatability of the supplement protocols. Each dose was mixed in an opaque bottle with additional water being added following initial consumption to ensure the entire supplement was ingested and none remained in the bottle.

Blood collection

Blood was collected prior to any activity at PRE, 24, 48, 72, and 96 h. The resting values were used to determine baseline levels for muscle damage markers. Blood was obtained from the antecubital vein into a 10 ml Vacutainer™ blood collection tube and allowed to clot at room temperature. The whole blood was centrifuged for 15 min at 2,500 rpm at room temperature with the serum being divided into Eppendorf™ tubes and frozen at −80°C for subsequent analysis.

Biochemical analysis

Serum from PRE, 24, 48, 72, and 96 h blood samples was analyzed for CK activity and Mb. CK was analyzed in duplicate using basic spectrophotometric techniques (Pointe Scientific, Canton, MI). Subsequently, 1,000 µl of reagent was pipetted into a polystyrene cuvette and prewarmed at 37°C for 5 min; 25 µl of sample was added to the reagent and incubated at 37°C for 2 min. Following the incubation period, absorbance at 340 nm was determined at three time points each separated by 1 min using a spectrophotometer (Genesys 5, Thermo Spectronic, Rochester, NY) with the mean absorbance difference used to yield results in IU/L. If the calculated activity was above 1,500 IU/L the sample was diluted 1:1 with saline and reanalyzed. Intra-assay CV was <3% across all samples. Normal reference range for males as detailed in the assay protocol is up to 160 IU/L.

Mb was analyzed in duplicate using a solid phase enzyme-linked immunosorbent assay (DRG International Inc., Mountainside, NJ). Serum was diluted tenfold and then 20 µl of diluted sample was pipetted into the appropriate well; 200 µl of Enzyme Conjugate Reagent was added to each well and then mixed for 30 s. The plate was incubated at room temperature for 45 min and then each well was rinsed five times with distilled water; 100 µl of TMB reagent was added to each well and incubated at room temperature for 20 min. The reaction was stopped by adding 100 µl of Stop Solution to each well. The absorbance of each well was determined by using a microplate spectrophotometer (µQuant, Bio-Tek Instruments, Inc., Winooski, VT) at a wavelength of 450 nm. Intra-assay CV was <5% across all samples. Normal reference range for males as detailed in the assay protocol is between 12 and 100 ng/ml.

Delayed-onset muscle soreness

The subject's perceived muscle soreness of the lower body was assessed using a VAS scale prior to blood collection at PRE, 24, 48, 72, and 96 h. Subjects subjectively rated their level of soreness during a maximal contraction of their quadriceps group by drawing an intersecting line across a continuum line extending from 0 cm (0 = no soreness) to 13 cm (13 = extreme soreness) (Buford et al. 2009). The scale was a continuous line and did not contain any segments or labels that would allow for quantification by the subject, thereby not allowing the subject to retain any perceived soreness value between testing sessions. Subjects were also not privy to their previous results and instructed to rate their soreness at that moment in time. The distance of each mark was measured from zero with the corresponding value being used as the perceived level of muscle soreness.

Dietary analysis

The subject's dietary intake was not standardized for this investigation; however, subjects were instructed to maintain their normal dietary habits for the duration of the study. Subjects in the L and P group were instructed to keep a 6-day food log, which began 48 h prior to the resistance exercise protocol and was continued until the final post-testing session. This was done to ensure that there were no significant differences between macronutrient intakes between experimental groups, particularly protein levels that could significantly alter total leucine intake during the study. The dietary food logs were evaluated using a dietary assessment food software program (Food Processor® SQL, ESHA Research, Salem, OR) to determine the average daily caloric (CAL) intake and macronutrient breakdown of carbohydrates (CHO), protein (PRO) and fat (FAT).

Statistical analyses

All statistical analyses were performed on SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA). Five separate repeated measure analysis of variance (ANOVAs) were used to determine whether isometric peak force, jump height, CK, Mb and muscle soreness values for the control group significantly changed during the testing period. If a significant time effect was observed, Tukey post hoc

analyses were performed to determine where significance was obtained. If the dependent variable did not change over time, it was excluded from further analyses. To determine the effect of the supplementation, five separate two [Group (L, P)] × 5 [Time (PRE, 24, 48, 72, 96 h)] factorial mixed design ANOVAs with repeated measures were used to analyze the isometric peak force, jump height, CK, Mb and muscle soreness. If a significant interaction were observed, separate repeated measure ANOVAs with Tukey post hoc analyses were performed to determine the within-group effects and independent *t* tests were used to determine the between group effects at each time point. If no significant interaction occurred, however, there was a main effect for time, data was collapsed across group to provide a marginal mean for the experimental groups and analyzed with a one-way repeated measure ANOVA. Tukey post hoc analyses were performed to determine the change in that variable across time. If no significant interaction occurred, however, there was a main effect for group, data was collapsed across time and analyzed with an independent *t* test. Analysis of dietary intake was done using independent *t* tests for each of the macronutrient variables. For all statistical measures, significance was set at $p \leq 0.05$. Isometric peak force and jump height were analyzed and presented as a percentage change from the PRE value. Serum CK and Mb absolute values were analyzed and presented as a percentage of each individual participant's maximum value (Jackman et al. 2010). This was done to account for the large inter-subject variability seen in the experimental groups. All values are expressed as mean ± SEM.

Results

Analyses on the control group data revealed that none of the dependent variables changed over the duration of the study (Table 2). These results confirm that the changes in the performance measures within the experimental groups were a result of the muscle damage sustained from the resistance-exercise protocol and not from other factors, such as accumulating fatigue. In addition, the performance measures did not result in any muscle soreness or additional muscle damage following the damage protocol.

The eccentric-based resistance exercise protocol resulted in significant alterations in muscle function. For squat isometric peak force there was a non-significant interaction between L and P groups, however, there was significant main effects for both time ($p < 0.001$) (Fig. 1a) and group ($p = 0.04$) (Fig. 1b). Isometric peak force was significantly decreased across all time point with the peak decrease occurring at the 24 h time point with the marginal mean for the two groups dropping from 2,147.1 ± 88.6 to 1,903.8 ± 93.3 N. When expressed relative to the Pre value, the main group effect showed the L groups isometric peak force was significantly higher than the P groups across all post-exercise time points. For the static jump height there was no interaction, no group effect ($p = 0.183$), however, there was a significant main time effect ($p < 0.001$) (Fig. 2). The marginal jump height means for the experimental groups were significantly lower than PRE (0.408 ± 0.009 m) at 24 h (0.371 ± 0.013 m) and 48 h (0.372 ± 0.011 m).

The eccentric-based resistance exercise protocol resulted in minimal changes in serum muscle damage markers. There was a significant main time effect for CK ($p = 0.001$) with both groups having a significant increase from Pre (274.8 ± 58.1 IU/L) to 24 h (777.7 ± 163.6 IU/L) (Fig. 3). There were no significant between group differences for CK. Similarly, there was a significant main time effect for Mb ($p = 0.005$) with both groups significantly increasing from Pre (42.0 ± 7.3 ng/ml) to 24 h (76.3 ± 6.9 ng/ml) (Fig. 4). There were significant changes in muscle soreness during the course of the experimental protocol. For muscle soreness, there was a significant main effects for both group ($p = 0.021$) and time ($p < 0.001$). Muscle soreness for the L and P group significantly increased following exercise at the 24, 48, 72 and 96 h (Fig. 5). The L group had significantly higher mean muscle soreness across all post-exercise time points. There were no significant differences ($p > 0.05$) for CAL, CHO, PRO or FAT intakes between the L and P group during the experimental period (Table 3).

Table 2 Control groups values for each of the dependent variables (mean ± SEM)

Time point	ISO peak force (N)	Jump height (m)	CK (IU/L)	Mb (ng/ml)	Soreness (cm)
Pre-exercise	2,101.6 ± 179.9	0.403 ± 0.023	359.9 ± 88.6	38.3 ± 3.0	1.29 ± 0.59
24 h	2,067.8 ± 164.4	0.392 ± 0.020	260.2 ± 54.3	32.8 ± 4.6	0.49 ± 0.14
48 h	2,066.7 ± 154.2	0.401 ± 0.024	233.4 ± 30.6	40.6 ± 9.2	1.29 ± 0.38
72 h	2,078.5 ± 153.8	0.397 ± 0.017	274.4 ± 41.3	42.6 ± 6.1	1.16 ± 0.33
96 h	2,103.3 ± 170.7	0.401 ± 0.020	289.4 ± 91.1	34.1 ± 5.3	1.11 ± 0.45

There were no significant differences between time points for any of the dependent variables ($p > 0.05$)

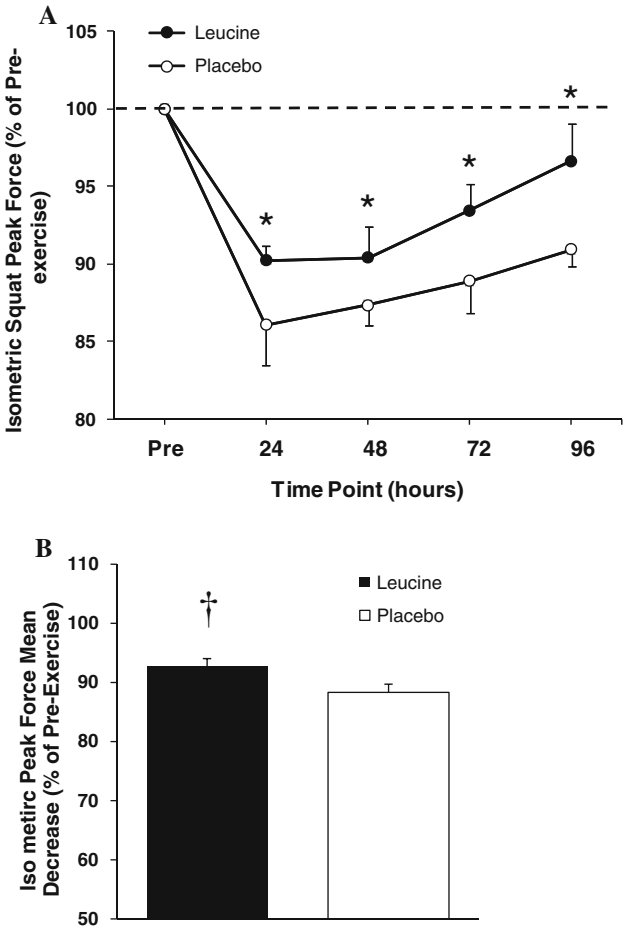

Fig. 1 Peak isometric force showed was no significant interaction, however, there were significant main effects for time ($p < 0.001$) and group ($p = 0.40$). **a** Changes in isometric peak force output pre- and post-resistance exercise protocol (mean ± SEM). *Asterisks* denotes a significant ($p < 0.05$) decrease in the marginal mean for both of the experimental groups from pre-exercise value. **b** Results for the group main effect for mean isometric peak force output across 24, 48, 72, and 96 h, expressed as a percentage of pre-exercise (mean ± SEM). *Dagger* denotes a significant difference from placebo group

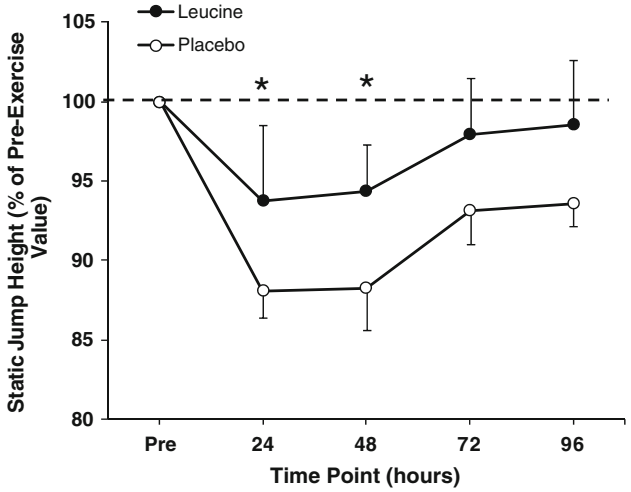

Fig. 2 Changes in static jump height pre- and post-resistance exercise protocol displayed a significant main time effect ($p < 0.001$). *Asterisks* denotes a significant ($p < 0.05$) decrease in the overall marginal mean for the experimental groups from pre-exercise value (mean ± SEM)

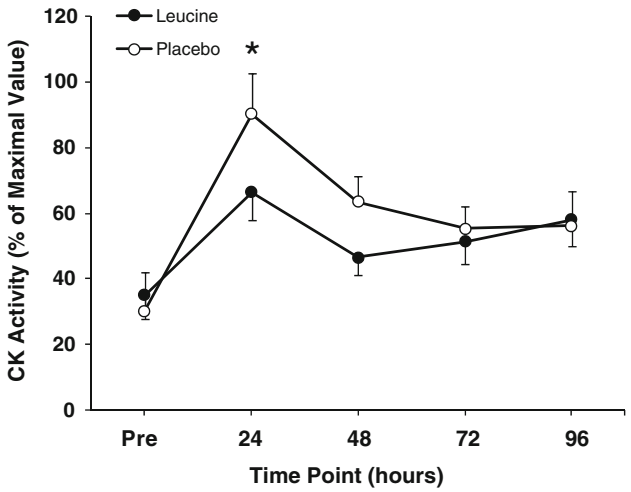

Fig. 3 Changes in CK activity pre- and post-resistance exercise protocol displayed a significant main time effect ($p = 0.001$) (mean ± SEM). Values are expressed as a percentage of each subject's maximum value. *Asterisks* denotes a significant ($p < 0.05$) increase in the overall marginal mean for the experimental groups from pre-exercise value

Discussion

The primary finding of this investigation is that high-dose leucine supplementation was unable to attenuate the increase in biochemical markers of muscle damage that follows eccentric-based resistance exercise, however, it may aid in the maintenance of isometric force output. Both experimental groups showed a similar decrease in isometric peak force across all time points; however, the L groups mean isometric squat value was significantly higher than the P groups following the exercise bout. This pattern for a maintenance of peak force output compared to pre-exercise values support those reported with a combined HMB and α-isoketocaproic acid supplement protocol (van Someren et al. 2005). Recently, Shimomura et al. (2010) reported that a single dose of BCAAs before unaccustomed squat exercise resulted in a significantly higher isometric peak force output in the knee extensors 3 days following exercise. Jackman et al. (2010) showed no effect of BCAA supplementation on knee extensor muscle function following eccentric damage. Similarly, Stock et al. (2010) reported no effect of leucine on squats to fatigue 72 h following exercise. The timing and dosage of the supplement may account for the discrepancy between the previous investigations and the current investigation, as both

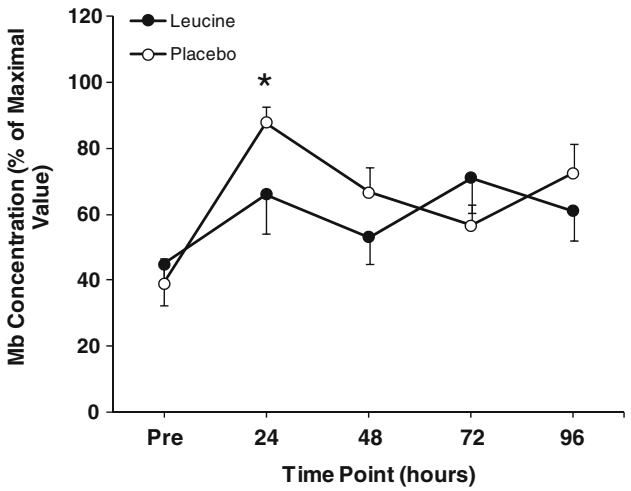

Fig. 4 Myoglobin concentration pre- and post-resistance exercise protocol displayed a significant main time effect ($p = 0.005$). *Asterisks* denotes a significant ($p < 0.05$) increase in the overall marginal mean for the experimental groups from pre-exercise value. Values are expressed as a percentage of each subject's maximum value (mean ± SEM)

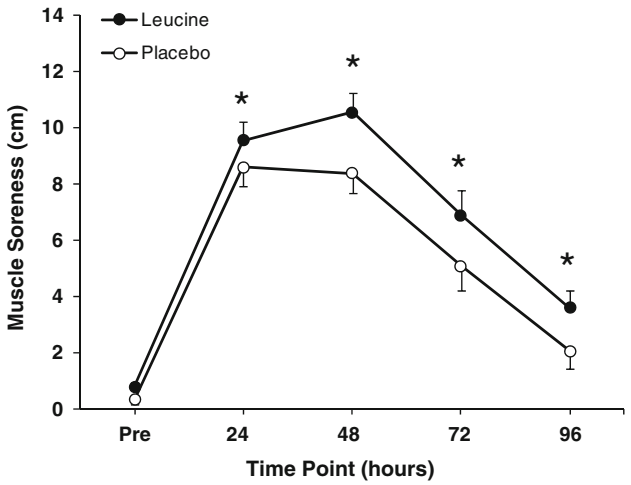

Fig. 5 Changes in delayed-onset muscle soreness showed was no significant interaction, however, there were significant main effects for time ($p < 0.001$) and group ($p = 0.021$). *Asterisks* denotes a significant ($p < 0.05$) increase in the marginal mean for both the experimental groups from pre-exercise value (mean ± SEM)

Table 3 Average daily intake of total calories, carbohydrates, protein, and fat determined by a 6-day food recall (mean ± SEM)

Group	CAL (kcal)	CHO (g)	PRO (g)	FAT (g)
Leucine	1,822.6 ± 191.1	228.2 ± 19.9	72.8 ± 8.6	64.6 ± 10.4
Placebo	1,869.5 ± 314.2	240.3 ± 38.7	72.5 ± 7.4	56.9 ± 8.7

There were no significant differences between experimental groups for dietary intake of any macronutrient ($p > 0.05$)

investigations used an acute supplementation design with relatively low doses of leucine during the exercise period (21.5–45 mg/kg leucine) (Jackman et al. 2010; Stock et al. 2010). Additionally, supplementation during the recovery period may be needed to see any effect of leucine on muscle damage, with acute amino acid supplementation being insufficient (Nosaka et al. 2006) unless given in a relatively high dose (Shimomura et al. 2010). Since orally supplemented leucine is able to activate protein synthesis in skeletal muscle (Anthony et al. 2000), this enhanced frequency of anabolic processes would appear to be a feasible method of accelerating the recovery process in physical performance. The application of these findings to other measures of muscle function may be limited due to contraction type that was assessed in the current investigation. Part of the rational for selecting an isometric squat as a measure of muscle function was to include the muscle groups that were used during the exercise protocol. This also allowed for the determination of changes in the cumulative force output from multiple muscle groups. Alternatively, leucine had no effect on dynamic muscle contractions, such as those used during the static jumps. These results support those recently reported that utilized a dynamic squat to evaluate muscle function (Stock et al. 2010). All subjects experienced a decrease in jump height from PRE to the 24 and 48 h time points. The effect of leucine may have such a minimal contribution to performance in such a biomechanically complex exercise making between group differences difficult to distinguish.

The eccentric-based resistance exercise protocol elicited minimal changes in indirect serum markers of muscle damage. There were no group differences for either CK or Mb between either experimental group. The non-significant increase at the later time points was most likely due to large inter-subject variation in their CK and Mb. Individual maximal responses varied from 189 to 4,835 U/L for CK and 45 to 480 ng/ml for Mb. This is a common occurrence in the literature (Baty et al. 2007; Cockburn et al. 2008; White et al. 2008), since there appear to be responders and non-responders to elevations in these biochemical molecules. When comparing the current exercise protocol to other protocols that used similar exercises, the damage response measured by CK were similar to those reported by Miyama and Nosaka (2004) but much less than those reported by Cooke et al. (2009). Due to their location within the cytosol, these markers are indicators of membrane disruption and are not necessarily representative of damage to the myofibrils.

As expected, the eccentric-based resistance exercise produced significant changes in perceived muscle soreness of the lower body. It is unclear why the L group on average experienced increased soreness at the post-exercise time points. These findings are in conflict with previous

investigations that either showed no effect of leucine or BCAAs on muscle soreness (Stock et al. 2010), or an attenuation in the severity of the soreness (Jackman et al. 2010; Shimomura et al. 2006). While the subjective nature of the measurement cannot be overlooked, one possible explanation might be an alteration in the inflammatory response elicited within the L group. It is well known that eccentric contractions result in an acute inflammatory response (Buford et al. 2009; Peake et al. 2005). The role of immunological changes and cytokine production on muscle soreness is still an area of debate, but it appears to some extent, that muscle soreness is moderately related to increases in certain inflammatory cytokines (Buford et al. 2009; MacIntyre et al. 2001). BCCA supplementation has been shown to alter the acute inflammatory response following exercise protocols that produce muscle damage (Bassit et al. 2002). Bassit et al. (2002) reported that BCAA supplementation stimulated the production of interleukin-2 and interferon-γ and a suppression of interleukin-4 following a triathlon. However, since immunological changes were not measured in this investigation, the effect of leucine on cytokine production and subsequent DOMS remains purely speculative. Inflammation is known to be a critical component during the muscle repair and regeneration periods (Smith et al. 2008), but the effect of leucine supplementation on these processes requires further investigation.

The results of this investigation show that leucine provides no protective effect on attenuating the immediate increase in biochemical markers of muscle damage following eccentric-based resistance exercise. Leucine may aid in the maintenance of isometric muscle function following muscle damage; however, the ergogenic effect was only significant when comparing the mean decrease at all time points and no single time point produced a significant interaction between the supplements. The physiological relevance of these findings may be trivial as there was no effect on dynamic muscle function during the static jump. Since everyday ambulation and movements rarely involve an isometric contraction, the ability to maintain force output during these type contractions may not translate into performance in more complex movements. Further investigations should focus on direct markers of myofibril damage, as well as some of the structures involved in the excitation–contraction coupling process responsible for force output. Additionally, examination of the effect of leucine supplementation on immunological changes following muscle damage may provide insight into the possible role in muscle recovery.

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