International Journal of Sport Nutrition and Exercise Metabolism, 2013, 23, 378-387

INTERNATIONAL JOURNAL OF
SPORT NUTRITION AND
EXERCISE METABOLISM
www.IJSNEM-Journal.com
ORIGINAL RESEARCH

Oral Creatine Supplementation Augments the Repeated Bout Effect

Kelle F. T. Veggi, Marco Machado, Alexander J. Koch, Sandro C. Santana, Sedison S. Oliveira, and Michael J. Stec

Purpose: We examined the effects of creatine supplementation on the response to repeated bouts of resistance exercise. ***Methods***: Young men (24.1 ± 5.2 yr) were divided into Creatine (CM, n = 9) and Placebo (PL, n = 9) groups. On day (D) 1 and D15, subjects performed four sets of bicep curls at 75% 1-RM to concentric failure. On D8-D13, subjects consumed either 20g/d creatine monohydrate or placebo. Muscle soreness and elbow joint range of motion (ROM) were assessed on D1-D5 and D15-D19. Serum creatine kinase activity (CK) was assessed on D1, D3, D5, D15, D17, and D19. ***Results***: The first exercise bout produced increases in muscle soreness and CK, and decreases in ROM in both groups ($p < .001$). The second bout produced lesser rises in serum CK, muscle soreness, and a lesser decrease in ROM (bout effect, $p < .01$ for all), with greater attenuation of these damage markers in CM than PL. CK levels on D17 were lower (+110% over D15 for CM vs. +343% for PL), muscle soreness from D15–19 was lower (–75% for CM vs. –56% for PL compared with first bout), and elbow ROM was decreased in PL, but not CM on D16 ($p < .05$ for all). ***Conclusions***: Creatine supplementation provides an additive effect on blunting the rise of muscle damage markers following a repeated bout of resistance exercise. The mechanism by which creatine augments the repeated bout effect is unknown but is likely due to a combination of creatine's multifaceted functions.

Keywords: muscle damage, monohydrate, phosphocreatine, kinase

Oral creatine supplementation enhances fatigue resistance and increases muscle strength through several mechanisms: increased glycogen (Nelson et al., 2001; Robinson et al., 1999; van Loon et al., 2004) and phosphocreatine availability (Harris et al., 1992; Hultman et al., 1996), increased expression of growth factors (Burke et al., 2008; Deldicque et al., 2005; Safdar et al., 2008; Willoughby et al., 2003), and faster phosphocreatine resynthesis (Greenhaff et al., 1994; Yquel et al., 2002). In addition, creatine supplementation may reduce postexercise inflammation/muscle damage (Bassit et al., 2008; Bassit et al., 2010; Cooke et al., 2009; Rahimi, 2011; Santos et al., 2004). Increased muscle creatine and phosphocreatine following supplementation may provide a protective effect via several mechanisms, including phosphocreatine's structural role in stabilizing the sarcolemmal phospholipid bilayer (Saks et al, 1993) and its ability to maintain intracellular ATP homeostasis (Wallimann et al., 1992) as well as creatine's role in regulating mitochondrial permeability (Dolder et al., 2003; O'Gorman et al., 1997) and its antioxidant effects (Sestili et al., 2006).

Unaccustomed exercise results in sarcomeric disruption and structural damage to the myofiber. This damage is characterized by reduced maximal force production, muscle soreness, decreased range of motion (ROM), increased activity of muscle serum proteins (e.g., myoglobin, creatine kinase ,CK), and overall decreased muscle function (reviewed in Clarkson & Hubal, 2002; Proske & Allen, 2005).

Several groups have assessed the effects of creatine supplementation on markers of muscle damage following stressful resistance (Cooke et al., 2009; Machado et al., 2009; Rahimi, 2011; Rawson et al., 2007; Rawson et al., 2001; Rosene et al., 2009; Warren et al., 2000) and endurance (Bassit et al., 2008; Bassit et al., 2010; Santos et al., 2004) exercise. Collectively, these studies indicate that creatine either reduces damage and enhances recovery from stressful exercise (Bassit et al., 2008; Bassit et al., 2010; Cooke et al., 2009; Rahimi, 2011; Rosene et al., 2009; Santos et al., 2004) or has no effect (Machado et al., 2009; Rawson et al., 2007; Rawson et al., 2001; Warren et al., 2000). Conflicting results could be related to differences between exercise protocols. For example, two studies that demonstrated no effect of creatine supplementation on muscle damage used protocols that elicited extensive muscle damage (e.g., 50–150 maximal eccentric contractions (Rawson et al.,

Veggi, Machado, Santana, and Oliveira are with the Laboratory of Physiology and Biokinetic, Universidade Iguaçu, Itaperuna, Brazil. Koch is with the Exercise Physiology Laboratory, Lenoir-Rhyne University, Hickory, NC. Stec is with the Dept. of Cell, Developmental, and Integrative Biology, University of Alabama, Birmingham, Birmingham, AL.

2001; Warren et al., 2000), perhaps overwhelming any protective effects of supplementation. Most studies that have found positive effects of creatine supplementation on muscle damage indicate that short-term (≤7 days) supplementation is sufficient to produce significant attenuation of muscle-damage markers (Bassit et al., 2008; Bassit et al., 2010; Cooke et al., 2009; Rahimi, 2011; Rosene et al., 2009; Santos et al., 2004). Rosene et al. (2009) found a greater retention in maximal isometric force in creatine-supplemented subjects after repeating the same eccentric exercise protocol after 30 days of supplementation. This study was the first to indicate that creatine supplementation can enhance muscle function after a repeated bout of damaging exercise.

It is well known that a repeated bout of eccentric-based exercise results in marked attenuation of damage compared with the initial bout. This protective adaptation is known as the repeated bout effect (RBE). The mechanisms by the RBE confers protection may include neural (e.g., more efficient motor unit recruitment), mechanical (e.g., increased cytoskeletal/intermediate filament proteins and muscle stiffness) and cellular (e.g., increased protein synthesis, increased stress proteins, adapted inflammatory response) adaptations following muscle damage (reviewed in McHugh, 2003; Nosaka et al., 2011). Although the RBE attenuates the decline in muscle function and increase in soreness associated with damaging exercise, it does not confer complete protection to muscle damage. Athletes training several times per week may be susceptible to muscle damage if training intensity is high and they are unaccustomed to the exercise. Therefore, a nutritional intervention that could decrease muscle damage and maintain function during repeated training sessions (i.e., augment the RBE) may benefit these athletes. The aim of this study was to examine the effects of short-term oral creatine supplementation on markers of muscle damage after repeated bouts of resistance exercise designed to induce minor muscle damage.

Methods

Subjects

Eighteen healthy, young men participated in this study. Subjects were not currently using drugs, dietary supplements, or anabolic steroids and were without joint, muscular, or cardiovascular diseases. None had a recent history (within 3 yr) of muscle or joint injuries. All subjects reported previous experience with resistance training but none had practiced this activity for >12 months before the experiment. All subjects were instructed not to perform any exercise throughout the testing and collection period. All received a list of supplements, drugs (e.g., NSAIDs), and procedures (e.g., massage, cooling, heat, etc.), that were to be avoided during the experiment. The purpose and procedures were explained to the subjects and informed consent was obtained according to the Declaration of Helsinki following approval from the local Ethics Committee.

Experimental Protocol

Subjects were randomized into Creatine (n = 9) or Placebo (n = 9) groups. Subjects' characteristics are displayed in Table 1. Two weeks before experimental sessions, two initial sessions were conducted to establish a reliable 1-RM (ICC, r = .81) for the biceps curl exercise; these initial sessions were separated by 72 hr The 1-RM procedures were performed to minimize possible muscle damage that could compromise the experimental sessions (e.g., subjects were only allowed up to three attempts to achieve their 1-RM, and a rest interval of at least 5 min was allowed between each attempt). For the experimental sessions, subjects reported to the laboratory on 11 separate days (D): D1–D5 for the first exercise bout and following assessments, D8 to receive creatine or placebo supplements, and D15–D19 for the second exercise bout and assessments. Height was measured on D1 with a wall-mounted stadiometer, and body mass was assessed with a calibrated balance scale on each day the subjects reported to the laboratory. On the days that subjects performed the exercise protocol (D1 and D15), blood was taken before the exercise bout, and range of motion (ROM) and muscle soreness were assessed before and immediately after the exercise bout. In addition, blood was taken again at 48 and 96 hr after both exercise bouts, and ROM and muscle soreness were assessed on the 4 consecutive days following the exercise bouts.

Resistance Exercise Protocol

On D1 and D15, subjects performed resistance exercises. Following a warm-up, subjects performed four sets of barbell biceps curls to concentric failure using 75% of their predetermined 1-RM, with 3 min rest between sets. Repetition cadence for each exercise was controlled with a digital sound signal (Beat Test & Training, CEFISE, Nova Odessa, Brazil) so that each repetition was completed in 4 s (two concentric and two eccentric). Total volume load for each set was calculated by multiplying the amount of weight lifted by total successful repetitions performed.

Many studies have employed eccentric muscle actions alone to induce muscle damage, often performed on an isokinetic dynamometer (Chen et al., 2007). The resistance-exercise bout performed in this experiment consisted of both concentric and eccentric muscle actions using isotonic equipment. This mode of exercise is more similar to the habitual training employed by athletes. Further, concentric actions alone have been found to induce significant muscle damage (Clarkson et al., 2002).

Supplementation

One week after the first exercise bout, subjects were given either creatine monohydrate or placebo supplements to ingest for 6 consecutive days (D8–13) before the second exercise bout. Subjects in the Creatine group ingested 5 g of creatine (powder form; Midway, Santos-SP, Brazil) and 5 g of dextrosol (New Millen Ltda, Cajamar-SP,

Table 1 Subject Anthropometrics, Nutritional, and Performance Characteristics

	Creatine (n = 9)	Placebo (n = 9)	P
Age (yr)	23.9 ± 5.5	24.3 ± 4.9	.86
Height (cm)	176 ± 4.0	178 ± 6.0	.54
Body mass (kg)	74.2 ± 3.7	74.5 ± 6.7	.90
Biceps curl 1-RM (kg)	42.7 ± 7.1	38.3 ± 4.4	.25
CHO (g)	300.8 ± 86.8	267.5 ± 88.7	.43
LIP (g)	65.7 ± 20.0	74.3 ± 28.7	.47
PTN (g)	121.0 ± 29.3	118.4 ± 48.4	.89
Total energy (kcal)	2279 ± 485	2213 ± 576	.79
PTN/Body Mass (g/kg)	1.64 ± 0.42	1.64 ± 0.78	.99

Note. CHO—carbohydrate; LIP—lipids; PTN—protein.

Brazil) four times per day, and subjects in the Placebo group ingested 10 g of dextrosol four times per day. Analyses from three independent laboratories confirmed that the creatine supplements were 99.9% pure, with no microbiological contaminants (Carvalho et al., 2011).

Food Intake Assessment

Food intake was assessed by three 24-hr dietary recalls undertaken on separate days (2 weekdays and 1 weekend day) using a visual aid photo album of real foods. Energy and macronutrient intake were analyzed by the Brazilian software Virtual Nutri Plus (Neves et al., 2011).

Range of Motion

Elbow joint angles were measured using a plastic goniometer while the subject was standing with the arm initially relaxed by his side. The elbow joint angles were measured when the subject attempted to fully extend his elbow joint (extended angle) and when the subject fully flexed his elbow joint in an attempt to touch his shoulder with his palm (flexed angle). Two measurements were taken for each angle, and the range of motion (ROM) was determined by deducting the flexed angle from the extended angle using the mean value of the two measures (ICC, r = .98). Landmarks used to measure the elbow joint angles were the lateral epicondyle of the humerus, the palpated distal end of the deltoid muscle, the midpoint between the styloid processes of the ulna and radius, and the styloid process of the radius. These sites were marked on the skin with a semipermanent ink marker to obtain consistent measures (Chen et al., 2007).

Muscle Soreness

Muscle soreness was assessed using a 100 mm visual analog scale (VAS) where the subject was instructed that 0 indicated no pain and 100 mm was an indication of unbearable pain. Subjects rated the soreness on the 100 mm line when the investigator palpated the elbow flexors (biceps brachii at 9–11 cm above the elbow crease). The same investigator conducted the muscle soreness assessment throughout the study, and the protocol was standardized such that the same pressure was applied to the sites using the tips of three fingers with a uniform circular movement while the subject's arm was placed on a table. The mean of the value of the two arms was used for further analysis.

Blood Collection and Analysis

Subjects provided blood samples following an 8-hr overnight fast before each exercise bout and 48 and 96 hr following each bout. Blood samples were taken from subjects in a seated position from an antecubital vein into plain evacuated tubes. Twenty minutes following collection, blood samples were centrifuged at 1600 g for 20 min. The serum was removed and the serum CK activity was analyzed with an enzymatic method at 37°C (CK-UV NAC-optimized; Biodiagnostica, Brazil) in a Cobas Mira Plus analyzer (Roche, Basel, Switzerland). The CK analyses were made in triplicate (ICC, r = .99).

Statistical Analysis

To determine the sample size, we used previously reported differences in serum CK activity before and 48 hr after a resistance exercise session (Machado et al., 2009). We calculated that nine subjects were needed to detect this association with a 2-tailed α = .05 and 1-b = 0.73 (Dupont & Plummer, 1990). The reliability of the CK assessments, ROM, and 1-RM loads were assessed with the intraclass correlation coefficient (ICC). Baseline dietary intake, anthropometric data, and 1-RM strength differences between the Creatine and Placebo groups were compared using unpaired t tests. In addition, change in body mass between groups throughout the study was assessed with a 2 × 11 (Group × Time) repeated measures analysis of variance (ANOVA). The volume completed

(Load × Repetitions) for each set was compared using a 2 × 2 × 4 (Group × Bout × Sets) repeated-measures ANOVA. Differences in serum CK activity between groups were compared using a 2 × 2 × 3 (Group × Bout × Time) repeated-measures ANOVA, and differences in muscle soreness and ROM were compared using 2 × 2 × 6 (Group × Bout × Time) repeated-measures ANOVA. The alpha level was set at $p < .05$ for all analyses. Significant main effects were further analyzed using pairwise comparisons with Bonferroni post hoc tests. Statistical analysis was completed using SPSS v17.0 for Windows (LEAD Technologies).

Results

Subject Characteristics

There was no difference in height, body mass, or 1-RM at baseline between the Creatine and Placebo, and nutrient intake did not differ between groups (all $p > .05$). There was a significant group x time interaction for body mass during the duration of the study ($p < .001$). Creatine increased body mass from baseline to postsupplementation (+1.9 kg), while Placebo's body mass remained stable (Figure 1). Body mass in Creatine remained elevated D13-D19.

Volume Load

Creatine lifted a greater total volume than Placebo at baseline ($p = .04$), however, there was no significant Group × Bout or Group × Bout × Set interactions ($p > .05$). Total volume lifted increased from the first to the second exercise bout in both groups, with an average increase of ~27% ($p = .006$, Table 2).

Creatine Kinase Activity

Serum CK activity increased 48 and 96 hr after the first exercise bout in both groups (time effect, p £ .001 for both), and there was no difference in the pattern of CK

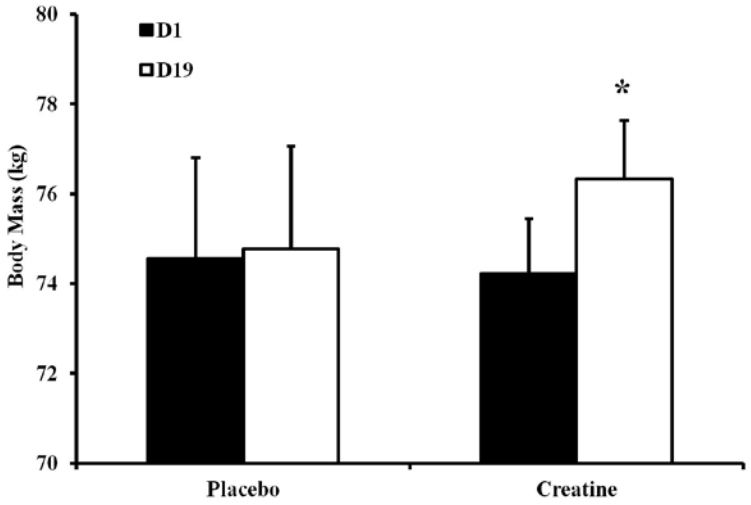

Figure 1 — Body mass significantly increased from baseline (D1) following supplementation in the Creatine group only (*$p <$ .05 for D13–19).

Table 2 Volume (kg; Load × Repetitions)

	Creatine (n = 8)		Placebo (n = 8)	
Sets	**First Bout**	**Second Bout**	**First Bout**	**Second Bout**
First	327 ± 83	341 ± 118	275 ± 46	298 ± 45
Second	191 ± 59	279 ± 93[a]	149 ± 19	251 ± 31[a]
Third	129 ± 39	174 ± 58[a]	98 ± 34	154 ± 26[a]
Fourth	85 ± 25	105 ± 34[a]	72 ± 21	86 ± 23[a]
Total	732 ± 178	899 ± 300*	593 ± 59	790 ± 102*

[a]Difference from first set ($p < .05$).

*Indicates significant increase in total volume from first to second bout.

activity between groups (Group × Time effect, $p = .96$). Compared with the first bout, both groups displayed reduced CK activity 48 hr after the second RE bout ($p < .001$). After the second exercise bout, the pattern of CK activity was significantly different between Creatine and Placebo (Group × Time effect, $p < .001$), with PLACEBO displaying higher CK activity than Creatine on D17 (+343% vs. +110%, respectively; Figure 2).

Muscle Soreness

Muscle soreness was significantly increased in both groups until D4 following the first exercise bout when compared with D1 post (time effect, $p < .001$), with no group differences through D5 (Group × Time effect, $p = .96$). Both groups significantly decreased soreness following the second exercise bout when compared with the first bout ($p < .01$), with soreness increased only at D17 in the Placebo group ($p = .02$). Creatine had a greater decrease in soreness from the first to second bout when compared with Placebo (average 75% vs. 56% decrease; $p < .05$; Figure 3).

Range of Motion

Range of motion at the right and left elbow was decreased in both groups after the first exercise bout (time effect, $p < .001$), with no difference between groups between D1 and D5 (right arm Group × Time effect, $p = .85$; left arm Group × time effect, $p = .58$), nor between D15 and D19 (right arm Group × Time effect, $p = .82$; left arm Group × Time effect, $p = .50$, Figure 4). Both groups increased right and left elbow ROM following the second bout compared with the first bout ($p < .01$). Although there was no Group × Time interaction, only Placebo decreased right elbow ROM from D15 to D16 ($p = .02$) and displayed

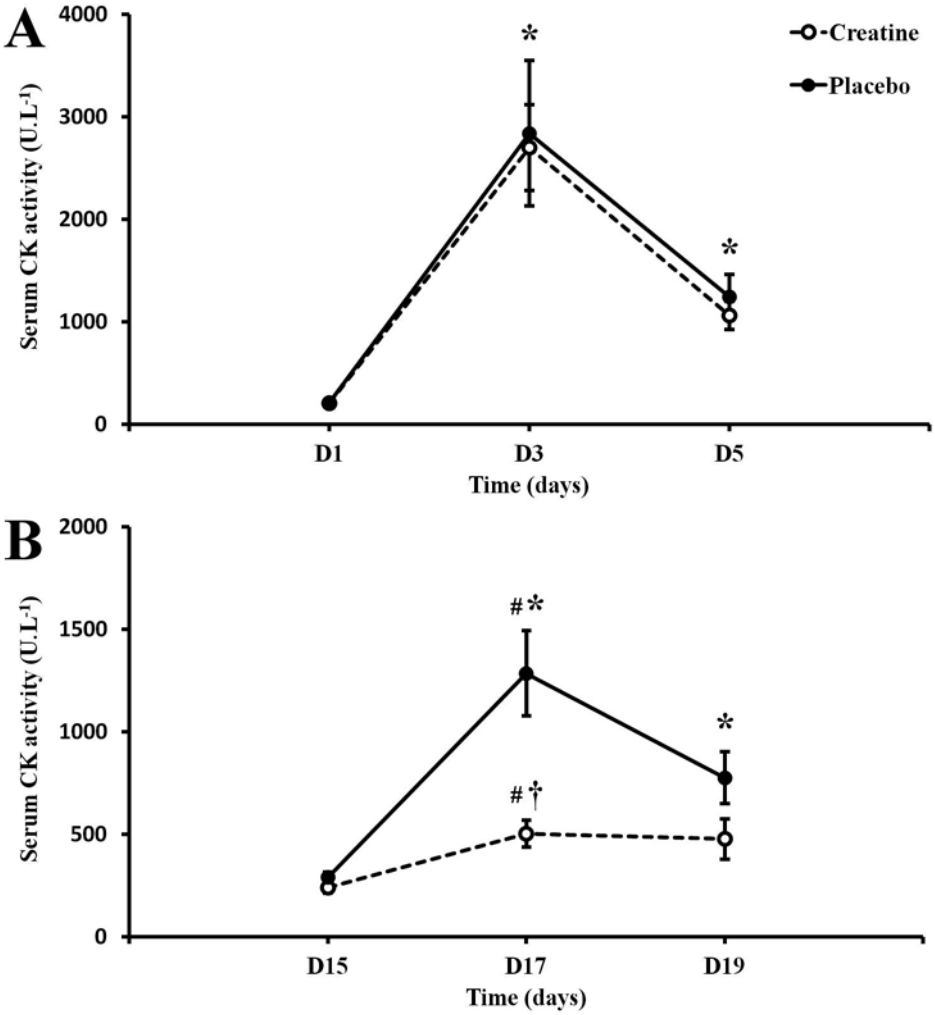

Figure 2 — Serum CK increased 48 and 96 hr following the first RE bout (A.) in both groups, but increased only in the Placebo group 48 hr following the second bout (B.; *$p < .01$). Both groups displayed an attenuated CK response to RE 48 hr after the second RE bout compared with the first bout (#$p < .001$), with the Creatine group showing significantly lower CK activity at this time point compared with Placebo (†$p < .001$).

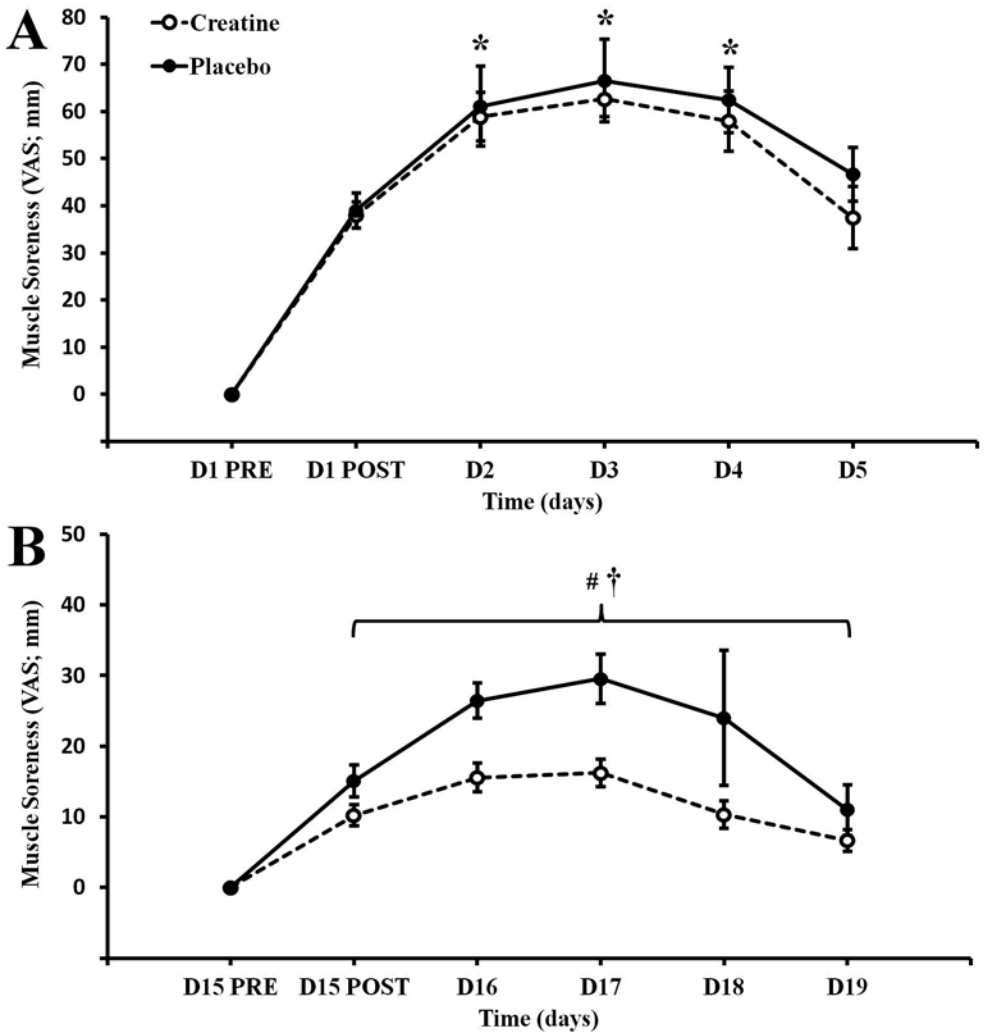

Figure 3 — *Muscle soreness increased following the first RE (A.) bout in both groups ($p < .01$), but only in the Placebo group 48 hr after the second bout (B.; D17, $p = .02$). Both groups had attenuated muscle soreness following the second bout compared with the first (#$p < .001$), but the Creatine group showed greater attenuation of soreness (†$p < .05$).

a trend toward decreasing left elbow ROM from D15 to D16 ($p = .06$). Creatine had no change at this time.

Discussion

Creatine supplementation has an additive effect on reducing muscle damage in response to repeated bouts of exercise. Both Creatine and Placebo reduced markers of muscle damage (e.g., serum CK, ROM, and muscle soreness) following a second bout of exercise, with Creatine showing significantly greater reduction in these markers. Although others have demonstrated conflicting evidence on whether creatine supplementation enhances recovery from acute muscle damage (Bassit et al., 2008; Bassit et al., 2010; Cooke et al., 2009; Machado et al., 2009; Rahimi, 2011; Rawson et al., 2007; Rawson et al., 2001; Rosene et al., 2009; Santos et al., 2004; Warren et al., 2000), we are the first to show that creatine supplementation exerts beneficial effects on reducing markers of muscle damage following repeated bouts of damaging exercise.

Creatine supplementation enhanced the RBE as assessed by decreased markers of muscle damage, but the precise mechanisms which provided this effect remain unknown. It is likely that several mechanisms could have played a role. Creatine supplementation increases intramuscular phosphocreatine content, which may stabilize the sarcolemma (Saks & Strumia, 1993). In addition, creatine may have reduced inflammation. In addition, Rahimi (Rahimi, 2011), recently demonstrated creatine's role as a free radical scavenger, showing that creatine supplementation reduced markers of oxidative DNA damage and lipid peroxidation after resistance exercise relative to a placebo.

Creatine supplementation can also alter cellular metabolism via increased intramuscular phosphocreatine (Harris et al., 1992; Hultman et al., 1996) and glycogen

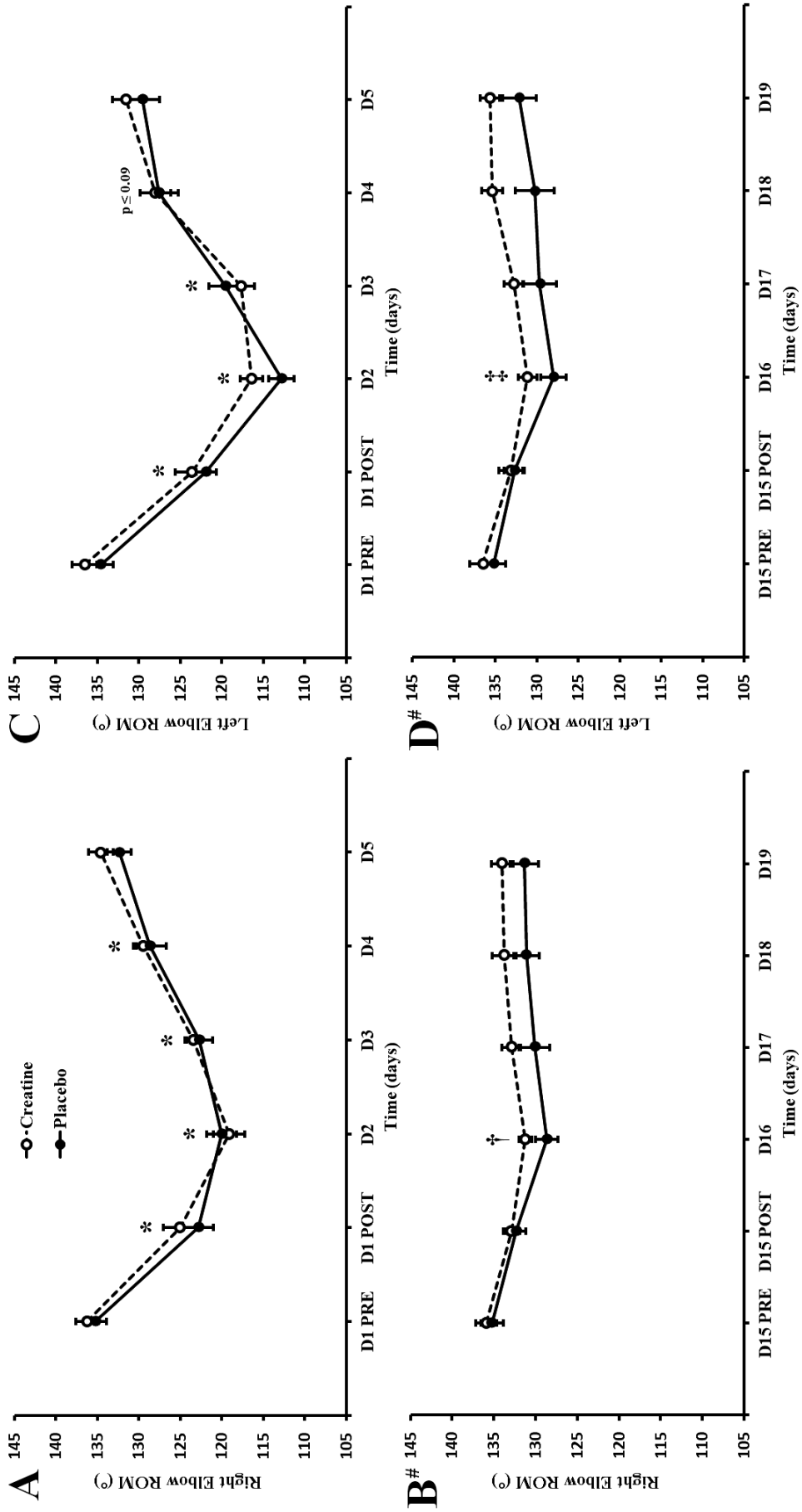

Figure 4 — *ROM decreased following the first RE bout in both groups (A., C.; $*p < .05$), but only decreased in the Placebo group 24 hr after the second bout (B., D.; $\dagger p = .02$; ‡trend toward decrease, $p = .06$). An overall increase in ROM was shown following the second RE bout when compared with the first ($\#p < .01$).

(Nelson et al., 2001; Robinson et al., 1999; van Loon et al., 2004) content. Increased intramuscular phosphocreatine and glycogen content can facilitate ATP homeostasis during times of stress, providing energy for efficient Ca^{2+}/ATPase pump activity in an effort to reduce Ca^{2+} influx and therefore reduce subsequent damage induced by increased intracellular Ca^{2+} (Minajeva et al., 1996). Though the aforementioned mechanisms are likely key players by which creatine supplementation may reduce muscle damage, several previous studies did not show any beneficial effect of creatine supplementation on muscle damage (Machado et al., 2009; Rawson et al., 2007; Rawson et al., 2001; Warren et al., 2000). Given the current findings, it is possible that these beneficial effects of enhanced creatine and phosphocreatine stores are relatively small, and are insufficient to attenuate robust muscle damage following a single bout of resistance exercise. However, when combined with a moderately damaging exercise protocol and the RBE, the effects of increased creatine and phosphocreatine content may have become apparent as seen by the attenuated rise of muscle damage markers after a second resistance bout.

Several studies have found creatine supplementation reduces postexercise markers of inflammation (Bassit et al., 2008; Santos et al., 2004). Specifically, creatine supplementation has been linked to lesser increases in TNF- α and PGE-2 (Bassit et al., 2008; Santos et al., 2004) and IL-1β and INF-α (Bassit et al., 2008) after long distance running. Thus it is possible that creatine supplementation may have reduced the extent of inflammation experienced during the exercise bout on D15, leading to the reduced CK, ROM, and muscle soreness.

Given the timing of creatine supplementation in the current study (7 d following the first exercise bout), it is unlikely that increased intramuscular creatine and PCr provided any immediate increases in protein synthesis following the first exercise bout. However, creatine supplementation has been shown to increase gene transcription and mRNA translation of several gene networks (e.g., cytoskeletal remodeling, protein and glycogen synthesis regulation, etc.; Safdar et al., 2008). It is possible that creatine supplementation could have promoted an increase in mRNA translation (i.e., protein synthesis) between the first and second exercise bouts which may have enhanced damage repair, particularly if cytoskeletal/myofibrillar mRNAs were selectively translated due to the first stimulus. The increase in body mass in Creatine vs. Placebo observed between D1 and D15 lends some support to this speculation, but we cannot determine if this increased body mass was caused by increases in protein content or intramuscular glycogen/water content.

Creatine supplementation enhances satellite cell myogenesis in vitro (Vierck et al., 2003) and enhances resistance training-induced increases in satellite cell and myonuclear addition in vivo (Olsen et al., 2006). Increased satellite cell number has been found up to 8 days after a single bout of damaging resistance exercise (Crameri et al., 2004), and it is likely that the damage protocol used in the current study produced similar increases in satellite cell number at this time. Satellite cells of Creatine subjects may have had a more robust differentiation capacity to contribute to myonuclear addition and generation of new fibers following the first exercise bout; thus enhancing myofiber repair in preparation for the following bout.

In conclusion, we have shown that creatine supplementation further reduced the presence of muscle damage markers (CK, ROM, muscle soreness) after a repeated bout of damaging resistance exercise, thereby enhancing the RBE. While the mechanisms leading to this response are unknown, sarcolemmal stabilization, reduced oxidative stress, reduced Ca^{2+} influx, and enhanced satellite cell activation/myogenesis are possibilities. The potential application of these results would be using creatine supplementation to enhance recovery between exercise sessions in athletes, particularly in training cycles where the athlete is adopting a new, unfamiliar exercise. Further experiments, employing trained subjects studied over longer training periods, are needed to verify the practicality of these results.

Acknowledgments

The authors would like to thank Pierre Augusto Silva for supplements preparation and the subjects for their participation in this study.

Conflict of Interest

There are no conflicts of interest among any of the authors of this manuscript. No external funding was received to develop this study. Study conducted at the Universidade Iguaçu (UNIG) Campus V.

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