Resistance exercise may cause muscle microdamage and caffeine may enhance this risk. The objective of this study was to evaluate the effect of caffeine supplementation on muscle injury markers in soccer players after resistance exercise. Fifteen male soccer players (18–20 years old) completed a placebo-controlled double blind (crossover design) study. The exercises were bench press, pullover, biceps curl, triceps curl, leg extension, and lying leg curls. Volume and intensity were 3 × 10 at 10RM each with a 2-minute rest interval controlled by digital sound signal. Blood samples were collected before and 24 hours after exercise to measure the levels of serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Caffeine or placebo was given in a randomized manner. The Wilcoxon test was used to compare treatment effects ($p < 0.05$). Resistance exercise caused an increase in all skeletal muscle markers. In caffeine session, serum CK level increased by 53.3%, LDH by 53.8%, AST by 33.1%, and ALT by 38.1%. In placebo session, the corresponding values were 65.2%, 48.2%, 38.8%, and 38.3%, respectively. There were no significant differences between the caffeine and placebo sessions. Caffeine supplementation ($\sim 4.5 \text{ mg} \cdot \text{kg}^{-1}$) did not affect muscle markers induced by exercise and did not augment the risk of muscle lesion.

**Keywords:** caffeine, creatine kinase, exercise, lactate dehydrogenase, transaminase

### Introduction

Caffeine is the most widely used psychoactive substance on earth. The general consensus of research findings indicates that caffeine (1,3,7-trimethylxanthine) has ergogenic effects by acting as a fatigue delayer and improving the contractile strength of cardiac and skeletal muscle (Stephenson 2008; Foad et al. 2008; Del Coso et al. 2008; De Hon & Courmans 2007; Armstrong et al. 2007; Schneiker et al. 2006; Avois et al. 2006). Caffeine also decreases muscular pain perception, effort perception, and the reaction time to a stimulus (Kalmar & Cafarelli 2004; Davis et al. 2003; Motl et al. 2003; Kruk et al. 2001). The effects of caffeine have been shown to be mediated by antagonism of adenosine receptors and inhibition of cyclic adenosine monophosphate–phosphodiesterase (cAMP–PDE), with some increased concentrations of intracellular cAMP and subsequent activation of the protein kinase A (PKA) pathway (Horrigan et al. 2006). Its use together with exercise activates both the hypothalamic-pituitary-adrenal axis and the autonomic nervous system, stimulating catecholamine and cortisol release (Bishop et al. 2005).

Resistance training can improve strength, hypertrophy, muscular power, muscular endurance, and health status. The training program can be manipulated to reach training goals and address individual goals (Kraemer & Ratamess 2004). It has been suggested that resistance exercise may cause muscle cell membrane disruption. This may be a consequence of both metabolic and mechanical causes. Indeed, exhausted muscle fibers exhibit increased membrane permeability following an increase
in internal free calcium ions, which promotes the opening of potassium channels and activation of proteolytic enzymes such as calpains and caspases (Branca et al. 2007; Nosaka et al. 2002; Clarkson & Hubal 2002). Exercise-induced muscle microinjury leads to cellular damage with membrane disruption and leakage to extracellular fluid and plasma. Creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been used extensively as markers for muscle microinjury (Pettersson et al. 2007; Mougios 2007; Chevion et al. 2003).

Bassini-Cameron et al. (2007) described synergistic effect of exercise and caffeine on muscle injury markers during stress conditions. Their subjects were submitted to an exercise protocol that simulated a soccer match, and blood specimens were collected immediately afterwards. Because many studies (Pettersson et al. 2007; Bishop et al. 2005; Nosaka et al. 2002) displayed a significant increase in the levels of muscle injury markers at 24–72 hours after exercise, data from Bassini-Cameron et al. (2007) were insufficient (but not negligible) and additional studies were necessary to verify caffeine’s hypothetical synergistic effect on exercise-induced microdamage.

Caffeine and caffeine-based substances have been increasingly used as ergogenic supplements by recreational and professional physical activity practitioners, but its effects on exercise-induced microdamage are still obscure. In the present study, we evaluated the effects of caffeine supplementation on resistance exercise-induced microdamage through serum analysis of muscle injury markers after resistance exercise.

Methods

Subjects

Soccer players (n = 15) participated in this investigation after giving verbal and written informed consent in accordance with federal and institutional guidelines. Their mean age, weight, height and VO2\textsubscript{max} were 18.4 ± 0.8 years, 71.8 ± 7.1 kg, 177.5 ± 4.6 cm, and 59.4 ± 13.3 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, respectively. They were nonsmokers, and used no medicinal drugs, dietary supplements, or anabolic steroids. The group was characterized by a similar lifestyle and had been closely monitored for 8 weeks during a pre-season and preparatory period, yielding a high degree of reproducibility within the group. The subjects were light caffeine consumers (<100 mg·d\textsuperscript{-1}), and all of them had previously participated in training sessions involving resistance exercise.

Experimental protocol

A double-blind, placebo-controlled experimental design was used, with all subjects serving as their own controls. Each subject performed two experimental trials separated by 2 weeks. No caffeine, xanthines, or other substance that could mask the results were ingested by the athletes for 12 hours before 10RM test and each blood collection. Ten minutes of warm up (joint mobilization and stretching) was carried out 35 minutes after receiving caffeine or placebo supplement.

Caffeine or placebo supplementation

In an individual randomized manner, caffeine (Purifarma, China) or lactose (Galena, Germany) was given to the group. Caffeine was given at a dose of 4.5 mg·kg\textsuperscript{-1}. This dose was chosen because it is within the supplementation range (3.0–9.0 mg·kg\textsuperscript{-1} body weight 30–60 minutes before exercise) shown to improve athletes’ performance (Graham 2001). For the control, subjects received one capsule of 500 mg lactose each (Bassini-Cameron et al. 2007). The supplements were ingested immediately after blood sample collection. The different supplements were indistinguishable so that the subjects did not know whether they were ingesting caffeine or lactose.

Test protocol

On the first day, subjects performed a 10RM test for each exercise. The 10RM tests were assessed in the same sequence of exercise sessions: bench press, pullover, military press, biceps curl, triceps curl, leg extension, and lying leg curls. Standard exercise techniques were followed for each exercise (NSCA 2008). To minimize possible errors in the 10RM tests, the following strategies were adopted: (1) subjects received standard instructions on the general routine of data assessment and the exercise technique for each exercise before testing; (2) the exercise technique of subjects during all test sessions was monitored and corrected as needed; and (3) subjects received verbal encouragement during the exercise sessions.

Two weeks after the above 10RM evaluation, resistance exercise session 1 (ES1) was assessed according to the same sequence as above. All exercises were performed for 3 sets of 10 repetitions at the predetermined individual load (10RM) for each exercise. Exercises were performed with a digital sound signal (Beat Test & Training, CEFISE, Brazil), which was adjusted to allow 2 seconds for each complete repetition and to have a 2-minute rest interval between sets. Exercise session 2 (ES2) took place 2 weeks after ES1, and the protocol was identical in both sessions.
Due to variations in exercise equipment design, we chose to describe the exercise force as arbitrary units (AU). This procedure was utilized in an attempt to simplify matters for research purposes. Subjects were allowed water *ad libitum* throughout their sessions.

**Data collection**

Venous blood samples were collected from the forearm with subjects in a seated position. The first sample (PRE) was collected before supplementation and the other sample (POST) was collected 24 hours after the first. Samples were centrifuged at 1500g for 10 minutes, the serum was separated and quickly frozen and stored at −70°C. From the serum samples, CK, LDH, ALT and AST were measured on an automated analyzer (Cobas Mira S Plus, Roche) with specific commercial kits (Biotécnica, Brazil).

**Statistical analysis**

The Kolmogorov-Smirnov test revealed that none of the variables studied were normally distributed. Thus, the nonparametric Wilcoxon test was applied to compare differences between trials. Significant differences were set at *p* < 0.05. Data are expressed as mean ± standard deviation and median (range).

The enzymes (LDH, AST, ALT) are generally taken to be clinically diagnostic of muscle and hepatocellular damage. Serum CK level seems to be the best indicator of exercise microdamage (Lazarim et al. 2008; Chevion et al. 2003). Therefore, serum kinetic changes in LDH, AST and ALT were used to verify CK changes. To examine the relationship between the effects of exercise and caffeine relative to enzyme kinetic changes, bivariate linear regression was performed. Individual kinetic coefficients (b[1]) between treatments were compared using the Wilcoxon test (*p* < 0.05).

**Results**

The 10RM performances of the subjects are shown in Table 1. All serum enzyme levels were significantly higher for POST compared with PRE (*p* < 0.05) in both sessions (Table 2). In the caffeine trial session, serum CK level increased by 53.3%, LDH by 53.8%, AST by 35.1%, and ALT by 38.1%, while in the placebo trial session, the corresponding values were 65.2%, 48.2%, 38.8%, and 38.3%, respectively.

Serum LDH, AST and ALT levels were plotted as a function of serum CK level (Figures 1–3). All analyses displayed a significant increase in serum enzyme levels (*p* < 0.05). No differences were found between angular coefficient by linear regression analysis between PRE and POST.

**Table 1. Subjects’ 10RM performance**

<table>
<thead>
<tr>
<th>Enzyme (AU)</th>
<th>Placebo</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Bench press</td>
<td>31.6 ± 9.9</td>
<td>35 (9–50)</td>
</tr>
<tr>
<td>Pullover</td>
<td>14.1 ± 4.8</td>
<td>12 (6–23)</td>
</tr>
<tr>
<td>Biceps curl</td>
<td>20.9 ± 5.8</td>
<td>21 (10–50)</td>
</tr>
<tr>
<td>Triceps curl</td>
<td>16.7 ± 5.6</td>
<td>18 (7–23)</td>
</tr>
<tr>
<td>Leg extension</td>
<td>48.5 ± 6.5</td>
<td>49 (35–62)</td>
</tr>
<tr>
<td>Lying leg curl</td>
<td>33.7 ± 8.8</td>
<td>31 (23–55)</td>
</tr>
</tbody>
</table>

*POST > PRE, *p* < 0.05. SD = standard deviation.

**Table 2. Serum enzyme levels before (PRE) and 24 hours after (POST) resistance exercise sessions, with a 2-week interval between caffeine and placebo supplementation**

<table>
<thead>
<tr>
<th>Enzyme (U·L⁻¹)</th>
<th>Placebo</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>CK Mean ± SD</td>
<td>412 ± 168</td>
<td>680 ± 259*</td>
</tr>
<tr>
<td>Median (range)</td>
<td>405 (170–649)</td>
<td>634 (364–1300)</td>
</tr>
<tr>
<td>LDH Mean ± SD</td>
<td>385 ± 54</td>
<td>570 ± 97*</td>
</tr>
<tr>
<td>Median (range)</td>
<td>368 (267–487)</td>
<td>540 (440–761)</td>
</tr>
<tr>
<td>AST Mean ± SD</td>
<td>23 ± 3</td>
<td>32 ± 78*</td>
</tr>
<tr>
<td>Median (range)</td>
<td>22 (18–28)</td>
<td>30 (26–49)</td>
</tr>
<tr>
<td>ALT Mean ± SD</td>
<td>20 ± 5</td>
<td>28 ± 4*</td>
</tr>
<tr>
<td>Median (range)</td>
<td>20 (10–30)</td>
<td>28 (20–36)</td>
</tr>
</tbody>
</table>

*POST > PRE, *p* < 0.05. SD = standard deviation.
The increase in serum enzyme levels suggest that the exercise protocol used in this study resulted in skeletal muscle injury. Mayhew et al. (2005) reported direct evidence of structural damage to muscle after a resistance exercise protocol, and this was associated with a significant increase in serum CK level post exercise. The efflux of these enzymes from muscle may occur as a result of increases in the permeability of myo-cellular membrane and/or intramuscular vasculature transient reorganization (Brancaccio et al. 2007; Peake et al. 2005).

The exercise protocol used in the present study induced an increase in serum CK level that was in the range proposed for athletes in other studies (Mougios 2007; Lazarim et al. 2008; Brancaccio et al. 2007). The results of this study demonstrated that serum CK level peaked at around 540 U/L 24 hours after the exercise sessions, and serum LDH reached a maximum of around 580 U/L. The time course for changes in these markers of muscle damage may also be dependent on exercise protocol and/or training status.

ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis, but physical exercise is known to cause transient elevations in serum transaminase activity (Pettersson et al. 2007; Chevion et al. 2003). In fact, total serum AST and ALT represents muscle and hepatic enzyme traffic into the circulation, and Pettersson et al. (2007) warned about imposing relevant restrictions on exercise prior to and during drug clinical studies. Linear regression analyses displayed a very close comportment (i.e. no significant differences were found in the angular coefficient) between LDH, AST and ALT against CK. These data indicate that the increases in the enzymes measured in this study resulted from muscle injury, in accordance with the results of previous studies (Bessa et al. 2008; Pettersson et al. 2007; Chevion et al. 2003).

Our results did not show any synergistic effect of caffeine and exercise on muscle injury. In contrast to data from the current study are data presented by...
Bassini-Cameron et al. (2007), which demonstrated a synergistic effect of caffeine and exercise, exposing athletes to risk of muscle injury. In their work, following a simulated soccer match, subjects performed a very intense exercise (the Yo-Yo test). If it is true that caffeine plays a role in delaying fatigue, then the caffeine group should perform better on the Yo-Yo test. Regrettably, the Yo-Yo performance data are not available in the Bassini-Cameron et al. (2007) paper. They justified the greatest increase in serum enzyme activity by muscular mechanical stress in caffeine-supplemented subjects. In our study, exercise performance was not different because the exercises were controlled by identical load, cadence and rest interval in both trials for all subjects (i.e. crossover experimental design). The two sessions (caffeine and placebo) were executed in the same conditions.

The window of peak enzyme activity described is 24–72 hours after exercise (Pettersson et al. 2007). Bassini-Cameron and colleagues (2007) reported a limitation in their study—blood samples were collected immediately after exercise—to explain the little increase in enzyme levels. Pettersson et al. (2007) reported that great interindividual variation in enzyme levels in the initial hours after exercise sessions was low after 24–72 hours. We collected the blood samples 24 hours after exercise and we used a crossover design to verify the effect of caffeine supplementation. This means that our results are within the window of peak enzyme activity, and each subject acts as his own control.

Data from the current study can be applied to resistance trained athletes undergoing resistance training. Caffeine supplementation did not increase muscle damage from a stressful trial of resistance exercise. Clinicians, researchers, strength and conditioning professionals and athletes should recognize that few studies have the statistical power to detect severe adverse events. Our results cannot be generalized to athletes who ingest caffeine for extended periods, to those who ingest caffeine at doses above what is recommended, or to athletes who are engaged in resistance training with an exaggerated eccentric component or plyometrics. Caffeine dose of ~4.5 mg·kg⁻¹ is proposed as an ergogenic aid and does not increase the risk to muscle integrity. Other collateral effects are described and our data suggest that the use of caffeine can be safe.

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References


