

## Caffeine Supplementation and muscle damage in soccer players

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The aim of this work was to evaluate the effect of caffeine supplementation and intermittent exercise on the muscle injury markers in soccer players. 15 male professional soccer players completed a placebo controlled double blind test protocol. 45 minutes before exercise, participants ingested 5.5 mg.kg<sup>-1</sup> body mass of caffeine (EXP, n=8) or placebo (CONT, n=7). The exercise was 12 sets of 10 sprints (20 m each) with 10 sec recovery time between sprints and 2 min between sets. Blood samples were collected before (PRE) and 48h after exercise (POST). Serum activity of CK, LDH, AST, and ALT were quantified. Serum enzyme activity was enhanced by exercise in both groups, without a synergistic effect of caffeine. The findings suggest muscle injury markers concentration increases after physical activities, but caffeine supplementation (as used in this study) has no influence upon muscle cellular integrity.

**Uniterms:** Muscle injury/markers. Transaminases. Physical exercise. Caffeine/supplementation. Soccer. Creatine kinase. Lactate dehydrogenase.

O objetivo do trabalho foi avaliar o efeito da cafeína e do exercício intermitente nos marcadores de lesão muscular em jogadores de futebol. 15 jogadores de futebol profissional completaram um estudo duplo-cego placebo controlado. 45 minutos antes do exercício, os participantes ingeriram 5.5 mg.kg<sup>-1</sup> do peso corporal de cafeína (EXP, n=8) ou placebo (CONT, n=7). O exercício consistiu em 12 séries de 10 sprints (com 20 m cada) com 10 segundos de recuperação entre os sprints e 2 min entre as séries. Amostras de sangue foram coletadas antes (PRE) e 48h depois do exercício (POST). As atividades séricas de CK, LDH, AST e ALT foram quantificadas. A atividade sérica de todas as enzimas aumentou em ambos os grupos, sem efeito sinérgico da suplementação de cafeína. Os achados confirmam que o exercício aumenta a atividade sérica das enzimas, mas a cafeína (como a usada neste estudo) não interfere na integridade da fibra muscular.

**Unitermos:** Lesão muscular/marcadores. Transaminases. Exercício físico. Cafeína/suplementação. Futebol. Creatina quinase. Lactato desidrogenase.

### INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is the most widely used behaviorally active substance on Earth. It was removed from the list of prohibited substances of the World Anti-Doping Agency (WADA) in 2004 and has been increasingly used as ergogenic supplement by soccer players (De Hon, Coumans, 2007). Supplementation with caffeine is also known to decrease muscular pain perception, effort perception, and the neuromuscular reaction

time which can further facilitate exercise performance (Graham, 2001; Altamari *et al.*, 2006).

Exercise Induced Micro-Injuries (EIMI) are described as muscle twitch, membrane disruption and subsequently inflammatory process after exercise session. This damage is evident by disruption of the normal banding patterns (i.e., alignment) of skeletal muscle and the broadening or complete disruption of sarcomere Z lines. EIMI increases serum activity of enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) (and others) which have been used as markers of muscle damage (Chevion *et al.*, 2003; Pettersson *et al.*, 2007; Brancaccio *et al.*, 2007).

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Bassini-Cameron *et al.* (2007) have described synergic effect of exercise and caffeine in soccer players muscle injury markers during stress conditions. In this study the soccer players were submitted to an exercise that simulates a soccer match and the blood specimens were collected immediately after exercise. Studies support that caffeine ingestion has a large effect on reducing leg-muscle pain during high-intensity exercise (Motl *et al.*, 2003; O'Connor *et al.*, 2004). Motl *et al.* (2003) have proposed that caffeine ergogenic effect is modulated by pain reduction. The hypoalgesic effect with effort perception altered could be the cause of a higher EIMI. In other hand, Pettersson *et al.* (2007) displayed a significant rise in muscle injury markers at 24-72 h after exercise, data blood samples obtained immediately after exercise are insufficient and additional measures are necessary to verify hypothetical caffeine synergic effect on EIMI.

Therefore, the purpose of this study was to examine in soccer players the effect of caffeine supplementation on muscle injury markers after intermittent exercise.

## METHODS

### Subjects

The study group included 15 soccer players (19±1 years old; 177±4 cm height; and 72±2 kg weight), healthy, non-smokers, who used no drugs, dietary supplements, or anabolic steroids, and participated voluntarily. The group was characterized by a similar lifestyle, yielding a high degree of reproducibility within the group. The subjects reported low daily caffeine consumption (i.e. <100 mg.d<sup>-1</sup>) and no hypersensitivity to caffeine. Written informed consent was obtained from the subjects, who were instructed as to the nature and procedures of the study.

### Experimental protocol

In a randomized double-blind, placebo-controlled design, the subjects were divided into 2 groups: experimental (EXP; n = 8) and control (CONT; n = 7). No caffeine, xanthines, or other substance that could mask the results were ingested by the athletes for 12 hours before blood collection. A morning blood specimen was collected 1h (PRE) after a standardized breakfast consisting of: bread (~50g), Minas' cheese (~20g) and skimmed milk (200 ml). Ten minutes of warm up (jogging, joint mobilization and stretching) were carried out 35 minutes after receiving the supplement (see below). The experimental conditions were in accordance with the norms of the BRAZILIAN NATIONAL HEALTH COUNCIL, under RESOLUTION

No. 196, promulgated in October 1996, referring to scientific research on human subjects.

### Diet supplementation

The different supplements were in indistinguishable capsules so that the subjects were not aware of the substance they were ingesting. Caffeine (*Jilin Shulan*, China) was given to the group EXP at a dose of 5.5 mg.kg<sup>-1</sup> in one 500 mg capsule, which also contained enough cellulose to fill the capsule (*Gujarat Microwax*, India). This dose was chosen because it is within the supplementation range shown (3.0-9.0 mg.kg<sup>-1</sup> body weight at 30-60 min prior exercise ingestion) to improve athletes' performance (Graham, 2001). The control group (CONT) received one capsule with 500 mg cellulose only. The supplements were ingested immediately after the blood sample collection.

### Test protocol

All subjects ran 12 sets of 10 maximum sprints at 20 meters each with 10 seconds passive recovery between each sprint and two minutes active recovery (walking) between sets. Between set 6 and set 7 the athletes rest for 15 minutes. The intensity of exercise was controlled by coaches in accordance with previous diagnostic tests (Yoyo Endurance Test L1). The athletes were allowed to ingest water *ad libitum* throughout their sprints.

### Data collection

Venous blood samples were collected from the forearm while the subjects were in a seated position. The first sample (PRE) was collected in the morning and another sample (POST) 48h after. After collection, the blood samples were divided in two tubes (one heparinized tube for hematological measures and the other was centrifuged for serum separation). Serum was quickly frozen and stored at -70°C. From each heparinized sample the following hematological measures were obtained; Hematocrit, Erythrocytes count, and Hemoglobin. From serum samples, creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). An enzymatic method at 37° C was used for enzymes activity analysis with commercial kits (BioTécnica - Brazil) in Cobas Mira Plus analyzer (Roche - Germany).

### Statistical analyses

Because the normal distribution was not verified,

the non-parametric Wilcoxon test for related samples was applied to compare PRE and POST values and Friedman test was used for compare EXP with CONT groups. Significant differences were set at  $P < 0.05$ .

## RESULTS

Table I summarizes the impact of exercise on serum enzymes activity. Initial CK, LDH, AST, and ALT values were above population values; which are most likely because all subjects were athletes actively involved in soccer training regimes. The serum concentrations of all enzymes increased significantly after exercise protocol ( $P < 0.05$ ). However, no significant differences were found between groups in their responses. Percentiles by serum enzyme activity are presented in Table II.

The hematocrit, erythrocytes and hemoglobin concentration remained stable and relatively homogeneous during the experimental protocol (data not shown).

There was no significant difference in performance time among the groups ( $P > 0.05$ ). Mean performance times were  $9023.1 \pm 22.2$  sec for the EXP group and  $9026.6 \pm 25.3$  sec for the CONT.

## DISCUSSION

Studies suggest that caffeine ingestion have hypoalgesic effect on muscle during high-intensity exercise (Motl *et al.*, 2003; O'Connor *et al.*, 2004) and the speculation that caffeine enhance the risk of muscle damage in soccer players, Because of this we analyzed four muscle damage makers before and 48 hours after an intense exercise and caffeine supplementation.

Our finding (increases in serum enzymes activity) supports the notion that the high intensity exercise protocol used in this study resulted in skeletal muscle injury (EIMI). Recently, Lazarim *et al.* (2009) measured plasma CK activity in 128 professional Brazilian soccer players during a real-life elite competition. The authors, using the International Federation of Clinical Chemistry (IFCC) recommended methodology, determined  $975 \text{ U.L}^{-1}$  as the upper limit value for professional soccer players. Our data corroborate the results found by the Lazarim *et al.* (2009) for serum CK activity in soccer players. We did not find other serum enzyme activity parameters for soccer players, but our data were similar with muscle damage markers alterations in other sports modalities (Chevion *et al.*, 2003;

**TABLE I** - Serum enzymes activity was measured pre and 48h post exercise and caffeine supplementation. Data are expressed as mean  $\pm$  SD \* Significantly high POST vs. PRE

	GROUP EXP (n=8)		GROUP CONT (n=7)	
	PRE	POST	PRE	POST
CK (U.L <sup>-1</sup> )	218.0 $\pm$ 129.4	685.3 $\pm$ 700.2*	219.7 $\pm$ 108.5	641.4 $\pm$ 765.4*
LDH (U.L <sup>-1</sup> )	168.1 $\pm$ 30.0	284.6 $\pm$ 74.1*	169.3 $\pm$ 45.8	286.2 $\pm$ 90.9*
AST (U.L <sup>-1</sup> )	26.5 $\pm$ 4.9	54.2 $\pm$ 11.0*	27.0 $\pm$ 4.2	58.9 $\pm$ 30.5*
ALT (U.L <sup>-1</sup> )	12.9 $\pm$ 2.4	16.0 $\pm$ 4.6*	12.2 $\pm$ 2.7	15.1 $\pm$ 2.4*

( $P < 0.05$ ).

**TABLE II** - Serum enzymes activity presented by pre and 48h post exercise and caffeine supplementation percentiles

		Quartiles PRE			Quartiles POST		
		25th	50th (Median)	75th	25th	50th (Median)	75th
GROUP EXP	CK	121.5	136.5	371.5	236.2	458.0	796.7
	LDH	140.5	170.5	184.7	219.7	278.5	360.7
	AST	22.7	26.5	29.5	46.2	52.0	58.2
	ALT	11.0	12.5	14.7	13.2	14.5	17.2
GROUP CONT	CK	120.0	220.0	343.0	236.0	343.0	679.0
	LDH	121.0	183.0	208.0	185.0	319.0	373.0
	AST	24.0	27.0	30.0	38.0	47.0	70.0
	ALT	9.0	13.0	14.0	12.0	15.0	18.0

Pettersson *et al.*, 2007; Brancaccio *et al.*, 2007). Serum LDH activity variation was similar to CK activity, and these data corroborate other studies (Pettersson *et al.*, 2007).

In contrast to our data, are the data presented by Bassini-Cameron *et al.* (2007), which demonstrate a synergistic effect of caffeine in addition to exercise. In that work, following a simulation of a soccer match, subjects performed a very intense exercise (Yoyo test). Because caffeine could play a role in delaying fatigue and presents hypoalgesic effect on muscle, this hypothesis should be reflected in a higher time to exhaustion in the caffeine group, the Yoyo's time to exhaustion data regrettably was not available in Bassini-Cameron *et al.* (2007) report. In the present study we controlled athletes' performance. It is important to recognize that other methodological aspects could have contributed to the differences in contrasting results too (higher dose, lower age and statistical analysis).

In the present study, it has been shown that intermittent exercise resulted in increases in ALT and AST levels. ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis, but physical exercise is known to cause transient elevations on serum transaminases activity. In fact, total serum AST and ALT represents muscle and hepatic enzyme traffic into circulation and Pettersson *et al.* (2007) warned about imposing relevant restrictions on exercise practice prior to and during drugs clinical studies.

During the present study, there were no differences (PRE vs. POST) in Hematocrit, Erythrocytes count, and Hemoglobin, which represents a lack in volumetric variation due to the exercise. This finding is important because hemoconcentration or hemodilution could have resulted in erroneous data interpretation (Del Coso *et al.*, 2008). It is interesting to note that Bassini-Cameron *et al.* (2007) observed significant hematocrit-hemoglobin increases pre-post exercise in their subjects ingesting caffeine. These authors, however, did not indicate whether their changes in muscle damage markers were adjusted for the hemoconcentration effects. We surmise our results advocate that the increase in the CK, LDH, AST, and ALT observed was caused by muscle stress and injury and not hemoconcentration.

In summary, this study indicates that intermittent exercise of a variety used with soccer training, induces raises serum injury muscle markers. This effect is not affected by caffeine (5.5 g.Kg<sup>-1</sup> body mass) supplementation.

## PRACTICAL APPLICATIONS

Caffeine is the most widely used behaviorally active substance on earth and it is proposed as ergogenic aid. Data

from the current study can be applied to trained athletes undergoing intermittent training. Caffeine supplementation did not increase muscle damage from a stressful bout of intermittent exercise. Clinicians, researchers, strength and conditioning professionals and athletes should recognize that few studies have the statistical power to detect severe adverse events. The results of the current study cannot be generalized to athletes ingesting caffeine for extended periods, to those ingesting caffeine in doses above the recommended, or to athletes engaged in resistance training. Caffeine dose at 5.5 mg.Kg<sup>-1</sup> is proposed as ergogenic aid but do not increases risk to muscle integrity. Other collateral effects are described, but our data can postulate the safe use of caffeine in the muscle integrity.

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