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EFFECT OF SHORT TERM CAFFEINE SUPPLEMENTATION AND INTERMITTENT EXERCISE ON MUSCLE DAMAGE MARKERS

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Abstract. Aim: To evaluate the effect of oral caffeine supplementation and strenuous intermittent exercise on muscle damage markers in soccer players. Materials and Methods: 15 male professional soccer players completed a placebo controlled double blind test protocol. At 45 min before exercise, participants ingested 5.5 $mg \cdot kg^{-1}$ body mass of caffeine (CAF, n=8) or cellulose (CEL, n=7). The exercise was 2 trials of 6 sets of 10 sprints (20 m each) with 10 s recovery time between sprints, 2 min between sets and 15 min between trials. Blood samples were collected before (PRE), 24, 48 and 72 h after exercise. Serum activity of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransaminase (AST), and alanine aminotrasaminase (ALT) were quantified. Results: Serum enzyme activity was enhanced by exercise in both groups, without a synergistic effect of caffeine. Conclusion: Our results suggest muscle damage markers increases after physical activities, but caffeine supplementation (5.5 $mg \cdot kg^{-1}$ body mass) has no influence upon serum enzymes reflective of muscle integrity and damage.

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Key words: Transaminases – Exercise – Caffeine – Soccer - Creatine kinase - Lactate dehydrogenase

Introduction

In 2004 caffeine (1,3,7-trimethylxanthine) was removed from the list of prohibited substances of the World Anti-Doping Agency (WADA) and since then has been increasingly used as ergogenic resource [5,10]. Bassini-Cameron *et al.* [1] describes a synergic effect between caffeine and exercise on muscle damage

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markers (MDM) in soccer players measured before and immediately after a simulated soccer match. In other hand, Pettersson *et al.* [15] and Machado *et al.* [12] reported a significant rise in muscle injury markers at only after 24-72 h after exercise. These investigators suggested data blood samples obtained immediately after exercise can be insufficient and additional measures are necessaries to verify hypothetical caffeine synergic effect on exercise-induced muscle damage.

Studies support that caffeine has a large effect on reducing leg-muscle soreness during high-intensity exercise [13,14]. Motl *et al.* [13] proposes that this caffeine ergogenic effect is caused by pain reduction (subjective perception). However, this hypoalgesic effect with effort perception (reduced pain) potentially could cause higher exercise-induced muscle damage, because pain is a signal to tissue protection and absence of signal augment damage possibility.

Caffeine have been used as ergogenic supplements in many sports, and are presently very popular with soccer players [5,8]. Soccer sports training typically involve a variety of physical exigencies as well as skill development activities. Furthermore it is currently very popular in soccer training to include stressful intermittent-interval training [7,16]. However, research on how this type of exercise training affects muscle damage responses is limited.

With the above issues in mind, the purpose of this study was to examine the effect of caffeine supplementation on muscle damage markers (MDM) in soccer players undergoing after strenuous intermittent exercise. Specifically, the intent was to examine the hypothesis that caffeine usage would result in enhanced levels of MDM following such stressful exercise.

Materials and Methods

Subjects: The study group included 15 soccer players (18±3 years old; 177±4 cm height; and 71±4 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. Written informed consent was obtained from the subjects, who were instructed as to the nature and procedures of the study. Because the habituation can change the caffeine effects [8], we select athletes who reported using less than 100 mg per day.

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.

Experimental protocol: In a randomized double-blind, placebo-controlled design, the subjects were divided into 2 groups: experimental (CAF; n=8) and control (CEL; n=7). No caffeine, xanthines, or other substance that could mask the results were ingested by the athletes for 12 h 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 min of warm up (jogging, joint mobilization and stretching) was carried out 35 min after receiving the supplement (see below).

Diet supplementation: The different supplements were in identical capsules so that the subjects were not aware of which substance they were ingesting. Caffeine (Jilin Shulan, China) was given to the group CAF at a dose of 5.5 $mg \cdot kg^{-1}$ 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-1 body weight at 30-60 min prior exercise ingestion) to improve athletes' performance [8,10]. The control group (CEL) received one capsule with 500 mg cellulose only. The supplements were ingested immediately after the blood sample collection.

Test protocol: All subjects ran 6 sets of 10 maximum sprints of 20 m each with 10 s passive recovery between each sprint and two min active recovery (walking) between sets. In addition between set 6 and set 7 the athletes rest for 15 min. The intensity of exercise was controlled by coaches in accordance with previous diagnostics tests. 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, 0) was collected in the morning and other samples 24, 48, and 72 h after. After collection, the blood samples were divided in two tubes (one heparinised tube for hematological measures and the other for serum). After centrifugation, the separated serum was quickly frozen and stored at -70°C. From each heparinised sample the following hematological measures were obtained; Hematocrit, Erythrocyte counts, and Hemoglobin. From serum samples, creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activity were measured. An enzymatic method was used for enzymes activity analysis with commercial kits (BioTécnica - Brazil) in Cobas Mira Plus analyzer (Roche - Germany).

Statistical analyses: The data were analyzed with a two-way (group by time) ANOVA with repeated measures where the groups where the CEL and CAF

treatments. Differences between means were identified by using the Tukey's post hoc procedure. Significant differences were set at 0.05.

Results

The anthropometric, hematological and performance characteristics between groups is identical (Table 1). The hematocrit, erythrocytes and hemoglobin concentration remained stable and relatively homogeneous during the experimental protocol.

Table 1
Participant characteristics

	CAF Group (n=11)	CEL Group (n=9)
Age (years)	18.8±0.3	18.9±0.3
Height (cm)	175.6±1.8	178.3±1.2
Weight (kg)	70.8±0.7	72.7±0.7
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	53.5±1.8	52.1±2.3
Hematocrit (%)	44.0±2.4	44.6±1.8
Eritrocytes ($x10^6/mm^3$)	4.6±0.3	4.9±0.4
Hemoglobin (g/dl)	14.1±0.8	14.3±0.4
Leukocytes (/mm³)	6741.9±554.7	7688.6±800.2
Basophiles (/mm³)	26.0±19.0	22.7±24.5
Eosinophiles (/mm³)	193.9±34.0	233.5±54.2
Neutrophiles(/mm³)	4113.4±625.1	4642.2±713.6
Lymphocytes (/mm³)	1861.7±224.5	2101.1±157.6
Monocytes (/mm³)	546.8±58.2	689.2±137.2

Mean ± SE

Serum CK activity increased after exercise (Fig. 1) but there was no significant effect between treatment groups (CAF vs. CEL) ($P>0.05$). Twenty-four hours after the PRE (0) blood collection the CK activity rose approximately 250% and remains elevated at 48 h ($P<0.05$). Activity at 72 h was elevated compared with the PRE value, but lower in comparison with 24-48 h values ($P<0.05$).

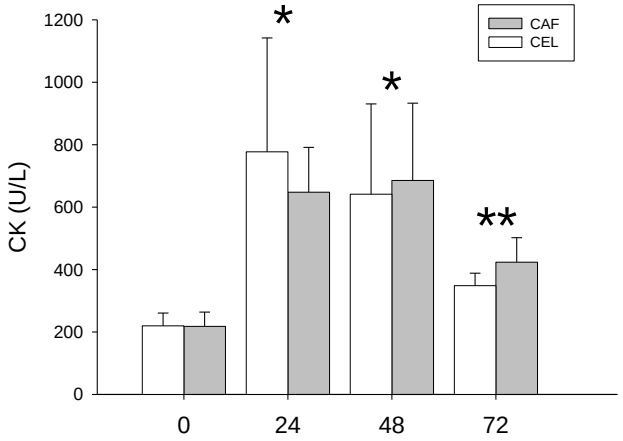

Fig. 1
Serum CK activity
Asterisk - Significantly different from pre-treatment within trial (P<0.05); Double Asterisk - Significant different from 0, 24 and 48h (P<0.05)

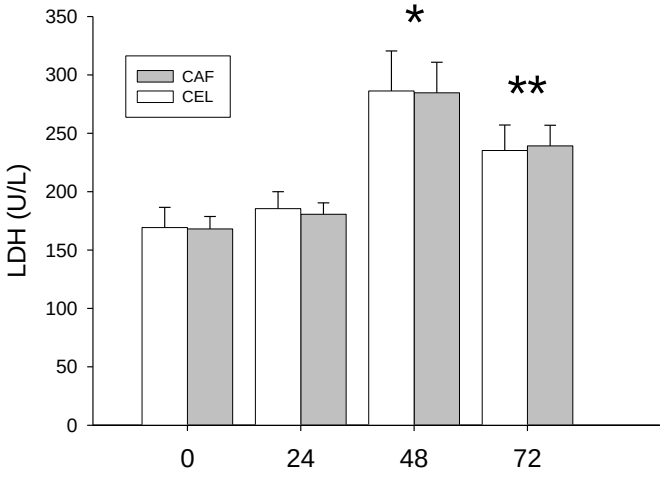

Fig. 2
Serum LDH activity
Asterisk - Significantly different from pre-treatment within trial (P<0.05); Double Asterisk - Significant different from 0, 24 and 48h (P<0.05)

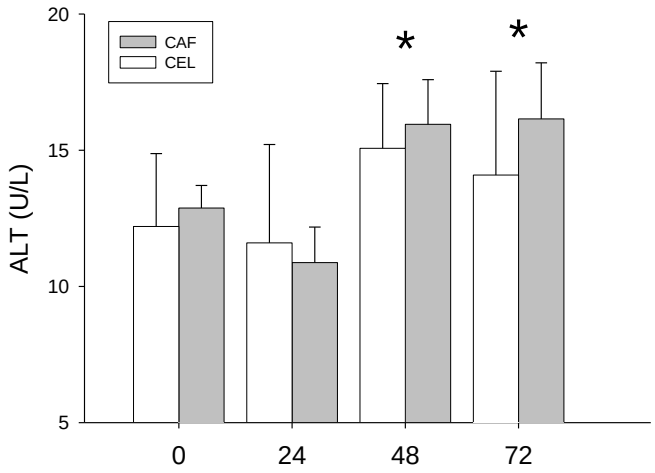

Fig. 3
Serum ALT activity
Asterisk Significantly different from pre-treatment within trial (P<0.05)

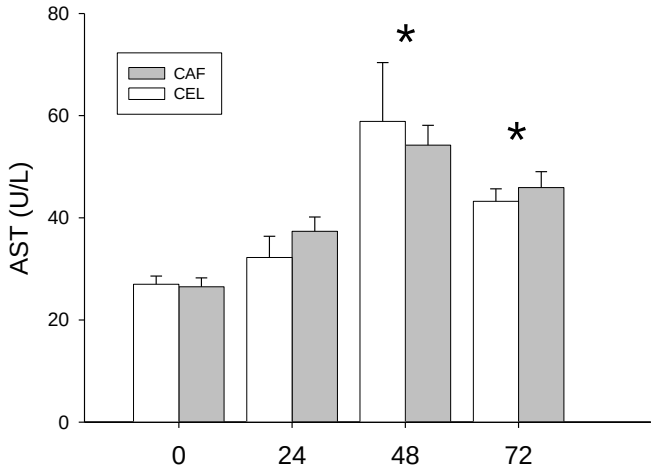

Fig. 4
Serum AST activity
Asterisk Significantly different from pre-treatment within trial (P<0.05)

Exercise caused significant elevations in LDH activity (Fig. 2), but again there were no differences between the treatment groups (P>0.05). At 48 and 72 h LDH was elevated from PRE values, but in addition at 72 h the values were significantly greater than 24 h and slightly lower than at 48 h (P<0.05).

Alanine aminotranferase activity were higher 48 and 72 h after exercise when compare with PRE value (P<0.05) and there were no significant differences between group responses (P>0.05, Fig. 3). At 48 and 72 h the enzyme activity was significantly greater than the PRE (0) values (P<0.05).

Serum AST activity significant increased in both groups at 48 and 72 h after the exercise session (P<0.05). The treatments (CAF and CEL) do not however alter the exercise-induced responses in AST activity (Fig. 4) (P>0.05).

Discussion

Previous reports suggest that caffeine ingestion affect muscle soreness during high-intensity exercise [13,14] and enhance the risk of muscle damage in soccer players [1], because of this we analyzed four MDM in soccer players before and 24, 48 and 72 h after strenuous intermittent exercise with and without caffeine supplementation. Contrary to other, we found there was no increased risk of muscle damage with caffeine supplementation.

Lazarim *et al.* [11] measures plasma CK activity in professional soccer players during one season and our data corroborate with the results found in that study. Furthermore, our data are similar with MDM alterations in other sports modalities too [2,4,12,15]. Specifically, our serum LDH activity variation was very similar with the Pettersson *et al.* [15] findings.

In contrast to our data are the data presented by Bassini-Cameron *et al.* [1], which demonstrate a synergistic effect of caffeine and exercise on MDM. In that study, subjects performed a very intense exercise test (Yoyo Recovery test) after a simulation of a soccer match, which could results in differing individual exercise performance outcomes. Caffeine could play a role on exercise-induced muscle soreness attenuation [14] and thus improve physical performance during exhaustive exercise [9,10]. Regrettably the performance on Yoyo was not reported in the Bassini-Cameron *et al.* [1] findings. This omission make interpretation of there data somewhat difficult because of the varying exercise level performed by their subjects. In the present study we controlled and standardized the athletes' performance and our results display non significant differences between groups. It is important, however, to recognize that exercises tests used in both studies were different and Bassini-Cameron's data is not negligible.

Our results showed that strenuous intermittent exercise resulted in increases in ALT and AST levels 48 h after exercise. 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 [3,12]. In fact, total serum AST and ALT represents muscle and hepatic enzyme traffic into circulation and Pettersson *et al.* [15] warned about imposing relevant restrictions on exercise practice prior to and during drugs clinical studies.

During the present study, there were no differences in Hematocrit, Erythrocyte counts, 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 [6]. It is interesting to note that Bassini-Cameron *et al.* [1] observed significant hematocrit-hemoglobin increases pre-post exercise in their subjects ingesting caffeine (control group do not has differences). These authors, however, did not indicate whether their changes in MDM were adjusted for the hemoconcentration effects. We surmise our hematological results advocate that the increase in the CK, LDH, AST, and ALT observed was caused by muscle stress and injury and not hemoconcentration.

In conclusion, this study indicates that strenuous intermittent exercise of a variety used with soccer training, induces raises MDM. This effect is not augmented by short-term caffeine (5.5 $g \cdot kg^{-1}$ body mass) supplementation. Future research is necessary to examine long supplementation periods and varying dosage levels.

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