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CAFFEINE INFLUENCES PERFORMANCE, MUSCLE PAIN, MUSCLE DAMAGE MARKER, BUT NOT LEUKOCYTOSIS IN SOCCER PLAYERS

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Abstract

Introduction: Caffeine is commonly used by athletes to improve exercise performance but the effects of caffeine supplementation on strenuous intermittent exercise performance, perceived muscle pain, muscle damage markers and immunological response in soccer players are not conclusive.

Objective: To evaluate the effect of caffeine supplementation on strenuous intermittent exercise performance, perceived muscle pain, muscle damage markers and immunological response in soccer players.

Methods: Twenty male professional soccer players completed a placebo-controlled double blind test protocol. At 45 min before exercise, participants ingested 5.5 mg·kg^{-1} body mass of caffeine (CAF, *n*=10) or cellulose (PLA, *n*=10). The exercise was 2 trials of 6 sets of 10 sprints (20m each) with 10s recovery time between sprints, 2 min between sets and 15 min between trials followed by a strenuous intermittent exercise (Yo-Yo intermittent recovery test; Yo-Yo IRT). The performance of Yo-Yo IRT, the perceived muscle pain before exercise protocol, after sprints and after Yo-Yo IRT were recorded. Blood samples were collected before, immediately, 24 and 48h after exercise for serum creatine kinase (CK) activity quantification and immunological cell count.

Results: Caffeine improved the performance during the strenuous intermittent exercise and reduced the perceived muscle pain along the exercise protocol (sprints and Yo-Yo IRT), without influence on muscle damage markers and immunological response.

Conclusions: Our results suggest that caffeine (5.5 mg·kg^{-1} BM) produce large reduction in pain resulting from strenuous intermittent exercise which is associated to the performance gains of soccer players. Additionally, the used caffeine dosage has no influence upon serum CK activity, reflective of muscle integrity and damage, and upon immunological cell count.

Key words: *adenosine antagonist, strenuous exercise, muscle pain, creatine kinase, immune system*

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 [1-4]. Bassini-Cameron et al. [5] described a synergic effect between caffeine and exercise on muscle damage markers (MDM) and leukocytes count in soccer players measured before and immediately after a simulated soccer match, demonstrating a greater increase of MDM and leukocytes after exercise for supplemented group, but blood samples were obtained only immediately after the exercise. Furthermore, Pettersson et al. [6] and Machado et al. [7] reported a significant rise in muscle injury markers after 24-72 h after exercise. These investigators suggested data blood samples obtained immediately after exercise can be insufficient and additional measures are necessary to verify hypothetical caffeine synergic effect on exercise-induced muscle damage.

Studies support that caffeine has a large effect on reducing leg-muscle pain during high-intensity exercise [8,9]. Motl et al. [8] 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 the absence of signal augment damage possibility.

Exercise induces an increase in sympathetic nervous system activity with subsequent increases in circulating hormones such as epinephrine, norepinephrine and cortisol [10]. The increases in circulating hormones may coincide with perturbations in immune cell circulation and function [11]. Additionally, resistance exercise-induced mechanical and metabolic stresses can lead to disruption of muscle cell membranes and release of pro-inflammatory cytokines and leukocytes [12]. The disruption of muscle cell

membranes increases membrane permeability and intracellular calcium ions, which promotes the activation of proteolytic enzymes [13-15].

Previous studies demonstrated that caffeine ingestion prior to exercise increased the inflammatory response, as demonstrated by greater increases in markers of muscle damage and leukocyte levels [5,16]. Several potential mechanisms exist by which caffeine may influence immune cell trafficking and function. Caffeine's role as an adenosine receptor antagonist could result in blockage of A2A adenosine receptors that are expressed on neutrophils. Additionally, a blockade of adenosine receptors could reduce perceptions of pain during exercise [17], allowing for a greater volume of work to be performed following caffeine ingestion. This could indirectly lead to a greater immune response, as a larger volume of work completed leads to a greater disturbance of homeostasis.

More directly, caffeine causes an increase in circulating catecholamines [18], which are largely responsible for the increase in leukocytes seen immediately post exercise via activation of β2 receptors located on leukocytes [11].

Caffeine has been used as an ergogenic supplement in many sports, and is presently very popular with soccer players [1,19]. 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 [20,21]. However, research on how this type of exercise training affects muscle damage responses is limited and results are controversial [5,7,22].

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

Materials and methods

Participants

The study group included 20 low caffeine–consuming professional 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. All procedures were approved by the local Ethics Committee according to the Declaration of Helsinki. 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 [19,23], we selected 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*=10) and control (PLA; *n*=10). 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 (T0) 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) was carried out 45 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 $mg \cdot kg^{-1}$ body weight at 30-60 min prior exercise ingestion) to improve athletes' performance [2,19]. The control group (PLA) 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 rested for 15 minutes. At the end, all the athletes had run the same distances. After the sprints, a Yo-Yo intermittent recovery test (Yo-Yo IRT) was carried out to drive the athletes to exhaustion. This test ended at different times for each athlete, and immediately afterwards blood was collected for laboratory analyses using a double blind procedure. The digital sound signal for Yo-Yo IRT was provided for Beat Test & Training (CEFISE, Brazil).

Data collection

Venous Blood samples (~3 mL) were withdrawn using a 5-cc syringe and a 25 gauge needle in a seated position. The first sample (T0) was collected in the morning and other samples immediately (T1), 24 (T24), 48h (T48) after. After collection, the sample was divided in two glass tubes (one with anti-coagulant eth-

ylenediaminetetraacetic acid (EDTA) for evaluation of hematological variables and the other was centrifuged for serum separation). The serum was quickly frozen and stored at -70°C. The Hematocrit (Hct) and Hemoglobin (Hb) values were used to calculate the changes in plasma volume via the Dill and Costill [24] method. All tubes were kept on ice until processed. The samples were centrifuged (4°C, 3000g) and the plasma or serum was decanted and frozen at -80°C for later analysis.

From the serum sample, creatine kinase (CK) activity was measured. An enzymatic method was used for enzymes activity analysis with commercial kits (BioTécnica - Brazil) in Cobas Mira Plus analyzer (Roche - Germany). From the EDTA sample, total leukocytes, basophils, eosinophils, neutrophils, lymphocytes and monocytes were counted through an automated hematology analyzer (Cobas Mira Plus analyzer, Roche - Germany).

Leg muscle pain ratings

Leg muscle pain intensity was measured before, after intermittent exercise and after Yo-Yo IRT by use of a previously described and validated category scale [8,9]. The scale has 12 categories from 0 to 10 with verbal anchors associated with the following numbers: 0 = no pain at all, 0.5 = very faint pain (just noticeable), 1 = weak pain, 2 = mild pain, 3 = moderate pain, 4 = somewhat strong pain, 5 = strong pain, 7 = very strong pain, and 10 = extremely intense pain (almost unbearable). No verbal anchors are given in association with numbers 6, 8 and 9. Pain ratings assessed by the category scale were significantly correlated (r = 0.79-0.94) with concurrent pain intensity assessed by a standard 10-cm visual analogue scale with verbal anchors of "no pain" and "worst possible pain" [25]. Subjects inform the perceived muscle pain before the exercise protocol, immediately after the sprints sets and immediately after the Yo-Yo IRT. The category scale was used in the present study both because of the evidence of its reliability and validity and because of its advantages over a visual analogue scale applied during exercise (e.g., no physical mark needs to be made on a sheet of paper). The participants were provided with previously described written and oral instructions on the use of the pain scale [25].

Statistical analyses

The data from serum CK activity, muscle pain and blood cell profile were analyzed with a two-way (group by time) ANOVA with repeated measures where the groups where the PLA and CAF treatments. The performance data were analyzed with Student T test. Significant ANOVA results were followed by appropriate post hoc tests with Bonferroni corrections. A significance level of $P < 0.05$ was used to identify statistical significance.

Results

The analysis of Yo-Yo IRT performance demonstrated a significant difference between groups ($P < 0.05$), with a better performance (~12.5%) to CAF group when compared to PLA group (Fig. 1).

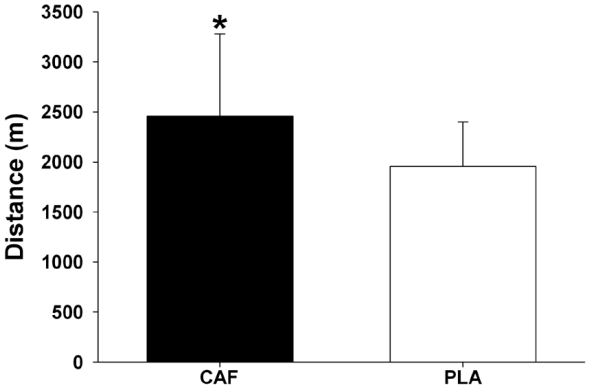

Fig. 1. *Performance (distance in meters) in the Yo-Yo IRT from caffeine (CAF) and placebo (PLA) groups (Data is presented as mean±SD). (*) Difference between groups (P < 0.05).*

Muscle pain data demonstrated a significant main effect for groups ($F_{1,9} = 12.24$, $P < 0.01$; observed power = 0.874; eta^2 = 0.576) and for time ($F_{2,18} = 262.15$, $P < 0.001$; observed power = 1.00; eta^2 = 0.976). Additionally, a significant group x time interaction ($F_{2,18} = 7.31$, $P < 0.001$ observed power = 0.891; eta^2 = 0.448) was observed. For both groups the perceived muscle pain increased along the task ($P < 0.01$), with higher values to PLA group when compared to CAF group immediately after sprint sets (POS1) ($P < 0.05$) and immediately after Yo-Yo IRT (POS2) ($P < 0.01$) (Fig. 2). We also observed that the perceived muscle pain was higher at POS1 measure when compared to POS2 ($P < 0.05$) for PLA group, what was not observed for CAF group ($P > 0.05$).

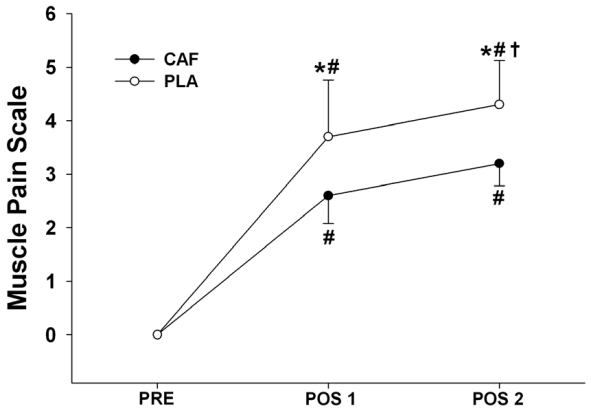

Fig. 2. *Muscle pain from caffeine (CAF) and placebo (PLA) groups before (PRE), immediately after sprints (POS1) and immediately after Yo-Yo IRT (Data is presented as mean±SD). (*) Significant difference between groups (P<0.05); (#) Significantly different from PRE (P<0.05)*

Table 1. *Hematological data (Mean±SD) from caffeine (CAF) and placebo (PLA) groups before (T0), immediately (T1), 24 (T24) and 48h (T48) after a strenuous exercise protocol*

	CAF Group(n=10)				PLA Group(n=10)			
	T0	T1	T24	T48	T0	T1	T24	T48
Hematocrit (%)	44.9±1.2	48.4±0.8§*	45.1±1.5	45.1±1.1	45.8±1.0	46.2±2.4	46.4±1.4	44.1±2.3
Erythrocytes ($x10^6/mm^3$)	5.1±0.3	5.4±0.4*	5.0±0.2	5.0±0.3	5.2±0.2	5.3±0.5	5.3±0.3	5.0±0.4
Haemoglobin (mmol/l)	8.9±0.3	9.5±0.4π	9.0±0.4	8.8±0.4	9.1±0.3	9.2±0.4	9.3±0.3	8.9±0.4
MCV (fl)	88.6±4.7	88.3±5.7	89.8±3.8	90.2±4.6	88.3±2.2	87.4±4.0	87.8±3.7	87.9±3.5
MCH (pg)	28.2±1.6	28.3±2.0	28.8±1.2	28.4±1.9	28.2±1.2	28.2±1.8	28.4±1.7	28.6±1.8
MCHC (%)	31.9±0.7	31.7±1.1	32.1±1.3	31.5±1.2	31.9±0.8	32.3±0.7	32.4±0.7	32.5±1.0

MCV = mean corpuscular volume; MHC = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration. (§) Significantly different from T0, T24 and T48 ($P < 0.05$); (π) Significantly different from T0 and T48; (Ŧ) Significant difference between groups ($P < 0.05$).

Hematological data are presented in Table 1. A significant difference between groups at the baseline measure (i.e., prior to the strenuous exercise protocol) was not observed. Hematocrit data demonstrated a significant main effect for time ($F_{3,27}$ = 15.36, P < 0.001; observed power = 1.00 ; eta^2 = 0.631) and a significant group x time interaction ($F_{3,27}$ = 13.79, P < 0.001; observed power = 0.987 ; eta^2 = 0.493), with an significant increase immediately after the strenuous exercise protocol for CAF group. Erythrocytes data, as well as haemoglobin concentration had a significant main effect only for time ($F_{3,27}$ = 11.93, P < 0.001; observed power = 0.999 ; eta^2 = 0.570 for erythrocytes ($F_{3,27}$ = 12.26, P < 0.001; observed power = 0.999 ; eta^2 = 0.577 for haemoglobin), increasing immediately after the strenuous exercise protocol only for the CAF group ($P < 0.05$).

The analyses of blood cell and plasma enzyme activity may be influenced by the plasma variations, as expected immediately after a strenuous exercise protocol like carried out here. Then, serum CK activity was analyzed with and without correction in function of plasma variation (Fig. 3). The results for serum CK activity without corrections by plasma volume demonstrated a significant main effect for time ($F_{3,27}$ = 26.17, P < 0.001; observed power = 1.00; eta^2 = 0.744) but there were not significant main effect for group ($F_{1,9}$ = 0.134, P > 0.05; observed power = 0.062; eta^2 = 0.015) or significant group x time interaction ($F_{3,27}$ = 0.700, P > 0.05; observed power = 0.178; eta^2 = 0.072). Similar results were found when serum CK activity results were corrected in function of plasma variation for time ($F_{3,27}$ = 26.50, P < 0.001; observed power = 1.00; eta^2 = 0.746), group ($F_{1,9}$ = 0.295, P > 0.05; observed power = 0.078; eta^2 = 0.032) and group x time interaction ($F_{3,27}$ = 0.812, P > 0.05; observed power = 0.202; eta^2 = 0.083).

The serum CK activity with and without correction in function of plasma volume increased along the analyzed time (i.e., T0-T48) for both groups ($P < 0.05$), but it is important to note that the CK data without correction of CAF group was significantly higher (7%) than corrected CK data at T1 ($P < 0.001$) (Result not shown in Fig. 3). Despite of plasma volume influences

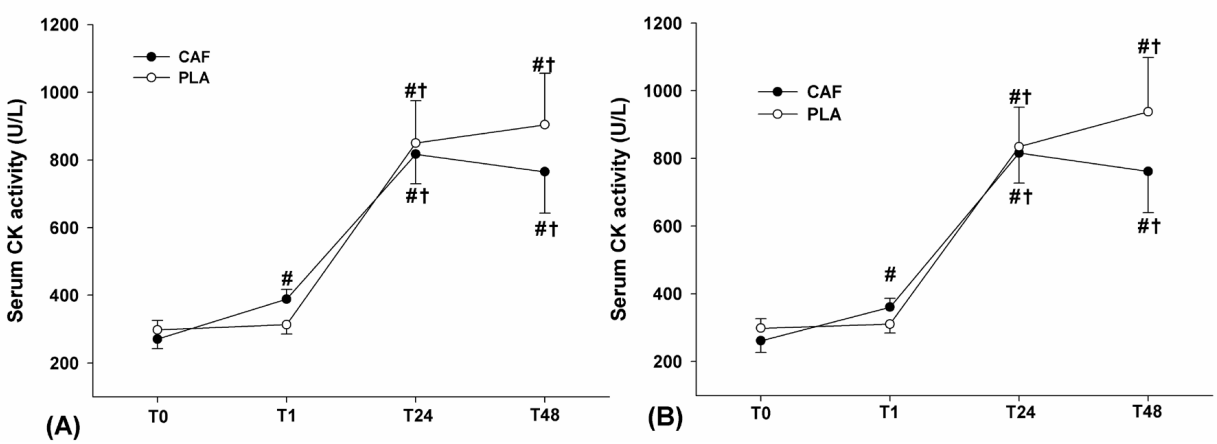

Fig. 3. *(A) Serum CK activity from caffeine (CAF) and placebo (PLA) groups before (T0), immediately after (T1), 24 (T24) and 48h (T48) after a strenuous exercise protocol. (B) Serum CK activity corrected in function of plasma variation from caffeine (CAF) and placebo (PLA) groups before (T0), immediately after (T1), 24 (T24) and 48h (T48) after a strenuous exercise protocol (Data is presented as mean±SD). (#) Significantly different from T0 (P<0.05); (†) Significantly different from T1 (P<0.05)*

the serum CK activity was not significantly different between groups ($P = 0.07$ and $P = 0.201$ for CK data with and without plasma volume correction). In addition, only CAF group demonstrated a significant difference between T1 and T0 independently of plasma volume correction of serum CK activity ($P < 0.01$ and $P < 0.001$ for CK data with and without plasma volume correction respectively).

The leukocytes count demonstrated a significant main effect for time ($F_{3,27} = 46.74$, $P < 0.001$; observed power = 1.00; $eta^2 = 0.839$), but a main effect for group ($F_{1,9} = 0.226$, $P > 0.05$; observed power = 0.071; $eta^2 = 0.024$) or group x time interaction ($F_{3,27} = 0.707$, $P > 0.05$; observed power = 0.180; $eta^2 = 0.073$) was not observed (Fig. 4). The number of leukocytes increased immediately after the strenuous exercise protocol ($P < 0.05$) for both groups similarly and return to the pre-exercise values 24h following the exercise.

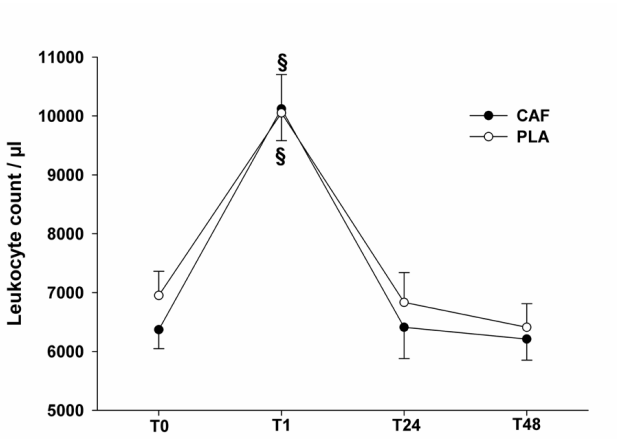

Fig. 4. *Total Leukocyte count from caffeine (CAF) and placebo (PLA) groups before (T0), immediately after (T1), 24 (T24) and 48h (T48) after a strenuous exercise protocol (Data is presented as mean±SD). (§) Significantly different from T0, T24 and T48 (P<0.05)*

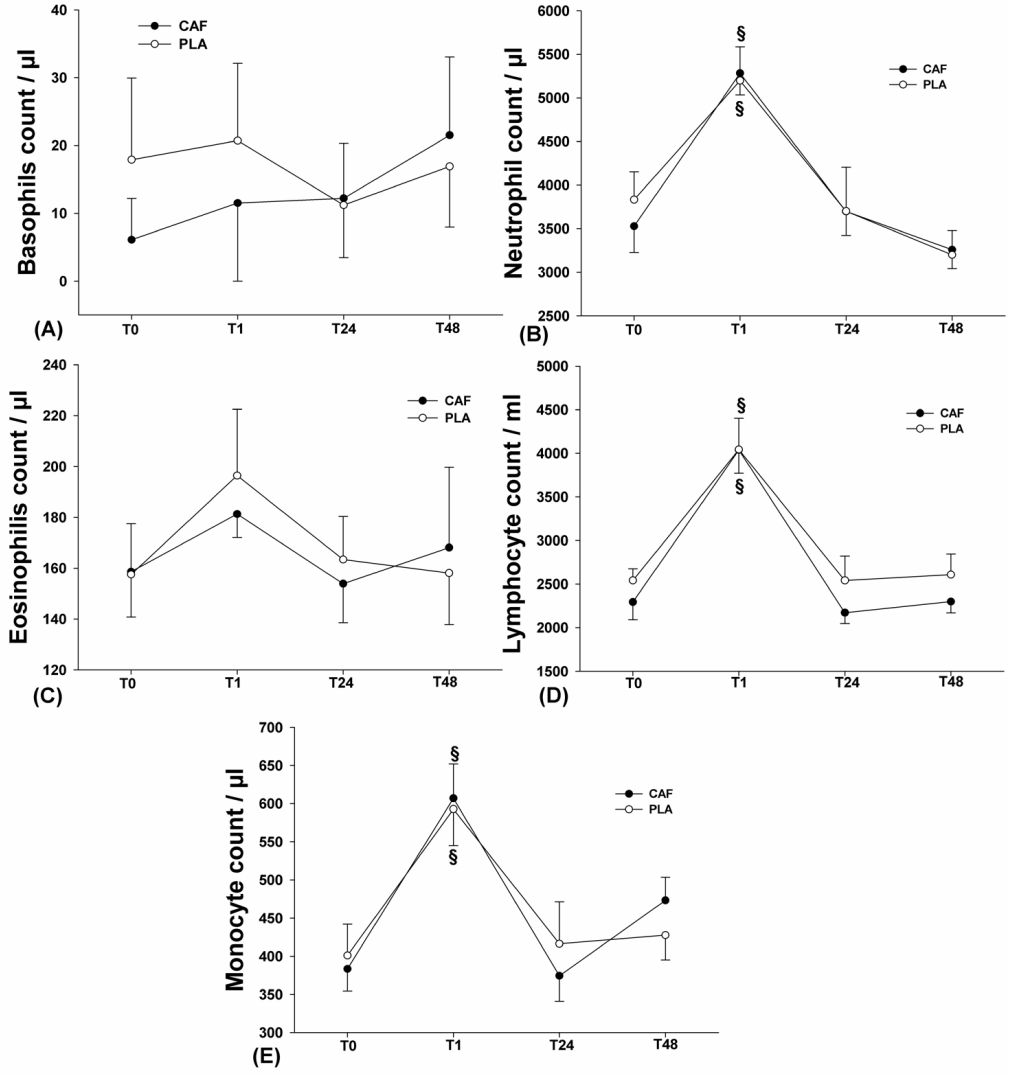

Fig. 5. *Basophils (A), neutrophils (B), eosinophils (C), lymphocytes (D) and monocytes (E) count from caffeine (CAF) and placebo (PLA) groups before (T0), immediately after (T1), 24 (T24) and 48h (T48) after a strenuous exercise protocol (Data is presented as mean±SD). (§) Significantly different from T0, T24 and T48 (P<0.05)*

The number of basophils, neutrophils, eosinophils, lymphocytes and monocytes are shown in Figure 5. The basophils and eosinophils count were not significantly affected by the strenuous exercise protocol and/or caffeine supplementations ($P > 0.05$ for group, time and group time interactions). But neutrophils, lymphocytes and monocytes count demonstrated a similar behavior with a significant main effect for time ($F_{3,27} = 20.54$, $P < 0.001$; observed power = 1.00; $eta^2 = 0.695$ for neutrophils / $F_{3,27} = 29.71$, $P < 0.001$; observed power = 1.00; $eta^2 = 0.768$ for lymphocytes / $F_{3,27} = 11.88$, $P < 0.001$; observed power = 0.999; $eta^2 = 0.569$ for monocytes), but a main effect for group ($F_{1,9} = 0.017$, $P > 0.05$; observed power = 0.052; $eta^2 = 0.002$ for neutrophils / $F_{1,9} = 0.780$, $P > 0.05$; observed power = 0.124; $eta^2 = 0.080$ for lymphocytes / $F_{1,9} = 0.0001$, $P > 0.05$ observed power = 0.050; $eta^2 = 0.0001$ for monocytes) or group x time interaction ($F_{3,27} = 0.521$, $P > 0.05$; observed power = 0.142; $eta^2 = 0.055$ for neutrophils / $F_{3,27} = 0.509$, $P > 0.05$; observed power = 0.140; $eta^2 = 0.054$ for lymphocytes / $F_{3,27} = 1.082$, $P > 0.05$; observed power = 0.259; $eta^2 = 0.107$ for monocytes) was not observed. We found an exercise-induced increase effect for neutrophils, lymphocytes and monocytes without influence of caffeine supplementation.

Discussion

Our results indicate that caffeine supplementation enhance the performance in Yo-Yo IRT and serum CK activity. Additionally, the immunological response to exercise is not influenced by caffeine supplementation.

Previous reports suggest that caffeine ingestion affect muscle pain during moderate intensity exercise [8,9] and enhance the risk of muscle damage in soccer players [5], because of this we analyzed four MDM in soccer players before and 24 and 48h after strenuous intermittent exercise with and without caffeine supplementation. We found a significant ergogenic effect, which was associated to a hypoalgesic effect, and refuted the hypothesis of an increased risk of muscle damage with caffeine supplementation.

The ergogenic effect of caffeine on endurance sports are well described, but in other modalities is still debatable, however Glaister et al. [26] found a significant improvement in performance in intermittent activities. Our findings corroborate the findings of Glaister et al. [26], the subjects underwent supplement had ~12.5% better performance than those who did not use the supplement. According to Motl et al. [8] and O'Connor [9], this may occur by decreasing the perception of pain induced by muscular exercise. We identify a significant difference in pain perception, where the subjects supplemented with caffeine reported lower values on the scale of muscle pain perception. It is important to note that previous studies used a moderate intensity exercise protocol [8,9,27] or a high intensity exercise protocol [28] limited to the duration of 30 minutes. Differently, we used an exercise protocol where the subjects were demanded to the maximum up to the exhaustion, which may determine a highest muscle pain.

Orally ingested caffeine by humans has effects on both the peripheral and the central nervous system because caffeine crosses the blood-brain barrier. Then, several possible mechanisms for the caffeine-induced reduction in muscle pain during exercise exist. The hypoalgesia might stem from caffeine acting on peripheral or central adenosine A_1 and/or A_{2a} receptors involved in the nociceptive system [29,30]. Alternatively, caffeine might indirectly influence the nociceptive system, for instance, by altering muscle sensory processes [31] or by acting on neurons involved in regulating the cardiovascular system [32].

Adenosine is a potent algesic agent, which is released during tissue injuries [33], and as caffeine is an adenosine antagonist, it is plausible that our findings resulted from caffeine attenuating nociceptive activity induced by adenosine in the periphery. As the adenosine receptors are widespread located on the nervous system and caffeine crosses the blood-brain barrier, many other ways of action could be postulated, as nociceptive afferent fibers, the dorsal horn of the spinal cord, and brain areas involved in pain such as primary and secondary somatosensory, insular, anterior cingulate, and prefrontal cortices [34,35]. The precise mechanism for the reduction in pain found in this experiment is uncertain because caffeine is a nonselective adenosine receptor antagonist with high affinity for both A_1 and A_2 Adenosine receptors present at different locations of nervous system [29].

Lazarim et al. [36] 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 [6,7,37,38].

In contrast to our data, Bassini-Cameron et al. [5] demonstrated a synergistic effect of caffeine and exercise on MDM. In that study, subjects performed a Yo-Yo IRT after a simulation of a soccer match, which could result in differing individual exercise performance outcomes. Regrettably the performance on Yo-Yo IRT was not reported in the Bassini-Cameron et al. [5] findings. This omission makes interpretation of these data somewhat difficult because of the varying exercise level performed by their subjects. It is important to note that caffeine could play a role on exercise-induced muscle pain attenuation [8,9] and thus improve physical performance during exhaustive exercise [2,39]. In the present study we controlled athletes' performance, which was better in caffeine supplemented subjects.

Many studies [37,40] verify great inter-individual differences on serum CK activity following exercises. This variability can explain the differences between groups on Bassini-Cameron et al. [5] study. The small sample, the immediately post exercise blood collection and statistical method can cause confusion on results interpretation. To avoid misinterpretations, we verify the serum CK activity dosages for a long time (up to 48 hours after the exercise) to verify real differences in supplemented and control groups and observed differences only immediately after exercise protocol. These results may indicate that the caffeine do not enhance the exercise-induced muscle damage risk because the peak of serum CK activity has been observed at 24-48h following the exercise [7,38]. It is possible that the immediately in serum CK activity result from a greater effort during the exercise protocol promoted by the ergogenic effect of caffeine. Then, it seems that caffeine supplementation does not act synergically with exercise to increase the exercise-induced muscle damage, since it was not observed differences between groups in serum CK activity at 24-48h following the exercise. The absence of synergic effect between caffeine supplementation and exercise to increase the exercise-induced muscle damage corroborate previous studies [7, 41].

During the present study, there were differences in Hematocrit, Erythrocyte counts, and Hemoglobin, which represents a lack in volumetric variation due to the exercise. This find is important because hemoconcentration or hemodilution could result in erroneous data interpretation [42]. It is interesting to note that Bassini-Cameron et al. [5] observed significant hematocrit-hemoglobin increases pre-post exercise in their caffeine supplemented subjects, but without differences for control group (placebo). However, these authors did not indicate whether their changes in MDM were adjusted for the hemoconcentration effects. Our findings also show that volumetric differences in the blood collection was present immediately after exercise, so we also noticed that volumetric differences may tend to overestimate the serum CK activity, which can lead to misinterpretations.

Increases in leukocytes and lymphocytes count, followed by a decrease to the baseline along the recovery time, are expected after a strenuous exercise [43-45]. It is hypothesized that changes in the circulating epinephrine, which is positively related to exercise intensity [46] and may be increased by caffeine supplementation (18), may induce leukocytosis owing to the $\beta 2$ receptors located on leukocytes [11]. Additionally, caffeine may opposite the inhibitory effect of endogenous Adenosine, biding A_{2a} receptors, over the polymorphonuclear leukocytes and lymphocytes [47]. Our results demonstrated an enhancement of total leukocytes and lymphocytes as expected, but fail to demonstrate an additional caffeine influence on blood cell content.

The absence of difference between groups on immunological response could be related to some limitations of the present study. Firstly, we have done only the total lymphocytes count, without discrimination of lymphocyte subpopulations. It is known that the exercise-induced muscle damage is associated to increase of NK cells and CD8+ T cells [48,49]. In addition, caffeine can influence blood cells activity, which was not measured here. Thereby, further studies are necessary to explain these topics.

In conclusion, this study indicates that short-term caffeine (5.5 $mg \cdot kg^{-1}$ body mass) supplementation improve the performance of the strenuous intermittent exercise of a variety used with soccer training, which may be related to the hypoanalgesic effect of caffeine. Additionally, caffeine supplementation did not influence the exercise-induced muscle damage and/or immunological response. Further researches are necessary to examine the response of lymphocytes subpopulations and the effect of long supplementation periods and varying dosage levels.

Declaration of interest

The author reports no conflicts of interest.

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F – Literature Search
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