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Original Article

Effect of a Single Dose of Caffeine Supplementation and Intermittent-interval Exercise on Muscle Damage Markers in Soccer Players

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This study examined the effect of caffeine supplementation on the white cell count and muscle damage marker responses to intermittent-interval exercise as performed by soccer players. Subjects ($n = 20$) completed a placebo-controlled double-blind test protocol. Forty-five minutes before exercise, participants ingested 4.5 $mg \cdot kg^{-1}$ body mass of caffeine (EXP) or placebo (CONT). Blood samples were collected before and after exercise to measure hematological parameters, serum creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) and γ-glutamyl transferase (γ-GT) activity. To compare differences among all variables, 2 (time) × 2 (group) repeated measures ANOVA (with Tukey's *post hoc* tests) was conducted. Exercise caused leukocytosis (38.5% and 36.1% in EXP and CONT, respectively), lymphocytosis (42.1% and 44.9%; $p < 0.05$) and neutrophilia (38.2% and 31.5%; $p < 0.05$) without an additional effect due to caffeine ($p > 0.05$). Also, serum CK and LDH activity were enhanced by exercise in both groups ($p < 0.05$), without a synergistic effect of caffeine. ALT, AST, AP and γ-GT serum activity was not modulated by exercise or caffeine. The findings demonstrate that white cells and muscle damage markers increase after intense intermittent exercise, but acute caffeine supplementation has no influence on immune responses or muscle cellular integrity. [*J Exerc Sci Fit* • Vol 7 • No 2 • 91–97 • 2009]

Keywords: caffeine, creatine kinase, exercise, leukocytosis, transaminases

Introduction

Caffeine (1,3,7-trimethylxanthine) is the most widely used behavioral influencing substance consumed on earth (Graham 2001). Relative to sport, it was removed from the list of prohibited substances by the World Anti-Doping Agency in 2004. This occurred even though there is general consensus from research findings that caffeine improves prolonged, continuous exercise performance (de Hon & Coumans 2007; Graham 2001). The ergogenic effects of caffeine are attributed to its action to delay fatigue and via an improvement in contractile strength of cardiac and skeletal muscle (Graham 2001). Supplementation with caffeine is also known to decrease muscular pain perception, effort perception, and neuromuscular reaction time, which can further facilitate exercise performance (Schneiker et al. 2006; Kalmar & Cafarelli 2004).

In addition to the above-noted effects, caffeine appears to influence the immune system response to exercise (Bassini-Cameron et al. 2007; Tsigos & Chrousos

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2002). Specifically, some research has demonstrated increased levels of leukocytosis and lymphocytosis after an exercise stress involving caffeine administration (Bassini-Cameron et al. 2007). These latter authors concluded that the effects of caffeine on white blood cell counts can lead to an aggravation of the muscle injury as a result of exercise. It is also important to note that caffeine use, along with exercise, leads to an enhanced activation of both the hypothalamic-pituitary-adrenal axis and the autonomic nervous system (Kalmar 2005; Graham 2001), which can also affect immune responses to exercise (Tsigos & Chrousos 2002). However, not all research is in agreement with these findings of caffeine influencing leukocytosis, lymphocytosis and muscle damage responses to exercise (Machado et al. 2008; Walker et al. 2007).

Caffeine- and xanthine-based substances have been used as ergogenic supplements in many sports, and are presently very popular with soccer players (de Hon & Coumans 2007; Graham 2001). Soccer sport training typically involves a variety of physical fitness conditioning as well as skill development activities (Krustrup et al. 2006; Stølen et al. 2005). Current popular soccer training includes a high degree of intermittent-interval training activities (Stølen et al. 2005). However, research on how this type of exercise training affects immune system and muscle damage responses is limited. Furthermore, the influence caffeine might have on these responses to such exercise is contradictory. Therefore, the present study was undertaken with a purpose of examining the effect of caffeine supplementation on the white cell count and muscle damage marker responses in soccer players to intermittent-interval exercise.

Methods

Subjects

The subjects were 20 healthy soccer players (mean age, 18.8 ± 1.0 years; mass, 73.0 ± 5.9 kg; height, 177 ± 7.0 cm; $\dot{V}O_{2max}$, 54.5 ± 1.9 mL · kg^{-1} · min^{-1}) who used no medicinal drugs, dietary supplements, or anabolic steroids. All voluntarily participated and gave written informed consent. The group was characterized by a homogeneous lifestyle based upon monitoring for 4 weeks during a pre-season period prior to the study beginning. The study protocol was approved by the local ethics committee.

Experimental protocol

In a randomized double-blind, placebo-controlled design, the subjects were divided into two groups: experimental (EXP; $n = 11$) and control (CONT; $n = 9$). No caffeine, xanthines, or other substance that could mask the results were ingested by the athletes for 12 hours before blood collection, i.e. there was a washout period (Graham 2001). A morning blood specimen (PRE) was collected 1 hour after a standardized breakfast (~100 g of bread, ~40 g of Minas cheese, and 200 mL of skimmed milk). Ten minutes of warm up (jogging, joint mobilization and stretching) was carried out 35 minutes after receiving the supplement (see below). These procedures were followed by the exercise protocol. Blood samples were then collected after (POST) the exercise protocol. The experimental design is displayed in Table 1.

Exercise protocol

All subjects ran six sets of 10 sprints at 20 meters each with 10 seconds of passive recovery between each sprint and 5 minutes of active recovery (walking) between sets. Total time was approximately 29 minutes in accordance with individual fitness status (minutes:seconds, minimum 29:24, maximum 29:30; with no significant differences between the EXP and CONT groups). The athletes were allowed to ingest water *ad libitum* throughout their sprints in order to reflect real life training situations.

Caffeine supplementation

The different supplements were in indistinguishable capsules so that the subjects were not aware of the substance they were ingesting. Caffeine (Purifarma, China) was given to the EXP group (dosage: 4.5 mg · kg^{-1} body mass in one 500 mg capsule), which also contained enough cellulose (Gujarat Microwax, India) to fill the capsule. This dose was chosen because it is within the supplementation range (3.0–9.0 mg · kg^{-1} body mass 30–60 minutes prior to exercise) shown to improve athletes' performance (Graham 2001). The CONT received one capsule containing 500 mg cellulose

Table 1. Experimental design

0 min	5 min	15 min	50 min	60 min	~89 min
PRE	Breakfast	Supplement	Warm-up	Exercise	POST

only. The supplements were ingested immediately after the PRE blood sample collection.

Environmental conditions

Subjects ran in a regular soccer field (grass) and dressed in habitual training uniform and shoes. During this study, the environmental temperature was 32 ± 1°C and air humidity was 57 ± 5% (on average). The subjects were well adapted to the environmental conditions and tolerated exposure without any undue distress.

Data collection

Blood samples were collected via venipuncture from the forearm vein while the subjects were in a seated position. As noted, the baseline sample (PRE) was collected in the morning while the next sample (POST) was collected immediately after the 60th sprint. 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, erythrocyte counts, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), leukocytes, basophiles, eosinophils, neutrophils (N bands and N segments), lymphocytes and monocytes.

From serum samples, creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) and γ-glutamyl transferase (γ-GT) activity were measured. An enzymatic method at 37°C was used for enzyme activity analyses using commercial assay kits (BioTécnica, Varginha, Brazil) in a Cobas Mira Plus analyzer (Roche, Mannheim, Germany).

Statistical analyses

Distribution testing (Kolmogorov-Smirnov) revealed that the variables studied were normally distributed. To compare differences among all variables, 2 (PRE *vs.* POST) × 2 (CONT *vs.* EXP) repeated measures ANOVA (with Tukey's *post hoc* tests) was conducted. To examine the relationship between the effect of exercise and caffeine relative to changes in enzyme kinetics, bivariate linear regression was performed. Individual kinetic coefficients (slope) between groups were compared with the Kolmogorov-Smirnov test ($p < 0.05$). All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Significant differences were set at $p < 0.05$. All data are expressed as mean ± standard deviation.

Results

Subjects from both groups had normal red and white cell counts in their baseline PRE blood samples and no differences were found between groups. These data are shown in Table 2. Hematocrit, erythrocyte counts and hemoglobin concentration remained stable and relatively homogeneous during the experimental protocol. There were also no changes in MCV, MCH or MCHC during the experiment (Table 2).

Table 2. Hematological variables*

	EXP group† (*n* = 11)		CONT group† (*n* = 9)	
	PRE	POST	PRE	POST
Hematocrit (%)	43.9 ± 1.2	44.1 ± 1.0	45.4 ± 1.5	44.8 ± 0.8
Erythrocytes (×10^6/mm^3)	5.0 ± 0.1	5.2 ± 0.1	5.1 ± 0.2	5.2 ± 0.1
Hemoglobin (g/dL)	14.4 ± 0.3	14.7 ± 0.2	14.5 ± 0.4	14.6 ± 0.2
MCV (fL)	88.3 ± 1.0	86.2 ± 1.6	88.5 ± 1.0	88.2 ± 1.2
MCH (pg)	29.0 ± 0.5	28.5 ± 0.4	28.3 ± 0.4	28.1 ± 0.4
MCHC (%)	32.8 ± 0.4	33.2 ± 0.7	31.9 ± 0.3	31.8 ± 0.4
Leucocytes (×10^3/mm^3)	6.5 ± 0.3	8.9 ± 0.6‡	7.0 ± 0.2	9.6 ± 0.3‡
Basophils (/mm^3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophils (/mm^3)	146.2 ± 14.7	174.4 ± 24.1	160.0 ± 12.8	156.7 ± 15.7
N bands (/mm^3)	118.2 ± 24.3	301.0 ± 33.2‡	111.2 ± 22.0	283.5 ± 33.0‡
N segments (/mm^3)	3227.4 ± 291.0	4324.6 ± 274.6‡	3329.9 ± 196.8	4243.0 ± 188.0‡
Lymphocytes (/mm^3)	2587.7 ± 115.8	3677.2 ± 338.2‡	3081.7 ± 187.2	4467.8 ± 170.2‡
Monocytes (/mm^3)	397.1 ± 31.8	492.0 ± 39.8‡	437.1 ± 37.6	494.4 ± 52.2‡

*Data presented as mean ± standard deviation; †no statistically significant differences were found between groups; ‡$p < 0.05$, PRE *vs.* POST. MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = MCH concentration; N = neutrophil.

Table 3. Changes in enzyme activity*

	EXP group† ($n = 11$)		CONT group† ($n = 9$)	
	PRE	POST	PRE	POST
CK (U/L)	529.4 ± 107.5	617.8 ± 109.0‡	401.8 ± 103.0	530.7 ± 127.4‡
LDH (U/L)	409.2 ± 24.8	584.6 ± 18.7‡	438.0 ± 26.6	664.7 ± 38.1‡
AST (U/L)	30.0 ± 1.4	31.9 ± 1.5	34.7 ± 2.0	35.2 ± 2.5
ALT (U/L)	20.5 ± 1.5	19.1 ± 1.3	17.9 ± 1.0	19.9 ± 1.7
AP (U/L)	89.5 ± 4.3	87.5 ± 3.3	79.9 ± 5.2	74.4 ± 5.9
γ-GT (U/L)	21.0 ± 1.9	21.5 ± 1.7	17.2 ± 2.6	17.5 ± 2.7

*Data presented as mean ± standard deviation; †no statistically significant differences were found between groups; ‡$p < 0.05$, PRE *vs.* POST. CK = creatine kinase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; AP = alkaline phosphatase; γ-GT = γ-glutamyl transferase.

Immediately after intermittent exercise, total leukocyte counts were 38.5% and 36.1% higher than pre-exercise values in the EXP and CONT groups, respectively ($p < 0.05$). Lymphocyte population also significantly increased in both groups compared to baseline values (42.1% and 44.9%, EXP and CONT, respectively; $p < 0.05$). However, no differences were found between groups for leukocytes and lymphocytes. Total neutrophil count rose 38.2% in the EXP group and 31.5% in the CONT group ($p < 0.05$); but there was no significant difference between the two groups. These data are shown in Table 2.

Table 3 summarizes the impact of exercise on serum enzyme activity. The PRE CK, LDH, AST, and ALT values were above population values according to Kratz and Lewandrowski (1998); which is most likely because all subjects were athletes actively involved in training regimes (Brancaccio et al 2007; Mougios 2007). Serum concentrations of CK and LDH increased significantly immediately after exercise ($p < 0.05$), whereas serum AST, ALT, AP and γ-GT levels did not change ($p > 0.05$). However, no significant differences were found between groups in their magnitude of enzymatic responses. Furthermore, all individual enzyme kinetics (regression slopes) between both groups were very similar and did not statistically differ from one another (see Figure).

Discussion

It has previously been reported that exercise increases circulating white cell counts because of their availability from blood storage sites, and the stimulus of tissue damage products, as well as hormonal interactions (Zaldivar et al. 2006; Scharhag et al. 2005). This finding is corroborated in the present study, as total leukocyte, neutrophil and lymphocyte blood populations rose after exercise. The magnitude of the observed leukocytosis, lymphocytosis and neutrophilia were similar to that reported in previous works (Zaldivar et al. 2006; Peake et al. 2005).

We also verify the prior finding of caffeine having no acute augmentation effect on immune cell counts following exercise (i.e. no significant differences between groups in POST measurements were found). These results are similar to the findings of Walker et al. (2007) in recreational male cyclists who performed exercise with caffeine supplementation. In contrast to the present data and that of Walker et al., Bassini-Cameron et al. (2007), demonstrated a synergistic action of caffeine and exercise, i.e. leading to a greater degree of leukocytosis, lymphocytosis and muscle damage. The exercise, caffeine dosage, blood sampling protocol, and randomization procedures of the present study and that of Bassini-Cameron et al. are very similar. Why we did not find similar results to these latter authors is unclear, but suggests further work on caffeine-exercise and immune responses is warranted.

During the present study, there were no differences (PRE *vs.* POST) in hematocrit, hemoglobin, MCV, MCH and MCHC, 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- to post-exercise in their subjects ingesting caffeine. Regrettably, these authors, however, did not indicate whether their changes in white cell population were adjusted for the hemoconcentration effects. We surmise that our results advocate that the increase in the white cell population observed was caused by muscle stress (see below) and/or injury and not hemoconcentration.

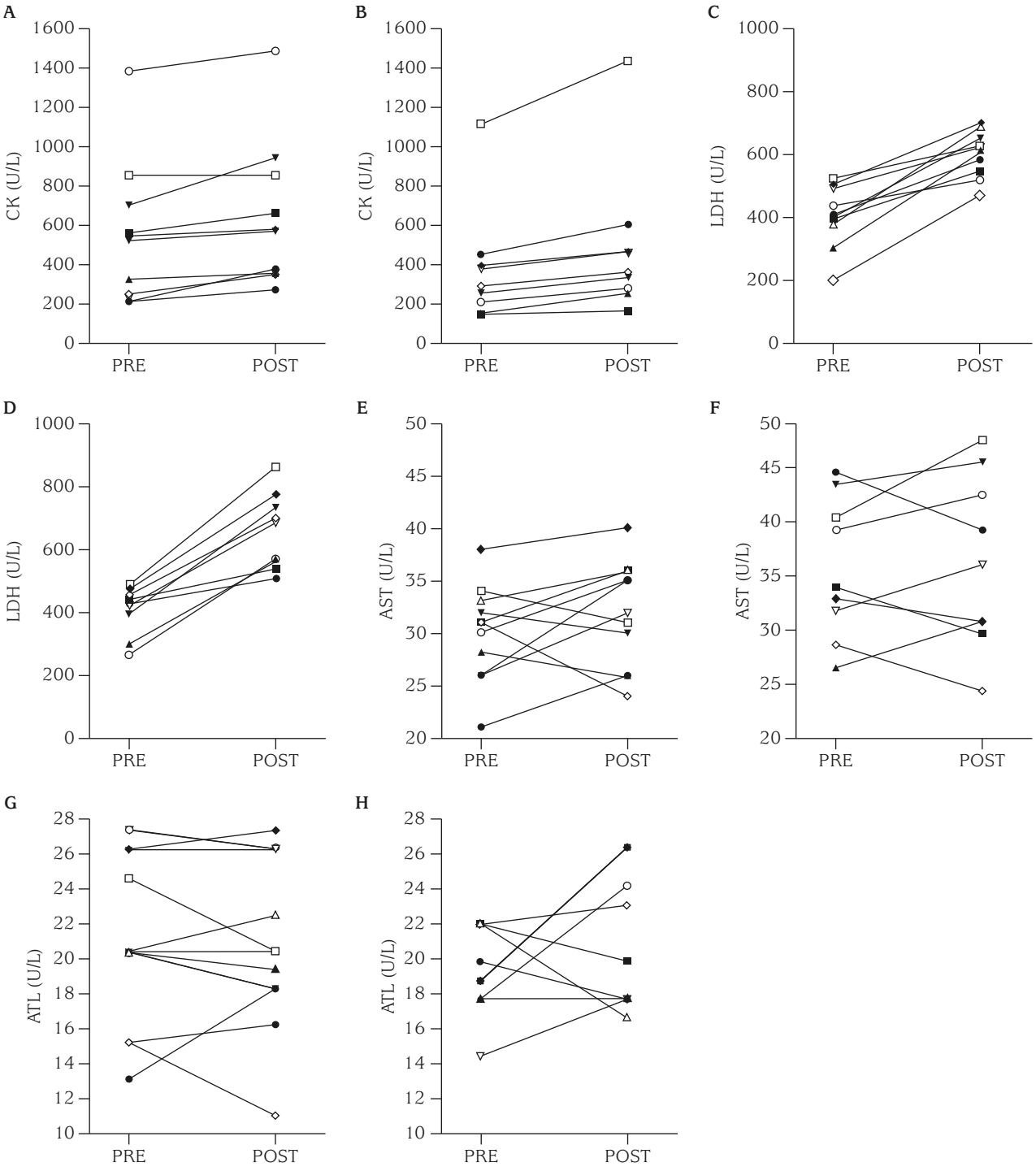

Fig. Individual enzyme kinetics. Each individual change in serum enzymes was plotted and bivariate linear regressions performed. (A) Creatine kinase (CK) concentration of EXP group. (B) CK concentration of CONT group. (C) Lactate dehydrogenase (LDH) concentration of EXP group. (D) LDH concentration of CONT group. (E) Aspartate aminotransferase (AST) concentration of EXP group. (F) AST concentration of CONT group. (G) Alanine aminotransferase (ALT) concentration of EXP group. (H) ALT concentration of CONT group. The Kolmogorov-Smirnov test with angular coefficients demonstrated non-significant differences between groups ($p > 0.05$).

The ultrastructural disruptions of muscle fibers, such as the breakage of exosarcomeric cytoskeleton proteins or the distortion of the alignment of the Z-disk induce the release of some growth factors known to affect immune cell activation and proliferation (Clarkson & Hubal 2002). In addition, sarcolemmal disruption occurs in the release of intracellular content. Enzymes such as CK, LDH, AST and ALT (all present in muscle fibers) are elevated in conditions when muscle damage is present (Pettersson et al. 2007). Our finding of increases in serum CK and LDH activity supports the notion that the high-intensity exercise protocol used in this study resulted in skeletal muscle injury.

The exercise-induced increase in serum CK activity was in the range reported for athletes in other studies (Brancaccio et al. 2007; Clarkson & Hubal 2002). The time course for changes in these markers of muscle damage may also be dependent on exercise protocol and/or training status. Previous studies demonstrate a peak in enzyme activity 24 hours after a session of exercise (Clarkson & Hubal 2002); however, many studies verify small but significant increases immediately afterwards and 1–3 hours after exercise (Bessa et al. 2008; Peake et al. 2005). Thus, our small (but significant) increase in enzyme activity may be associated with the shorter time course between the termination of exercise and blood collection, or perhaps reflect the function of the athletes' adaptations to the muscular exertion associated with soccer training.

The CK and LDH changes noted may reflect not only a loss of muscle cell membrane integrity, but also hepatocyte cellular disruption (Clarkson & Hubal 2002). The ALT and AST enzymes are two of the most reliable markers of hepatocellular injury or necrosis; although physical exercise is also known to cause transient elevations in these serum transaminases (Pettersson et al. 2007). To discern hepatic influence on serum enzyme responses, we also measured γ-GT and AP, which are dependable hepatocellular injury markers (Pettersson et al. 2007). Neither the transaminases nor γ-GT and AP displayed any significant alterations immediately after the exercise session. These results lead us to postulate that there was no hepatic injury as a result of intermittent exercise or caffeine supplementation. Our ALT and AST findings are at odds with those of Bassini-Cameron et al. (2007), which could be due to the slight methodological differences between the studies or the issue of hemoconcentration as noted earlier.

We feel that the findings from the current study can be applied to most soccer players undergoing similar training—acute caffeine supplementation does not increase muscle damage from stressful intermittent exercise. Clinicians, researchers, coaches and athletes should recognize, however, that few studies have the statistical power to detect severe adverse events. Furthermore, our results should not be generalized to athletes ingesting caffeine for extended periods of time, or to those ingesting caffeine in dosages above what is recommended, or to athletes engaged in training with an exaggerated eccentric component or plyometrics. Most certainly though, our data support that caffeine used as an ergogenic aid at a dose of $4.5\,mg \cdot kg^{-1}$ body mass does not increase the risk of damage to muscle integrity. Nonetheless, our findings are limited somewhat by the constraints of our study protocol and design (e.g. blood sampling).

In summary, this study indicates that intermittent-interval exercise of a variety used with soccer training augments the white blood cell population and increases serum muscle damage markers. This effect is not significantly affected by acute caffeine supplementation at a dosage of $4.5\,g \cdot kg^{-1}$ body mass. Furthermore, markers of hepatocellular injury were not significantly affected by this form of exercise or the level of caffeine supplementation used.

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