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Caffeine Does Not Augment Markers of Muscle Damage or Leukocytosis Following Resistance Exercise

Marco Machado, Alexander J. Koch, Jeffrey M. Willardson, Frederico C. dos Santos, Victor M. Curty, and Lucas N. Pereira

Purpose: The purpose of this study was to evaluate the effects of caffeine ingestion before a resistance exercise session on markers of muscle damage (CK, LDH, ALT, AST) and leukocyte levels. ***Methods:*** Fifteen soccer athletes completed two resistance exercise sessions that differed only in the ingestion of caffeine or a placebo preworkout. ***Results:*** CK concentration increased significantly following the caffeine session (415.8 ± 62.8 to 542.0 ± 73.5) and the placebo session (411.5 ± 43.3 to 545.8 ± 59.9), with no significant differences between sessions. Similarly, LDH concentration increased significantly following the caffeine session (377.5 ± 18.0 to 580.5 ± 36.1) and the placebo session (384.8 ± 13.9 to 570.4 ± 36.1), with no significant differences between sessions. Both sessions resulted in significant increases in the total leukocyte count (caffeine = 6.24 ± 2.08 to 8.84 ± 3.41; placebo = 6.36 ± 2.34 to 8.77 ± 3.20), neutrophils (caffeine = 3.37 ± 0.13 to 5.15 ± 0.28; placebo = 3.46 ± 0.17 to 5.12 ± 0.24), lymphocytes (caffeine = 2.19 ± 0.091 to 2.78 ± 0.10; placebo = 2.17 ± 0.100 to 2.75 ± 0.11), and monocytes (caffeine = 0.53 ± 0.02 to 0.72 ± 0.06; placebo = 0.56 ± 0.03 to 0.69 ± 0.04), with no significant differences between sessions. ***Conclusion:*** Ingestion of caffeine at 4.5 mg·kg^{-1} did not augment markers of muscle damage or leukocyte levels above that which occurs through resistance exercise alone.

Keywords: exercise performance, exercise physiology, exercise training, physical performance, resistance training, strength training

Caffeine (ie, trimethylxanthine) might be the most widely consumed pharmacologic and psychoactive substance, and is used by competitive and noncompetitive athletes as a legal ergogenic aid.[1] Previous studies and reviews have indicated that caffeine increased work output and continuous endurance time to exhaustion.[2,3] Other studies demonstrated that caffeine delayed fatigue and increased the contrac-

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tile force of cardiac and skeletal muscle.[3–8] Caffeine also decreased the perception of muscular pain, perceived exertion, and the reaction time to a stimulus.[9,10]

Several physiological and psychological mechanisms have been proposed through which caffeine may exert its ergogenic effects. Caffeine increases the activation of the sympathetic nervous system and decreases the perception of pain through binding and blocking of adenosine receptors. Increasing the activity of the sympathetic nervous system effects energy utilization (eg, increased lipolysis, glycogen sparing), muscle contractile processes (eg, increased intracellular calcium, improved Na^+-K^+ ATPase pump activity), hormone release (eg, catecholemines, β endorphins, cortisol), and cognition (eg, increased mental alertness).[11] The effects of caffeine ingestion before endurance exercise have been commonly studied.[3,12,13] However, less is known concerning the effects of caffeine ingestion before resistance exercise.

Resistance exercise induces an increase in sympathetic nervous system activity with subsequent increases in circulating hormones such as epinephrine, norepinephrine, and cortisol.[14] The increases in circulating hormones may coincide with perturbations in immune cell circulation and function.[15] Additionally, resistance exercise-induced mechanical and metabolic stresses can lead to disruption of muscle cell membranes and release of proinflammatory cytokines and leukocytes.[16] The disruption of muscle cell membranes increases membrane permeability and intracellular calcium ions, which promotes the activation of proteolytic enzymes.[17–19]

Previous studies demonstrated that caffeine ingestion before exercise increased the inflammatory response, as demonstrated by greater increases in markers of muscle damage and leukocyte levels.[12,20] 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 $A2_A$ adenosine receptors that are expressed on neutrophils. In addition, a blockade of adenosine receptors could reduce perceptions of pain during exercise,[21] 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,[23] which are largely responsible for the increase in leukocytes seen immediately post exercise via activation of β_2 receptors located on leukocytes.[15] Bassini-Cameron et al[20] hypothesized that the fatigue delaying effects of caffeine may augment the extent of muscle damage during intense exercise. The effects of caffeine ingestion before resistance exercise on markers of muscle damage and immune responses have not been fully elucidated. Therefore, the purpose of the current study was to evaluate the effect of caffeine ingestion before resistance exercise on markers of muscle damage and leukocytosis.

Methods

Subjects

Fifteen male soccer athletes were asked to participate (age = 18.4 ± 0.8 y, height = 177.5 ± 4.6 cm, mass = 71.8 ± 7.1 kg). Subjects had not used any dietary supplements (eg, creatine) or drugs (eg, anabolic steroids) that may confound the outcomes of the current study. Subjects were characterized by a similar lifestyle and had been

closely monitored for 8 weeks during preseason and preparatory training cycles; all were light caffeine consumers (ie, < 100 mg·d^{-1}) and had previously participated in experiments involving resistance exercise. Written informed consent was obtained from the subjects, who were instructed concerning the procedures of the study. The experimental procedures were approved by the local Institutional Review Board.

A double-blind, placebo-controlled experimental design was used, with all subjects serving as their own controls. All subjects performed two resistance exercise sessions that were separated by one week. Each subject was given a comprehensive list of caffeine containing foods and drinks and was asked to abstain from these products during the 12 h before the experimental sessions. In randomized order, caffeine or a placebo was ingested in the form of indistinguishable capsules, so that the subjects were not aware of which substance was ingested. The caffeine (Purifarma, China) was administered at 4.5 mg·kg^{-1} and the placebo consisted of 500 mg of lactose (Galena, Germany). Before each exercise session, subjects ingested the caffeine or placebo immediately preceding the blood draws; this was followed by 45 min of inactivity, and then a 10-min warm-up. The warm-up consisted of aerobic activity and light static stretching.

Screening

Before the experimental exercise sessions, subjects were tested on two separate days to determine a 10-repetition maximum (ie, 10-RM) for the bench press, pullover, biceps curl, triceps extension, leg extension, and prone leg curl. All exercises were performed on resistance machines (Righetto, Brazil). To minimize possible errors in the 10-RM assessments, the following strategies were employed (a) on each day the exercise testing proceeded in the same sequence as described above, (b) all subjects received standard instructions on exercise technique, (c) exercise technique was monitored and corrected as needed, and (d) all subjects received verbal encouragement.

Exercise Bouts

Following the 10-RM assessments, two resistance exercise sessions were performed seven days apart in the same sequence as described above. The two resistance exercise sessions differed only in the ingestion of caffeine or a placebo preworkout. All exercises were performed for three sets of 10 repetitions with the predetermined 10-RM; a 2-min rest interval was instituted between the sets and exercises. The repetition cadence was controlled with a digital sound signal (Beat Test & Training, CEFISE, Brazil) that was adjusted so that each repetition was completed in approximately 2 s. A spotter gave minimal assistance if necessary so that ten repetitions were completed on all three sets for each exercise. Therefore, the volume of work (load × sets × repetitions) was equalized between the experimental sessions.

Blood Collection and Analysis

Venous blood samples were collected from the forearm with subjects in a seated position. The first sample (PRE) was collected preworkout and the second sample (POST) was collected immediately postworkout. After collection, the sample was divided in two glass tubes (one with anticoagulant ethylenediaminetetraacetic 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. From the serum sample, creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were 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 measured.

Statistical Analyses

The reliability of the 10-RM assessments was determined through calculation of intraclass correlation coefficients. A 2 (PRE vs. POST) $\times$ 2 (caffeine versus placebo) repeated-measures ANOVA (with Tukey post hocs) was conducted to compare differences for all variables. Significant differences were determined based on an alpha level of less than 0.05. All statistical analyses were performed using SPSS 15.0 (LEAD Technologies, Inc., IL, USA).

Results

The 10-RM assessments for each exercise demonstrated high reliability: bench press (intraclass $r = .92$), pullover (intraclass $r = .80$), biceps curl (intraclass $r = .73$), triceps extension (intraclass $r = .87$), leg extension (intraclass $r = .84$), and prone leg curl (intraclass $r = .86$).

The CK concentration increased significantly following the caffeine session (415.8 ± 62.8 to 542.0 ± 73.5; $P = .045$) and the placebo session (411.5 ± 43.3 to 545.8 ± 59.9; $P = .028$), with no significant differences between sessions ($P = .463$; see Figure 1). Similarly, the LDH concentration increased significantly following the caffeine session (377.5 ± 18.0 to 580.5 ± 36.1; $P = .043$) and the placebo session (384.8 ± 13.9 to 570.4 ± 36.1; $P = .003$; see Figure 1), with no significant differences between sessions ($P > .05$). There were no significant increases following either session in ALT (caffeine 20.0 ± 4.0 to 22.0 ± 3.0, $P = .752$; placebo 20.0 ± 5.0 to 22.0 ± 3.0, $P = .550$) and AST (caffeine 23.0 ± 4.0 to 24.0 ± 3.0, $P = .797$; placebo 23.0 ± 4.0 to 24.0 ± 3.0, $P = .727$) concentrations.

Both sessions resulted in significant increases in the total leukocyte count (caffeine = 6.24 ± 2.08 to 8.84 ± 3.41, $P = .0002$; placebo = 6.36 ± 2.34 to 8.77 ± 3.20, $P = .0073$), neutrophils (caffeine = 3.37 ± 0.13 to 5.15 ± 0.28, $P = .00001$; placebo = 3.46 ± 0.17 to 5.12 ± 0.24, $P = .00001$), lymphocytes (caffeine = 2.19 ± 0.091 to 2.78 ± 0.10, $P = .00001$; placebo = 2.17 ± 0.100 to 2.75 ± 0.11, $P = .00001$), and monocytes (caffeine = 0.53 ± 0.02 to 0.72 ± 0.06, $P = .0042$; placebo = 0.56 ± 0.03 to 0.69 ± 0.04, $P = .0006$), with no significant differences between sessions ($P > .05$). Eosinophils did not significantly increase (caffeine = 0.20 ± 0.01 to 0.19 ± 0.02, $P = .2005$; placebo = 0.19 ± 0.02 to 0.21 ± 0.02, $P = .3701$) following either session.

Discussion

Previous studies have demonstrated elevations in circulating levels of leukocytes following exercise.[16,18,23,24] Increases in circulating levels of epinephrine, and to a lesser extent, norepinephrine during exercise are thought to be the major mechanisms
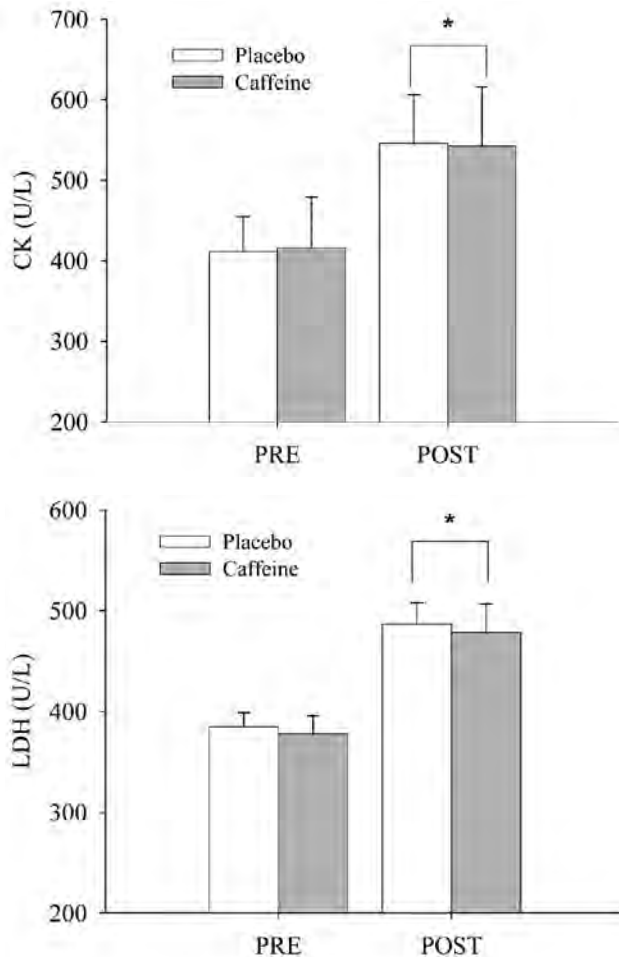
Figure 1 — Effect of exercise and caffeine ingestion on acute serum CK (top) and LDH (bottom) activity. *P < .05, PRE vs POST.

mediating the immediate postexercise rise in leukocytes.[15] The immune system plays a role in skeletal muscle repair, so the degree of exercise induced microtrauma might be reflected in the circulating levels of leukocytes. A recent review by Tidball[16] described many mechanisms by which leukocytes are involved in the inflammatory response induced by exercise. For example, leukocytes may act directly (eg, phagocytosis) or indirectly (eg, cytokine release) in the process of muscle tissue regeneration and subsequent hypertrophy.

In the current study, the ingestion of caffeine before resistance exercise did not augment circulating levels of leukocytes above those which occurred through resistance exercise alone. These results were consistent with Walker et al[12] who demonstrated that cortisol levels were not augmented when 6.0 mg·kg⁻¹ caffeine

was ingested before cycling exercise at 65% of V_{O2max} for 120 min. Conversely, the results of the current study were not consistent with Bishop et al[23] who demonstrated that epinephrine and lymphocyte levels were augmented when caffeine was ingested at 6.0 mg·kg⁻¹ before cycling exercise at 70% of V_{O2max} for 90 min. These inconsistencies might be accounted for due to lower dosage of caffeine in the current study and the potential for differences in caffeine tolerance between subjects.

Elevations in circulating epinephrine are thought to be a primary mechanism behind exercise-induced alterations in leukocyte levels,[15] and caffeine ingestion raises epinephrine levels.[23] It is unfortunate that we did not measure epinephrine in the current study, as it would have strengthened our understanding of the role of caffeine on the leukocyte response to resistance exercise. From the current data, we can only state that caffeine at a dose of 4.5 g·kg⁻¹ did not alter leukocyte levels in response to resistance exercise.

The increases in total leukocyte count, in addition to serum CK and LDH concentrations, supports the notion that the resistance exercise protocol used in the current study resulted in skeletal muscle microtrauma. The efflux of CK and LDH from skeletal muscle may occur as a result of increases in the permeability of the muscle cell membrane and/or the temporary reorganization of the intramuscular vasculature.[25,26] The exercise induced increase in serum CK concentration was in the range demonstrated by athletes in a previous study (ie, 82 to 1083 U·L⁻¹).[26]

In the current study, serum CK and LDH concentrations peaked at 580 U/L and 485 U/L immediately following exercise. Previous studies demonstrated that the peak enzyme concentrations were reached within 24 to 48 h; however, small but significant increases occurred immediately postworkout.[18,26] The higher acute responses demonstrated in the current study versus the delayed responses demonstrated in other studies[18,26] might be due to the types of exercises examined; downhill running on a treadmill at 60% of V_{O2max} for 45 min[18] or cycling at a workload of 1.5 kp (60 rpm) for 90 min.[26] The total body circuit used in the current study, which incorporated moderate intensity sets to voluntary exhaustion, may have represented a greater threat to the maintenance of homeostasis.

The current study demonstrated that resistance exercise resulted in increased LDH and CK concentrations, but not in ALT and AST concentrations. ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. Previous studies have demonstrated acute increases in the concentrations of these enzymes following exercise.[28,29] Pettersson et al[28] demonstrated increased concentrations of AST and ALT 48 h following a resistance exercise session. We cannot rule out the possibility that in the current study, increased concentrations of these enzymes may have occurred if subjects had been tracked over longer time periods.

In contrast to the results of the current study, Bassini-Cameron et al[20] demonstrated that caffeine ingestion augmented circulating levels of leukocytes. Subjects in the Bassini-Cameron et al study participated in a simulated soccer match that was followed by the Yoyo test. However, because caffeine ingestion may have delayed fatigue; subjects could have performed a higher volume of work during the Yoyo test following ingestion of caffeine. A higher volume of work, rather than ingestion of caffeine per se, may have resulted in higher levels of circulating leukocytes. Unfortunately, Bassini-Cameron et al did not report the Yoyo performance results. An advantage of the current study was that during each resistance exercise session (ie, caffeine or placebo) the volume (load × sets × repetitions) of work was equalized so that the true effects of caffeine could be delineated.[24]

In summary, the current study demonstrated that moderate intensity sets stimulated increased concentrations of skeletal muscle enzymes and leukocytes. The key finding was that the ingestion of caffeine at 4.5 g·kg^{-1} did not augment markers of muscle damage or leukocyte levels. In a study of elite athletes, Turncliffe et al[30] found that average daily caffeine intake was approximately 0.85 mg·kg^{-1}. Thus based on the current data, it appears that the ingestion of caffeine at normal dietary levels may not cause greater muscle cell injury above that which occurs through resistance exercise alone. However, if caffeine were ingested at doses high enough to provide an ergogenic effect (≥ 6.0 mg·kg^{-1}), it remains possible that an adverse effect on muscle damage or leukocyte levels could become apparent. In addition, a more intense exercise bout would likely have elicited more muscle damage and it is possible that caffeine may have exerted a different effect in the hormonal milieu following a more intense bout.

Conclusion

Caffeine might be the most widely used stimulant on earth. Data from the current study can be applied to resistance trained athletes when performing moderate intensity sets. Caffeine supplementation did not augment muscle damage from a stressful bout 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.

The results of the current study cannot be generalized to athletes ingesting caffeine for extended time periods, to those ingesting caffeine in higher dosages, or to athletes engaged in resistance exercise with an exaggerated eccentric component or plyometrics. The ingestion of caffeine at 4.5 mg·kg^{-1} does not appear to cause greater muscle cell injury above that which occurs through resistance exercise alone. Future studies should examine physiological responses with caffeine ingestion over longer time periods and with variations in resistance exercise prescriptive variables.

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