Original Article

Effects of acute caffeine on muscle damage biomarkers and time to exhaustion after a single session of resistance exercises followed by exhaustive incremental test in long-distance runners

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ABSTRACT

The present study was designed to investigate the acute effect of caffeine on muscle damage biomarkers (creatine kinase, lactate dehydrogenase, creatine kinase MB, and myoglobin) measured before, immediately after, and 24 h after a single session of resistance exercises followed by exhaustive incremental test. In addition, the effect of caffeine intake on time to exhaustion during exhaustive incremental test was determined. Fifteen male long-distance runners (30.67 ± 3.40 yrs.) performed two consecutive trials (7 days apart). Athletes were assigned randomly either to ingest caffeine (6 mg/kg) 1 h prior to exercise or placebo using a double-blind crossover design. Each trial consisted of 5 resistance exercises followed by exhaustive incremental test. Blood samples were collected before, immediately, and 24 h after each trial. The independent *t* test of data showed no significant differences in biomarkers of muscle damage at all time points between trials ($p > .05$). Using paired sample *t* test, data revealed that caffeine increased the time to exhaustion (45.78 ± 2.42 min) during exhaustive incremental test compared to the placebo (43.83 ± 2.21 min) ($p = .001$). In conclusion, 6 mg/kg of caffeine 1 hour prior to resistance exercises followed by exhaustive incremental test had no effect on muscle damage biomarkers in long-distance runners probably due to mechanical stress precisely affected fast twitch fibres rather than slow twitch fibres. However, the increased time to exhaustion due to caffeine consume may attributed to dampened pain sensation.

Keywords: Ergogenic; Eccentric action; Myoglobin; Ryanodine receptor; Sarcomere.

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INTRODUCTION

It has been shown that elite endurance athletes engaged in resistance exercise within training session to enhance maximal speed (Taipale et al., 2014) and improve running economy (Burt, Lamb, Nicholas, & Twist, 2014; Vikmoen et al., 2016). However, both resistance exercise and prolonged running, particularly when the acceleration is increased at the end of a race, require a high load eccentric contractions (Owens, Twist, Cobley, Howatson, & Close, 2019) that may produce exercise-induced muscle damage (EIMD) (Mackey & Kjaer, 2017). Of relevance, tensile force generated during eccentric contractions can induce disruption of intramuscular structural proteins (*i.e.* z-discs degradation) (Hume, Cheung, Maxwell, & Weerapong, 2004; Owens et al., 2019), sarcomere disruption, and filament tears (Burt et al., 2014; Naclerio, Larumbe-Zabala, Cooper, Jimenez, & Goss-Sampson, 2014). This leads to leakage of intramuscular proteins into blood stream, such as creatine kinase (CK) and lactate dehydrogenase (LDH), associated with localized swelling (Owens et al., 2019). Additionally, it has been shown that myoglobin (Del Coso et al., 2013; Naclerio et al., 2014; Ramos-Campo et al., 2016), CK MB, a cardiac marker used to assist diagnosis of an acute myocardial infarction and/or damage, and cardiac troponins are the more specific biomarkers of both skeletal and cardiac muscle damage during strenuous and prolonged exercise (Lippi et al., 2008; Ramos-Campo et al., 2016). Consequently, functional symptoms will occur including delayed-onset muscle soreness (DOMS) (Marquez-Jimenez et al., 2018), which may increase with motion (Hume et al., 2004), reduced maximal strength (Marques-Jimenez et al., 2018), and decreased range of motion (Mackey & Kjaer, 2017).

Reduction in force production resulting from muscle damage may last a days after training session (Taipale et al., 2014). This could result in muscle soreness, that may increase with high CK accumulation, and subsequent alteration of excitation-contraction coupling system (Burt et al., 2014; Naclerio et al., 2014), contributing to poor performance.

Ergogenic substances have been reported to improve performance. Caffeine, a natural alkaloid, is the most frequently consumed pharmacological substance in the world (Nawrot et al., 2003; Vieira et al., 2017). It acts as an adenosine receptor antagonist (Nawrot et al., 2003) and central nervous system (CNS) stimulant (da Costa Santos et al., 2011; Vieira et al., 2017). In addition, caffeine can activate protein kinase A (PKA) pathway (Horrigan, Kelly, & Connor, 2006) by its ergogenic effect on inhibition of cyclic adenosine monophosphate-phosphodiesterase and by increased levels of intracellular cAMP (Horrigan et al., 2006; Machado, Zovico, & da Silva, 2008). During exercise, caffeine might elicit the hypothalamic-pituitary-adrenal axis, resulting in increased catecholamines release (Bishop, Fitzgerald, Potter, Scanlon, & Smith, 2005) and subsequent increased energy expenditure. Moreover, caffeine may rescue the damaged muscle fibres, probably through the robust antioxidant effect of its metabolites that reduce oxidative stress (Cechella et al., 2014).

Several studies have been demonstrated that caffeine (4.5 - 9 mg/kg) had no effect on muscle damage biomarkers following resistance exercise (Machado et al., 2008; Nosaka, Newton, & Sacco, 2002; Soleimani, Shakerian, & Ranjbar, 2017; Zarghami-Khameneh & Jafari, 2014). In contrast, there are studies reported that caffeine (5, 6, and 9 mg/kg) intake could enhance endurance exercise (Glaister, Muniz-Pumares, Patterson, Foley, & Mcinnes, 2015; Lee, Lin, & Cheng, 2011; Wu & Lin, 2010), particularly prolonged and exhaustive exercise (Wu & Lin, 2010). To our knowledge, however, there is no research investigated the effects of caffeine on biomarkers of muscle damage following both resistance exercise and exhaustive performance in long-distance runners. Consequently, the aims of the present study were to investigate the effect of acute caffeine (6 mg/kg) 1 h prior to a single session of resistance exercises followed by exhaustive incremental test on muscle damage biomarkers (CK, LDH, CK MB, and myoglobin) measured before, immediately after,

and 24 h after a session, and to determine the effect of caffeine on time to exhaustion during exhaustive incremental test, compared to placebo. We hypothesized that caffeine may enhance exhaustive incremental performance without attenuation of muscle damage biomarkers due to mechanical stress may affect performance during resistance exercise rather than prolonged running.

METHODS

Participants

Fifteen male long-distance runners who were familiarized with resistance exercise and exhaustive incremental test participated in this study. Demographic characteristics are described in Table 1. Women were excluded from the study due to menstruation and/or utilization of oral contraceptive pills may affect caffeine clearance (Astorino et al., 2008). Any athlete who reported a history of injury such as sprain, DOMS, or if they ingested any ergogenic aids 48 prior to beginning of the study that might have affected performance was excluded from participation. Background information for medical vital factors was used to assess athletes for inclusion. Athletes were light coffee consumer (1 - 2 cup per day; < 100 mg/day). Participants were provided with written informed consent that explained the benefits and any risk associated with participation in the study. The experimental procedures were approved by the Local Scientific Ethics Committee (process 04/2019/M A).

Table 1. Descriptive characteristics of the 15 participants.

Variables	Mean ± SD
Age (years)	30.67 ± 3.40
Height (cm)	176.87 ± 3.16
Weight (kg)	67.87 ± 2.64
BMI (kg/m2)	21.63 ± .80
Resting HR (bpm)	56.07 ± 2.09
VO2max (ml/kg/min)	59.40 ± 4.83
Training volume (min/week)	444.0 ± 71.89
Training experience (years)	8.67 ± 3.40

Study design

Athletes were randomly assigned to either caffeine (CAF) or placebo (PLA). Before data collection, caffeine and placebo were coded. A double-blind crossover design was used, as neither examiners nor athletes were aware of experimental intervention. The trials (CAF and PLA) were separated by one week to allow a complete recovery. Each trial consisted of 5 resistance exercises (bench press, biceps curl, triceps pushdown, leg press, and leg extension) followed by treadmill exhaustive incremental test. Both trials were performed at the same time of the day (8.00 AM) to avoid circadian interference. Before beginning a trial, each athlete visited the laboratory three times over 7 days period. In the first visit, they performed a one repetition maximum (1-RM) test for each exercise and were submitted to an exhaustive incremental test. A familiarization with these tests were performed during the second and third visits. Athletes were instructed to refrain from any intake of coffee, green tea, and soft drinks one day prior to each trial. They were asked to abstain from high intensity exercise 72 prior to each trial, and too fast for at least 3 hours prior to trials. Athletes were asked to drink 500 ml of water 2 h prior to beginning of a trial to prevent any possible dehydration. All resistance exercises and exhaustive incremental test during both trials were performed in at 21 - 23 ˚C and 51- 52% relative humidity.

Supplementation

Athletes ingested an opaque capsule containing either 6 mg/kg of caffeine (CAF trial) or 6 mg dextrose with 150 mL water (18 ˚C) one hour before each trial. The dose was chosen due to supplementation range (3 - 6 mg/kg body mass) has previously been shown to raise plasma caffeine levels (Graham, 2001) and improve performance.

Experimental protocol

After one hour of supplementation ingestion (CAF or PLA) while setting at the laboratory, athletes initially warmed up on the leg curl (30 - 45 kg) machine and French press (8 - 15 kg) for 2 sets × 10 repetitions at a load of 50% of the estimated 1-RM, with 2 min recovery between sets and exercises, and muscles stretching for 3 minutes. Thereafter, they begun a trial, which initially performed a resistance exercise test consisted of bench press, biceps curl, triceps pushdown, leg press, and leg extension. Rest period were 5 min after completion of these exercises. Athletes then performed exhaustive incremental test on treadmill until volitional exhaustion. Athletes returned one week later and reported the same experimental protocol after ingesting the other intervention. They were asked to answer some questions about symptoms or problems regarding their health status. The entire trial took roughly 90 min.

Resistance exercise

After one hour of ingestion either caffeine or placebo, athletes completed 5 resistance exercises: bench press, biceps curl, triceps pushdown, leg press, and leg extension. Each exercise technique was performed based on the guidelines of the national strength and the American College of Sports Medicine (ACSM, 2010). All athletes received the guidelines for each exercise before the beginning of the trials. Athletes received verbal encouragement during performance. They performed 3 sets of 10 repetitions at 60% 1-RM. Rest periods were two min between exercises and sets. Determination of 1-RM ensued according to the method of Willoughby et al. (2014). Upper- and lower-body 1-RM tests were performed using bench press, biceps curl, triceps pushdown, leg press, and leg extension. The 1-RM test started by a warm-up that consisting of 10 repetitions at estimated 50% 1-RM. Following a 2-min recovery, a load of 60% of estimated 1-RM was used to perform 5 repetitions. Thereafter, the weight gradually increased until the athletes could perform only one repetition in a proper technique, with 2 min recovery in between each successful attempt. Pilot testing showed no difference in 1-RM bench press ($t = 1.63$, $p = .22$), biceps curl ($t = 0.54$, $p = .71$), triceps pushdown ($t = 0.46$, $p = .59$), leg press ($t = 1.53$, $p = .19$), and leg extension ($t = 0.77$, $p = .84$). The tests were measured over 2 days.

Exhaustive incremental test

The exhaustive incremental test was carried out on a treadmill (Techno-Gym, Lifefitness-6322, USA). Athletes began the test with a standardized 5 min warm-up at 7 km/h. Then, they initially ran at 8 km/h. Every 5 minutes, the speed was increased 1 km/h until volitional exhaustion. Athletes received verbal encouragement to continue running as long as possible. Running tolerance was defined as the incapacity to maintain a normal step cadence on treadmill's belt. At this point, the examiner presses on the emergency button, and record the time.

Blood samples collection and analysis

The blood samples were collected before (pre), immediately after (post), and 24 h after (post 24 h) trial. All biomarkers variables were stored in plane tube and centrifuged at 5000 RPM for 5 min. CK and LDH were analysed using (Roche Diagnostics GmbH, Mannheim, Germany), while CK MB, and myoglobin were assayed by (Elecsys 2010, Roche, Germany). The reference ranges of variables were as follows: 38 - 197 U/L for CK, 200 - 400 U/L for LDH, 7 - 6 ng/mL for CK MB, and 28 - 72 ng/mL for myoglobin.

Statistical analysis

Shapiro-Wilk test was applied to check for normal distribution. All the analysed variables (muscle damage biomarkers) were normally distributed (p > .05). Independent *t* test was utilized to analyse the differences in muscle damage biomarkers (CK, LDH, CK MB, and myoglobin) at the 3 time points between trials. A one-way ANOVA with *post hoc* Bonferroni corrections was utilized to determine differences among the 3 time points within the trial. Time to exhaustion during exhaustive incremental test was analysed using Paired sample *t* test. Statistical analyses were carried out by SPSS version 23.0. Descriptive statistics are reported as Mean ± SD. Differences were considered statistically significant at $p < .05$.

RESULTS

Table 2 illustrates the effect of caffeine or placebo on muscle damage biomarkers before, immediately after, and 24 h after trial. Independent *t* test showed no significant differences between trials in CK, LDH, CK MB, and myoglobin before the beginning of resistance exercises (F = 0.073, $p = .789$; F = 0.069, $p = .795$; F = 0.024, $p = .879$; F = 2.530, $p = .123$, respectively). Data also demonstrated no significant differences between trials in all biomarkers immediately after the exhaustive incremental test (F = 0.008, $p = .930$; F = 0.021, $p = .885$; F = 0.403, $p = .531$; F = 0.008, $p = .931$, for CK, LDH, CK MB, and myoglobin, respectively). In addition, caffeine failed to make significant differences in CK, LDH, CK MB, and myoglobin after 24 hours of completion of a single session compared to placebo (F = 0.026, $p = .874$; F = 0.102, $p = .752$; F = 1.008, $p = .324$; F = 0.020, $p = .890$, respectively). A one-way ANOVA with *post hoc* Bonferroni revealed significant differences in muscle damage biomarkers between two points (before and immediately after) within a trial ($p < .05$), and between the points of immediately after and 24 h after within a trial ($p < .05$).

Table 2. Results (mean ± SD) of biomarkers of muscle damage before (pre), immediately after (post), and 24 h after (post-24 h) a single session consisted of resistance exercises followed by exhaustive incremental test in 15 long-distance runners.

Variables	PLA trial			CAF trial		
	Pre	Post	24 h	Pre	Post	24 h
CK (U/L)	167.2 ± 19.4	289.6 ± 21.8[a]	394.5 ± 18.4[b]	169.0 ± 17.9	291.9 ± 21.4[a]	385.6 ± 13.3[b]
LDH (U/L)	295.3 ± 25.9	390.3 ± 27.3[a]	339.2 ± 21.0[b]	293.6 ± 24.8	392.3 ± 26.3[a]	328.0 ± 10.8[b]
CK MB (ng/ml)	4.4 ± 0.2	4.9 ± 0.3[a]	5.9 ± 0.2[b]	4.3 ± 0.2	4.8 ± 0.3[a]	5.6 ± 0.1[b]
Myoglobin (ng/ml)	38.5 ± 5.6	101.2 ± 25.2[a]	63.1 ± 12.3[b]	39.6 ± 3.9	103.2 ± 25.7[a]	54.1 ± 9.6[b]

[a] Significant difference after exercise between pre and immediately-post within a trial. [b] Significant differences after exercise between post and post-24 h within a trial. No significant differences were detected for any of the biomarkers of muscle damage between trials. Significant level was set at p < .05. PLA Placebo, CAF Caffeine, CK Creatine kinase, LDH Lactate dehydrogenase, CK MB Creatine kinase MB.

Paired sample *t* test showed that the time to exhaustion was significantly longer in CAF trial (45.78 ± 2.42 min), with an effect size = 1.0 compared to PLA (43.83 ± 2.21 min) (t = -8.32, $p = .001$) (Figure 1). Athletes performed in the range of 40.22 min to 47.44 min (16 km/h - 17 km/h) in the PLA trial and 42.31 min to 50.12 min (16 km/h - 18 km/h) in the CAF trial.

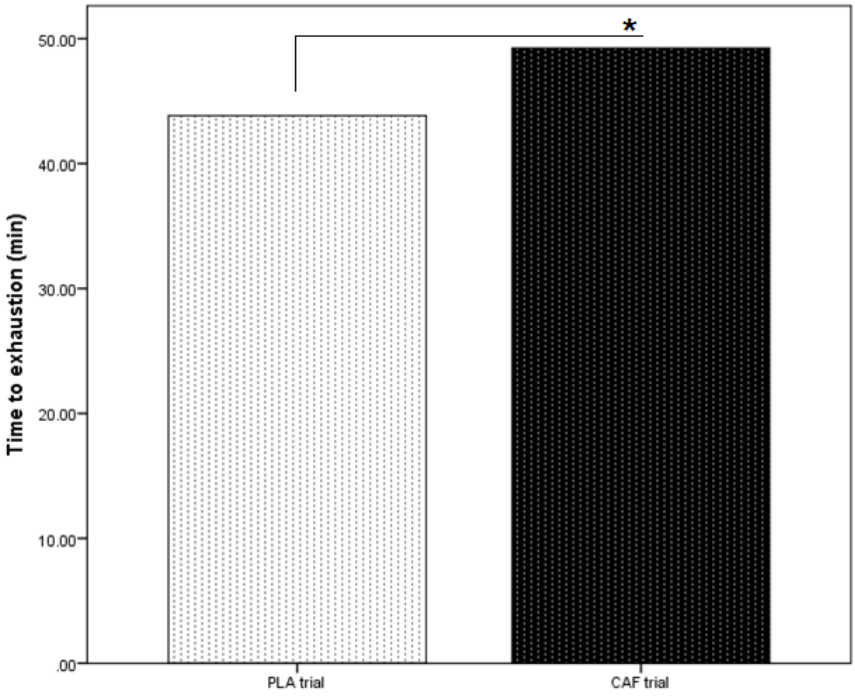

Figure 1. Time to exhaustion during exhaustive incremental test following resistance exercises in both trials (CAF and PLA). Time to exhaustion was significantly longer in CAF trial (45.78 ± 2.42 min) compared to PLA (43.83 ± 2.21 min) (t = -8.32, p = .001).

DISCUSSION

The results of this study indicated that an acute ingestion of 6 mg/kg of caffeine 1 h prior to a single session of both resistance exercises and exhaustive incremental test significantly failed to protect muscle fibres from damage biomarkers including CK, LDH, CK MB and myoglobin immediately after and 24 h after the session, but significantly increased the time to exhaustion compared to the placebo trial.

Regarding the resistance exercises, similar to our findings several studies have shown that caffeine had no effect on muscle damage biomarkers following resistance exercise (Machado et al., 2008; Nosaka et al., 2002; Wu & Lin, 2010). This result may be a consequence of mechanical stress and metabolic perturbations (Machado et al., 2008). Mechanical stress produced by eccentric muscle actions (Owens et al., 2019), which can induce muscle damage (Mackey & Kjaer, 2017), occurs at high rate during prolonged running (Owens et al., 2019; Taipale et al., 2014) and resistance exercise (Owens et al., 2019). In addition, high volume of resistance exercise could induce cellular membrane disruption (Machado et al., 2008), resulting in increased influx of calcium into skeletal muscle cell, which activate proteolytic enzymes (i.e. caspase and calpain) (Nosaka et al., 2002) that are responsible for apoptosis and inflammation. Further, it has been thought that fast-twitch fibres predispose to damage rather than slow-twitch fibres because resistance exercise activates the faster motor units (Owens et al., 2019). This could be explained by our findings that demonstrated similar biomarkers levels of muscle damage between PLA and CAF trials, particularly between immediately and 24 h post exercise. Another explanation in which biomarkers of muscle damage appeared after resistance exercise, regardless of caffeine consumption, is that EIMD might induce macrovascular stiffness (Barnes, Trombold, Dhindsa, Lin, & Tanaka, 2010), resulting in changes in vascular extensibility during exercise. In

this regard, Caldwell et al. (2016) demonstrated macrovascular dysfunction during moderate eccentric muscle damage in 13 unaccustomed eccentric EIMD, but without caffeine intervention.

The result of our study agreed with the findings of Machado et al. (2008) who found that resistance exercises (bench press, pullover, biceps curl, triceps curl, leg extension, and lying leg curls) caused increases in skeletal muscle markers including CK, LDH, aspartate aminotransferase (AST), alanine aminotransferase (ALT), but were not significant between caffeine (4.5 mg/kg) and placebo in male soccer players. Soleimani et al. (2017) reported that caffeine loading (2 g per day for 2 weeks) had no significant effect on serum high-sensitivity C-reactive protein (hs-crp) and CK after exhaustive aerobic exercise in 22 overweight college students. They suggested that inflammation markers may be diminished as a result of adaptation to exercise. Zarghami-Khameneh & Jafari (2014) showed that different doses of caffeine (6 and 9 mg/kg) 1 h prior to endurance exercise until exhaustive had no effect on total serum CK and LDH at both immediately and 24 h after exercise in male volleyball players compared to placebo. However, Wistar rats who received either tap water or caffeine (1 mg/ml) before swimming for about 40 min/day, 5 days per week for total of 30 days, demonstrated that serum CK was significantly reduced in the sedentary caffeine group (787.3 ± 230.3 U/L), trained control (775 ± 232.3 U/L), and trained caffeine (379.5 ± 110.5 U/L) compared to sedentary control (1610.2 ± 276.5 U/L) (da Costa Santos et al., 2011).

In the current study, CK levels reached to 291 ± 21 U/L immediately post and 385 ± 13 U/L 24 hours post exercise in the CAF trial compared to PLA (289 ± 21 U/L, 394 ± 18 U/L, respectively). These values seem to be slightly higher compared to findings of Lippi et al. (2008), who observed, without caffeine treatment, that CK levels reached 176 ± 32 U/L and 227 ± 29 U/L 24 after a 21-km run compared to pre-exercise (127 ± 25 U/L) in healthy-trained Caucasian males. This variance might be attributed to the characteristics of participants. Basically, the elevations of biomarkers following exercise are usually higher in untrained and/or recreationally trained players than competitive athletes. Importantly, it has been shown that muscle damage biomarkers, such as myoglobin, LDH, and CK were increased more than 5-fold immediately after strenuous exercise (Zainudin, Caszo, Knight, & Gnanou, 2019). In addition, the value of muscle damage biomarkers could depend on several aspects including the type of working skeletal muscle, type of contractions, characteristic of marker, exercise intensity, duration of training session, training level, and time of recovery.

On the other side, some studies have reported beneficial effect of caffeine on muscular strength and endurance (Beck et al., 2006; Woolf, Bidwell, & Carlson, 2008), but the others have failed (Astorino, Rohmann, & Firth, 2008; Machado et al., 2008). Trexler et al. (2016) reported improved repetitions to fatigue for leg press and bench press in coffee (8.9 g, yielding 303 mg caffeine) compared to caffeine (300 mg, yielding 3-5 mg/kg) and placebo in resistance-trained men. But they observed that the sprint test (5, 10 s cycling ergometer) that was completed following resistance exercise was improved in the caffeine trial compared to coffee and placebo. However, they did not measure any signs of muscle damage. Similarly, Duncan, Stanley, Parkhouse, Cook, & Smith. (2013) found that caffeine ingestion (5 mg/kg) 1 h prior to resistance-strength endurance enhanced repetitions to failure in caffeine trial (19.3 ± 3.7 rep) compared to placebo (18.5 ± 3.7 rep) in 11 resistance-trained individuals (9 males, 2 females). What is more important that our protocol was not designed to repetitions to fatigue to maintain participants' ability to complete the incremental test. Astorino et al. (2008) reported no differences (12% and 11%) in total weight lifted to failure during moderate (60% 1-RM) bench press and leg press after 1 h of caffeine (6 mg/kg) ingestion in 22 resistance-trained men compared to placebo. Grgic & Mikulic (2017) showed that 6 mg/kg of caffeine intake 1 h prior to exercise enhanced muscular strength for barbell squat (+2.8%) and muscular power that assessed by seated medicine ball throw (+4.3%) but not muscular endurance which represented repetitions of back squat and bench press (60% 1-RM) to momentary muscular failure in 17 resistance-trained men compared

to placebo. They observed that the rating of perceived exertion due to decreased pain feelings was significantly lower with caffeine intake. In a study conducted with Assault™ supplement containing caffeine and other gradients, Spradely et al. (2012) observed that Assault™ improved muscular endurance for leg press (13 ± 6 rep) compared to placebo (11 ± 3 rep). They suggested that caffeine contained in supplement could increase energy expenditure and delayed onset of fatigue.

Surprisingly, our results showed that caffeine increased time to exhaustion during exhaustive incremental treadmill test following resistance exercises compared to PLA. The explanation of this result might be attributed to beneficial binds of caffeine to ryanodine receptor (Lee et al., 2011), which elicit calcium kinetics from sarcoplasmic reticulum (Owens et al., 2019; Trexler et al., 2016), potentiating sustained contraction cycle, and subsequently improved performance. Additionally, the inhibition of adenosine uptake due to caffeine consumption (Azevedo, Silva-Cavacante, Gualano, Lima-Silva, & Bertuzzi, 2016; Machado et al., 2008; Nawrot et al., 2003) activate release of excitatory neurotransmitter (Glaister et al., 2015; Owens et al., 2019), namely dopamine (Reolands & Meeusen, 2012). This excitation is thought to alter pain sensation (Duncan et al., 2013; Glaister et al., 2015; Graham, 2001; Tarnopolsky, 2010), decrease rating of perceived exertion (Astorino et al., 2008; Duncan et al., 2013), and thus delay the onset of fatigue (Tarnopolsky, 2010). Lee et al. (2011) found that caffeine (6 mg/kg) + creatine (.3 g/kg/day for 5 days) increased time to exhaustion on a cycling ergometer (1087.2 ± 123.9 s) compared to creatine + placebo (1040.3 ±96.1 s) and base trial (1009.2 ± 86.0 s) in 12 active men. However, they suggested that although caffeine could delay the onset of fatigue during endurance exercise, it might lead to accumulation of blood lactate concentration.

Another proposed mechanism through which the caffeine increased time to exhaustion is the activation of calcium/calmodulin protein-dependent kinase-ß (CaMKKß) and the phosphorylation of AMP-activated protein kinase (AMPK) systems (de Costa Santos et al., 2011). These pathways related to caffeine ingestion are involved in increased energy expenditure via increased glycogen resynthesis and fat oxidation and thus increased plasma free fatty acids (FFAs) (de Costa Santos et al., 2011; Spriet, 2014). Of relevance, Wu & Lin (2010) suggested that the elevation in FFAs levels after caffeine ingestion might be attributed to decreased growth hormone in response to resistance exercise. Moreover, activation of sodium/potassium ATPase pump (Trexler et al., 2016) as a result of caffeine consumption represents the other potential mechanism for increased time to exhaustion in the CAF compared to PLA trial. In addition to this, the improvement of performance during exhaustive incremental test in the current study could also be attributed to the ergogenic effect of caffeine that may reduce conduction block-induced muscular fatigue (Tarnopolsky, 2010). This phenomenon occurs due to increased plasma potassium concentration during endurance exercise. A limitation of the present study was that cardiac troponins, ALT, and AST were not measured so we recommended a further research to measure these variables.

CONCLUSION

Our data do not support the ingestion of caffeine (6 mg/kg) 1 h prior to exhaustive incremental test followed by resistance exercises to significantly protect muscle fibres from damage using bench press, biceps curl, triceps pushdown, leg press, and leg extension. All biomarkers of muscle damage including creatine kinase, lactate dehydrogenase, creatine kinase MB, and myoglobin were not different between caffeine (CAF) and placebo (PLA) trials. After completion resistance exercises, however, caffeine consumption increased the time to exhaustion during exhaustive incremental test in long-distance runners compared to the placebo. This may be attributed to dampened pain sensation during performance.

AUTHOR CONTRIBUTIONS

Author's name	*Conception and design of the study*	*Data collection*	*Data interpretation*	*Drafting the article and/or its critical revision*	*Final approval of the version to be published*
Mohammad Fayiz AbuMoh'd	√		√	√	√
Nabil Shamrokh		√			√
Ahmed S. Bataineh		√			√
Ramzi Al-Horani	√			√	√

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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REFERENCES

Astorino, T. A., Rohmann, R. L., & Firth, K. (2008). Effect of caffeine ingestion on one-repetition maximum muscular strength. Eur J Appl Physiol, 102, 127-132. https://doi.org/10.1007/s00421-007-0557-x

Azevedo, R., Silva-Cavacante, M. D., Gualano, B., Lima-Silva, A. E., & Bertuzzi, R. (2016). Effects of caffeine ingestion on endurance performance in mentally fatigued individuals. Eur J Appl Physiol, 116, 2293-2303. https://doi.org/10.1007/s00421-016-3483-y

Barnes, J. N., Trombold, J. R., Dhindsa, M., Lin, H., & Tanaka, H. (2010). Arterial stiffness following eccentric exercise-induced muscle damage. J. Appl. Physiol., 109, 1102-1108. https://doi.org/10.1152/japplphysiol.00548.2010

Beck, T. W., Housh, T. J., Schmidt, R. J., Johnson, G. O., Housh, D. J., Coburn, J. W., & Malek, M. H. (2006). The acute effects of a caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities. Journal of Strength and Conditioning Research, 20(3), 506-510. https://doi.org/10.1519/00124278-200608000-00008

Bishop, N. C., Fitzgerald, C., Potter, P. J., Scanlon, G. A., & Smith, A. C. (2005). Effect of caffeine ingestion on lymphocyte counts and subset activation in vivo following strenuous cycling. Eur J Appl Physiol, 93, 606-613. https://doi.org/10.1007/s00421-004-1271-6

Burt, D. G., Lamb, K., Nicholas, C., & Twist, C. (2014). Effects of exercise-induced muscle damage on resting metabolic rate, sub-maximal running and post-exercise oxygen consumption. European Journal of Sport Science, 14(4), 337-344. https://doi.org/10.1080/17461391.2013.783628

Caldwell, J. T., Wardlow, G. C., Branch, P. A., Ramos, M., Black, C. D., & Ade, C. J. (2016). Effect of exercise-induced muscle damage on vascular function and skeletal muscle microvascular deoxygenation. Physiological Reports, 4(22), 1-12. https://doi.org/10.14814/phy2.13032

Cechella, J. L., Leite, M. R., Dobrachinski, F., da Rocha, J. T., Carvalho, N. R., Duarte, M. M. M. F., Soares, F. A. A., Bresciani, G., Royes, L. F. F., & Zeni, G. (2014). Moderate swimming exercise and caffeine supplementation reduce the levels of inflammatory cytokines without causing oxidative stress in tissues of middle-aged rats. Amino acid, 46(5), 1187-1195. https://doi.org/10.1007/s00726-014-1679-1

da Costa Santos, V. B., Ruiz, R. J., Vettorato, E. D., Nakamura, F. Y., Juliani, L. C., Polito, M. D., & Martin Siqueira, C.. P. C. (2011). Effects of chronic caffeine intake and low-intensity exercise on skeletal muscle of Wistar rats. Exp Physiol, 96(11), 1228-1238. https://doi.org/10.1113/expphysiol.2011.060483

Del Coso, J., Salinero, J. J., Abian-Vicen, J., Gonzalez-Millan, C., Garde, S., Vega, P., & Perez-Gnzalez, B. (2013). Influence of body mass loss and myoglobinuria on the development of muscle fatigue after a marathon in an environment. Appl. Physiol. Nutr. Metab., 38(3), 286-291. https://doi.org/10.1139/apnm-2012-0241

Duncan, M. J., Stanley, M., Parkhouse, N., Cook, K., & Smith, M. (2013). Acute caffeine ingestion enhances strength performance and reduces perceived exertion and muscle pain perception during resistance exercise. European Journal of Sport Science, 13(4), 392-399. https://doi.org/10.1080/17461391.2011.635811

Glaister, M., Muniz-Pumares, D., Patterson, S. D., Foley, P., & Mcinnes, G. (2015). Caffeine supplementation and peak anaerobic power output. European Journal of Sport Sciences, 15(5), 400-406. https://doi.org/10.1080/17461391.2014.962619

Graham, T. E. (2001). Caffeine and exercise: metabolism, endurance and performance. Sports Med, 31(11), 785-807. https://doi.org/10.2165/00007256-200131110-00002

Grgic, J., & Mikulic, P. (2017). Caffeine ingestion acutely enhances muscular strength and power but not muscular endurance in resistance-trained men. European Journal of Sport Science, 17(8), 1029-1036. https://doi.org/10.1080/17461391.2017.1330362

Horrigan, L.A., Kelly, J. P., & Connor, T. J. (2006). Immunomodulatory effects of caffeine: friend of foe?. Pharmacol Ther, 111, 877-892. https://doi.org/10.1016/j.pharmthera.2006.02.002

Hume, P. A., Cheung, K., Maxwell, L., & Weerapong, P. (2004). DOMS: An overview of treatment strategies. International SportMed Journal, 5(2).

Lee, C. L., Lin, J. C., & Cheng, C. F. (2011). Effect of creatine plus caffeine supplements on time to exhaustion during an incremental maximum exercise. European Journal of Sport Science, 12(4), 338-346. https://doi.org/10.1080/17461391.2011.573578

Lippi, G., Schena, F., Salvagno, G. L., Montagnana, M., Gelati, M., Tarperi, C., Banfi, G., & Guidi, G. C. (2008). Acute variation of biochemical markers of muscle damage following a 21-km, half-marathon run. The Scandinavian Journal of Clinical & Laboratory Investigation, 68(7), 667-672. https://doi.org/10.1080/00365510802126844

Machado, M., Zovico, P. V. C., & da Silva, D. P. (2008). Caffeine does not increase resistance exercise-induced microdamage. J Exerc Sci Fit, 6(2), 115-120.

Mackey, A. L., & Kjaer, M. (2017). The breaking and making of healthy adult human skeletal muscle in vivo. Skeletal Muscle, 7(1), 101-124. https://doi.org/10.1186/s13395-017-0142-x

Marquez-Jimenez, D., Callega-Gonzalez, J., Arratibel-Imaz, I., Delextrat, A., Uriarte, F., & Terrados, N. (2018). Influence of different types of compression garments on exercise-induced muscle damage markers after a soccer match. Research In Sports Medicine, 26(1), 27-42. https://doi.org/10.1080/15438627.2017.1393755

Naclerio, F., Larumbe-Zabala, E., Cooper, R., Jimenez, A., & Goss-Sampson, M. (2014). Effect of a carbphydrate-protein multi-ingredient supplement on intermittent sprint performance and muscle damage in recreational athletes. Appl. Physiol. Nutr. Metab., 39, 1151-1158. https://doi.org/10.1139/apnm-2013-0556

Nawrot, P., Jordan, S., Eastwood, J., Rotestein, J., Hugenjoltz, A., & Feeley, M. (2003). Effects of caffeine on human health. Food Additives and Contaminations, 20(1), 1-30. https://doi.org/10.1080/0265203021000007840

Nosaka, K., Newton, M., & Sacco, P. (2002). Muscle damage and soreness after endurance exercise of the elbow flexors. Med Sci Sports Exerc, 34, 920-927. https://doi.org/10.1097/00005768-200206000-00003

Owens, D. J., Twist, C., Cobley, J. N., Howatson, G., & Close, G. L. (2019). Exercise-induced muscle damage: what is it, what cause it and what are the nutritional solutions?. European Journal of Sport science, 19(1), 71-85. https://doi.org/10.1080/17461391.2018.1505957

Ramos-Campo, D. J., Avila-Gandia, V., Alacid, F., Soto-Mendez, F., Alcaraz, P. E., Lopez-Roman, F. J., & Rubio-Arias, J. A. (2016). Muscle damage, physiological changes, and energy balance in ultra-endurance mountain-event athletes. Appl. Physiol. Nutr. Metab, 41, 872-878. https://doi.org/10.1139/apnm-2016-0093

Reolands B, & Meeusen R. (2012). Caffeine, dopamine and thermoregulation. Eur J Appl Physiol, 112: 1979-1980. https://doi.org/10.1007/s00421-011-2127-5

Soleimani, A., Shkerian, S., & Ranjbar, R. (2017). The effect of caffeine supplementation on serum sensitivity C-reactive protein and creatine kinase after exhausted aerobic exercise in active overweight university students. Journal of Birjand University of Medical Sciences, 24(2), 84-93.

Spradely, B. D., Crowley, K. R., Kristina, C. T., Kendall, K. L., Fukuda, D. H., Esposito, E. N., Moon, S. E., & Moon, J. R. (2012). Ingestion a pre-workout supplement containing caffeine, B-vitamins, amino acids, creatine, and beta-alanine before exercise delays fatigue while improving reaction time and muscular endurance. Nutrition & Metabolism, 9(28), 1-9. https://doi.org/10.1186/1743-7075-9-28

Spriet, L. L. (2014). Exercise and sport performance with low doses of caffeine. Sports Med, 44(Suppl 2), S175-S184. https://doi.org/10.1007/s40279-014-0257-8

Taipale, R. S., Schumann, M., Mikkola, J., Nyman, K., Kyrolainen, H., Nummela, A., & Hakkinen, K. (2014). Acute neuromuscular and metabolic responses to combined strength and endurance loadings: the "order effect" in recreationally endurance trained athletes. Journal of Sports Sciences, 32(12), 1155-1164. https://doi.org/10.1080/02640414.2014.889842

Tarnopolsky, M. A. (2010). Caffeine and creatine use in sport. Ann Nutr Metab, 57(suppl 2), 1-8.

Trexler, E. T., Smith-Ryan, A. E., Roelofs, E. J., Hirsch, K. R., & Mock, M. G. (2016). Effects of coffee and caffeine anhydrous on strength and sprint performance. European Journal of Sport Science, 16(6), 702-710. https://doi.org/10.1080/17461391.2015.1085097

Vieira, J. M., Carvalho, F. B., Gutierres, J. M., Soares, M. S. P., Oliveira, P. S., Rubin, M. A., Morsch, V. M., Schetinger, M. R., & Spanevello, R. M. (2017). Caffeine prevents high-intensity exercise-induced increase in enzymatic antioxidant and NA-K-ATPase activities and reduction of anxiolytic like-behaviour in rats. Redox Report, 22(6), 493-500. https://doi.org/10.1080/13510002.2017.1322739

Vikmoen, O., Raastad, T., Seynnes, O., Bergstrom, K., Ellefsen, S., & Ronnestad, B. R. (2016). Effects of heavy strength training on running performance and determinants of running performance in female endurance athletes. PLoS ONE, 11(3), 1-18. https://doi.org/10.1371/journal.pone.0150799

Willoughby, D. S., Spillane, M., & Schwarz, N. (2014). Heavy resistance training and supplementation with the alleged testosterone booster NMDA has no effect on body composition, muscle performance, and serum hormones associated with the hypothalamic-pituitary-gonadal axis in resistance-trained males. Journal of Sports Science and Medicine, 13, 192-199.

Woolf, K., Bidwell, W. K., & Carlson, A. G. (2008). The effect of caffeine as an ergogenic aid in anaerobic exercise. International Journal of Sport Nutrition and Exercise Metabolism, 18(4), 412-429. https://doi.org/10.1123/ijsnem.18.4.412

Wu, B., & Lin, J. (2010). Caffeine attenuates acute growth hormone response to a single bout of resistance exercise. Journal of Sports Science and Medicine, 9, 262-269.

Zainudin, H., Caszo, B. A., Knight, V. F., & Gnanou, J. V. (2019). Training induced oxidative stress-derived DNA and muscle damage in triathletes. Eurasian J Med, 51(2), 116-120.

Zarghami-Khameneh, A., & Jafari, A. (2014). The effect of different doses of caffeine and a single bout of resistance-exhaustive exercise on muscle damage indices in male volleyball players. Journal of Kashan University of Medical Sciences, 18(3), 220-228.

