Caffeine intake improves intense intermittent exercise performance and reduces muscle interstitial potassium accumulation

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Mohr M, Nielsen JJ, Bangsbo J. Caffeine intake improves intense intermittent exercise performance and reduces muscle interstitial potassium accumulation. J Appl Physiol 111: 1372–1379, 2011. First published August 11, 2011; doi:10.1152/japplphysiol.01028.2010.—The effect of oral caffeine ingestion on intense intermittent exercise performance and muscle interstitial ion concentrations was examined. The study consists of two studies (S1 and S2). In S1, 12 subjects completed the Yo-Yo intermittent recovery level 2 (Yo-Yo IR2) test with prior caffeine (6 mg/kg body wt; CAF) or placebo (PLA) intake. In S2, 6 subjects performed one low-intensity (20 W) and three intense (50 W) 3-min (separated by 5 min) one-legged knee-extension exercise bouts with CAF and without (CON) prior caffeine supplementation for determination of muscle interstitial K⁺ and Na⁺ with microdialysis. In S1 Yo-Yo IR2 performance was 16% better (P < 0.05) in CAF compared with PLA. In CAF, plasma K⁺ at the end of the Yo-Yo IR2 test was 5.2 ± 0.1 mmol/l with no difference between the trials. Plasma free fatty acids (FFA) were higher (P < 0.05) in CAF than PLA at rest and remained higher (P < 0.05) during exercise. Peak blood glucose (8.0 ± 0.6 vs. 6.2 ± 0.4 mmol/l) and plasma NH₃ (137.2 ± 10.8 vs. 113.4 ± 13.3 µmol/l) were also higher (P < 0.05) in CAF compared with PLA. In S2 interstitial K⁺ was 5.5 ± 0.3, 5.7 ± 0.3, 5.8 ± 0.5, and 5.5 ± 0.3 mmol/l at the end of the 20-W and three 50-W periods, respectively, in CAF, which were lower (P < 0.001) than in CON (7.0 ± 0.6, 7.5 ± 0.7, 7.5 ± 0.4, and 7.0 ± 0.6 mmol/l, respectively). No differences in interstitial Na⁺ were observed between CAF and CON. In conclusion, caffeine intake enhances fatigue resistance and reduces muscle interstitial K⁺ during intense intermittent exercise.

fatigue; potassium; free fatty acids; repeated exercise; muscle ion kinetics

CAFFEINE is classified as part of the methylxanthine family of drugs and is commonly consumed by athletes as an ergogenic aid (45), since it was removed from the World Anti-Doping Agency list of prohibited substances (46). A caffeine ingestion of 3–6 mg/kg body mass has been shown to improve fatigue resistance during various types of exercise such as time trial cycling (35), sprint running (20, 44), and repeated sprint running (11). In trained athletes caffeine improved 1 km cycling performance (47) whereas caffeine had no effect on either muscle strength and power (4, 47) or 200-m swimming (40) in trained athletes. In addition, no effect of caffeine intake was seen in power output during intense cycling exercise (24). In a study by Stuart et al. (44) caffeine administration 1 h before a simulated rugby union game improved passing accuracy and 30-m sprint speed. Thus the performance-enhancing effects of caffeine administration appear to be specific to the type of exercise performed, but the effect on repeated intense exercise has not been determined. The Yo-Yo intermittent recovery test, level 2 (Yo-Yo-IR2), is well-described (7, 31, 37) and has been shown to have a highly anaerobic nature, and test performance correlates with the amount of intense exercise performed in team sports (7). Thus, to examine the effect of caffeine intake on intense intermittent exercise as observed in team sport, the Yo-Yo IR2 test can be applied.

Caffeine has a number of physiological effects. It been shown to elevate the catecholamine levels (22, 24, 32, 44), the systemic FFA (21), NH₃ concentrations, sarcoplasmatic reticulum Ca²⁺ handling (1, 17) as well as lower the respiratory exchange ratio (RER) values (5). Caffeine may also increase the muscle Na⁺-K⁺-ATPase activity directly or indirectly through an elevated catecholamine response, which may delay fatigue if accumulation of potassium in the muscle interstitium is involved in fatigue (27, 36, 39, 42). Indeed, Lindinger et al. (32) and Graham and colleagues (23) demonstrated that high doses (6 and 9 mg/kg body wt) of caffeine administration decreased plasma K⁺ concentration during prolonged exercise, but not during repeated 30-s maximal exercise bouts (24). However, the effect of caffeine on plasma potassium is controversial, and no data exist about the effect of caffeine on muscle interstitial potassium and sodium.

Thus the aim of the present study was to examine the impact of caffeine intake on performance and physiological response, including changes in muscle interstitial ion concentrations, during repeated intense exercise.

MATERIALS AND METHODS

Subjects

The study consists of two studies (S1 and S2). The participants in S1 were eight males and four females with an age of 23.1 ± 0.6 yr, height of 179.0 ± 3.0 cm, and body mass of 72.6 ± 3.7 kg. They were all active in team sports at a subelite level and were familiar with intense intermittent exercise. In S2 six habitually active male subjects (age: 25.9 ± 1.0 yr; height: 183.0 ± 2.8 cm; body mass: 83.8 ± 3.8 kg) participated. The subjects in S2 had a maximal oxygen uptake of 54.0 ± 1.7 ml O₂·kg⁻¹·min⁻¹. All subjects were informed of risks associated with the experiment before giving their written consent to participate. The study conforms to the code of Ethics of World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

Experimental Design

In S1 the subjects performed the Yo-Yo intermittent recovery test, level 2 (Yo-Yo IR2), until exhaustion (31) on two separate occasions separated by 6 days. The tests were carried out at the same time of the...
day. Fluid and food intake as well as activity level was noted the day before the first experimental day and mimicked before the second day. Seventy minutes before the test either caffeine (CAF; MERCK, Darmstadt, Germany) or placebo (PLA; dextrose) was taken orally in a gelatin capsule (6 mg/kg body wt; 436 ± 22 mg in total). The blood glucose concentration was unaffected by the placebo intake. The experiment was randomized, double-blinded, and counterbalanced.

In S2 the subjects performed a control (CON) and a caffeine (CAF) trial separated by 80 min. For each trial the subjects performed a one-legged knee-extensor exercise protocol consisting of two 20-W exercise periods separated by 10 min of passive recovery, then after 10 min of recovery the subjects completed three 3-min exercise bouts (EX1, EX2, and EX3) at an intensity of 50 W interspersed by 5 min of passive recovery (Fig. 1). The first 20-W period was carried out to obtain a higher reliability of the interstitial K⁺ measurements (39) and only the second 20-W period was included. The CON trial was completed first, and after 10 min of recovery caffeine was administered orally in a gelatin capsule at a concentration of 6 mg/kg body wt corresponding to 516 ± 21 mg in total. Then the subject rested for 70 min before starting the CAF-trial, which is sufficient for the muscle to recover from the exercise performed in CON. The CON and the CAF trial were performed on the same day to ensure that the microdialysis probes were placed in the same position. The CON trial was performed before the CAF trial to avoid possible effects of caffeine in the CON trial. None of the subjects were heavy coffee drinkers.

**Experimental Protocol**

**S1.** All subjects were familiar with the Yo-Yo IR2 test and had performed at least two familiarization trials. On the experimental day the subjects reported to the laboratory in the morning after consuming a light meal. The subjects were instructed to avoid intake of caffeine containing food and drinks on the day of the experiment as well as alcohol consumption and heavy physical activity on the day before the experiment. Thus no caffeine was ingested in the last 12–15 h before the experiment. A catheter (Venflon Pro, 18G) was inserted into an antecubital vein ~30 min before the exercise protocol was initiated for frequent blood sampling before, during, and after the test. The first 5 min of the Yo-Yo intermittent recovery test, level 1 (Yo-Yo IR1), was used as warm-up before the Yo-Yo IR2 test. This procedure has been shown to markedly elevate the muscle temperature (30, 31). The Yo-Yo IR2 test was initiated 2 min after the warm-up. A blood sample was taken at rest, after the warm-up period, after 160, 280, 440, 600, and 760 s, and at exhaustion in the Yo-Yo IR2 test, as well as 1, 2 and 5 min into recovery.

**S2.** One-legged knee-extensor exercise was carried out on a modified Krogh ergometer permitting the exercise to be confined to the quadriceps muscle (3). The exercise is performed as knee extensions from an angle of 90° to ~170° while flywheel momentum repositions the relaxed leg during the flexion. EMG recordings have demonstrated that the hamstring muscles are passive, while the quadriceps muscles are active in the extension phase (3). To familiarize the subjects with the exercise ergometer the subjects completed 3–4 preexperimental sessions. The subjects were trained to keep a constant kicking frequency of 60 kicks/min (by visual feedback from a display showing the frequency), and to confine the exercise to the quadriceps femoris muscle (by verbal and visual feedback based on online force recordings).

On the experimental day the subjects reported to the laboratory in the morning and followed the same guidelines as described in S1. Six microdialysis probes were inserted parallel to the muscle fibers of the vastus lateralis muscle in the experimental leg under local anesthesia (lidocaine, 1 ml of 20 mg/ml) as previously described (27, 36, 39). The probes were custom design (length of microdialysis membrane 30–40 mm, outer diameter 0.22 mm) as described by Hellsten et al. (25). The perfusate was Ringer acetate containing (in mM) 130 Na⁺, 2 Ca²⁺, 4 K⁺, 1 Mg²⁺, and 30 Ac⁻. The exact perfusate K⁺ concentration was determined in each experiment. In addition, the perfusate contained 3 mM of glucose and 201Tl (activity < 7,000 Bq/ml). The probes were flushed and connected to a pump (CMA 102). Then they were perfused at a rate of 2 µl/min for 1 h and 5 µl/min for the remaining part of the experiment. In addition, a catheter
Physiological Response During the Yo-Yo IR2 Test

Plasma $K^+$ was $3.6 \pm 0.1$ and $3.9 \pm 0.1$ mmol/l at rest in CAF and PL, respectively, and rose ($P < 0.05$) during the Yo-Yo IR2 test to $5.2 \pm 0.2$ and $5.9 \pm 0.4$ mmol/l at exhaustion, respectively, with no differences between the two trials (Fig. 2A). Also peak plasma potassium in CAF and PL was not different ($5.3 \pm 0.2$ and $5.9 \pm 0.3$ mmol/l, respectively). In CAF blood lactate increased ($P < 0.05$) from 1.0 to $0.1$ to $8.6 \pm 0.7$ mmol/l during the test with no differences between the two trials (Fig. 2B). The peak blood lactate concentration was $11.2 \pm 0.6$ and $10.8 \pm 0.8$ mmol/l in CAF and PL, respectively.

Blood glucose was $5.8 \pm 0.6$ mmol/l before the test in CAF, which tended to be higher ($P = 0.06$) than in PL ($4.5 \pm 0.5$ mmol/l; Fig. 2C). The blood glucose levels at exhaustion were $6.4 \pm 0.5$ mmol/l in CAF, which was not significantly different from PL ($4.9 \pm 0.3$ mmol/l). Peak blood glucose level during the test was $8.1 \pm 0.6$ mmol/l in CAF and higher ($P < 0.01$) than in PL ($6.2 \pm 0.5$ mmol/l). Plasma $NH_3$ was $32.7 \pm 5.5$ and $30.0 \pm 3.1$ mmol/l at rest in CAF and PL, respectively, and increased ($P < 0.05$) during the Yo-Yo IR2 test to $76.7 \pm 9.8$ and $94.0 \pm 18.4$ mmol/l, respectively, with no differences between trials (Fig. 2D). However, 5 min into recovery the plasma $NH_3$ was $137.2 \pm 10.8$ mmol/l in CAF, which was higher ($P < 0.05$) than in PL ($105.1 \pm 10.7$ mmol/l). At rest plasma FFA was $490 \pm 77$ mmol/l in CAF, which was higher ($P < 0.05$) than in PL ($167 \pm 31$ mmol/l; Fig. 2E), and remained higher ($P < 0.05$) in CAF than in PL during the entire experiment (Fig. 2E).

Muscle Interstitial Potassium and Sodium

At rest [$K^+]_{\text{int}}$ was $4.3 \pm 0.2$ and $4.2 \pm 0.5$ mmol/l in CAF and CON, respectively. During the 20-W knee-extensor exercise [$K^+]_{\text{int}}$ was lower ($P < 0.05$) in CAF than CON and remained lower ($P < 0.05$) during the 10-min recovery period (Fig. 3A). At rest [$Na^+]_{\text{int}}$ was $134 \pm 6$ and $134 \pm 3$ mmol/l in CAF and CON, respectively. No changes in [$Na^+]_{\text{int}}$ were observed with exercise during the 20-W period and no statistical differences were found between CAF and CON (Fig. 3B).

Before EX1 [$K^+]_{\text{int}}$ was not significantly different in CAF and CON, but during EX1 the levels were lower ($P < 0.05$) in CAF than CON with peak values being $5.7 \pm 0.3$ and $7.5 \pm 0.7$ mmol/l, respectively (Fig. 4A). In the recovery period after EX1 [$K^+]_{\text{int}}$ remained lower ($P < 0.05$) in CAF than CON. During and after EX2 [$K^+]_{\text{int}}$ was lower ($P < 0.05$) in CAF than CON (Fig. 4A). Also during EX3 [$K^+]_{\text{int}}$ was lower ($P < 0.05$) in CAF than CON with peak values being $5.5 \pm 0.3$ vs. $7.3 \pm 0.3$ mmol/l, respectively (Fig. 4A).

 [$Na^+]_{\text{int}}$ did not change during the 50-W exercise periods and no differences were found between CAF and CON, although [$Na^+]_{\text{int}}$ in CAF tended to be higher than in CON ($P = 0.07$; Figs. 3B and 4B).

Plasma Potassium and Metabolites During Knee-Extensor Exercise

Venous plasma $K^+$ was $3.7 \pm 0.1$ and $3.9 \pm 0.0$ mmol/l at rest in CAF and CON, respectively. After the 20-W exercise plasma $K^+$ became lower ($P < 0.05$) in CAF than in CON ($3.8 \pm 0.1$ and $4.2 \pm 0.1$ mmol/l, respectively). Plasma $K^+$ was also lower ($P < 0.05$) in

RESULTS

Yo-Yo IR2 Performance

Yo-Yo IR2 performance was $607 \pm 55$ m in CAF, which was $16\%$ better ($P < 0.05$) than in PLA ($523 \pm 55$ m).
CAFEINE compared with CON after EX1 (4.0 ± 0.2 and 4.3 ± 0.0 mmol/l, respectively) but not after EX2 and EX3. At rest plasma Na⁺ was 138 ± 1 and 135 ± 2 mmol/l in CAF and CON, and no changes were observed during exercise in either CAF or CON.

Plasma FFA was 437 ± 103 and 124 ± 41 mmol/l at rest in CAF and CON, respectively (P = 0.07). After the 20-W exercise bout plasma FFA in CAF was higher (P < 0.05) than in CON (726 ± 179 and 200 ± 64 mmol/l, respec-
performing better after caffeine intake. In support, Stuart et al. (44) substantiated an elevated performance level during repeated sprint tests performed during a simulated rugby game in high-level players after caffeine intake. Moreover, others have shown improved high-intensity work during a 90-min simulated soccer game (18) and simulated tennis (26). Thus caffeine ingestion seems to elevate fatigue resistance during team sport-relevant, high-intensity intermittent exercise. A number of studies have used short-term all-out exercise protocols to investigate possible performance-enhancing effects of caffeine (8, 12, 24, 48). These studies using maximal sprinting exercise lasting 15–30 s showed no performance-enhancing effects of caffeine administration, indicating that the ergogenic effects of caffeine do not appear to have an impact on performance during these short-term, single-exercise bouts. In contrast, a large number of observations using both laboratory and field-based exercise protocols report an elevated fatigue resistance during exhaustive intense exercise trials lasting from 1 to 15 min (8, 16, 17, 48). Caffeine appears to be especially ergogenic...
for speed endurance exercise ranging 1–3 min (15). Thus the performance-stimulating effect of caffeine seems to occur in relatively short-term intense exercise with a marked anaerobic component indicating that caffeine stimulates physiological mechanisms counteracting this specific type of fatigue.

Fatigue development during high-intensity intermittent exercise is complex, but an altered resting membrane potential has been proposed as a central candidate (19, 34, 41). The main contributor to the depolarized sarcolemmal potential is a marked efflux of K⁺ from the muscle cell (34) and a concomitant accumulation in the muscle interstitium (27, 36, 39, 42). The Yo-Yo IR2 test has been substantiated to exert a major stimulus on the anaerobic energy systems, and venous K⁺ concentrations reach the approximate levels (31) observed by others at the point of fatigue during intense exercise lasting a few minutes (6, 38). In the present study no differences in plasma K⁺ between CAF and PLA were observed, but in CAF it tended to be lower during the entire trial (Fig. 2A). In support of this Crowe and coworkers (14) found a significant decrease in plasma K⁺ after caffeine intake, and others have demonstrated a similar pattern during prolonged exercise (24, 32). However, any fatiguing effect of elevated extracellular K⁺ is exerted in the interstitium of the exercising muscles. Therefore, in S2 the effect of caffeine administration on interstitial K⁺ was studied, and [K⁺]ᵢₑ₅ was markedly lower during and after both moderate-intensity exercise and repeated intense exercise (Figs. 3A and 4A). Thus caffeine intake attenuates the accumulation of interstitial K⁺ during repeated intense exercise, which may delay fatigue as seen in S1. It should be noted that the average values of a level of 7–8 mmol/l is not expected to cause fatigue (27, 36, 38, 39) and are lower than observed in other studies where exhaustive intense exercise has been examined. In addition, in S1 plasma potassium was not different between the caffeine and placebo trial, despite that a marked difference in [K⁺]ᵢₑ₅ was found in S2, which suggests that plasma K⁺ during whole body exercise is not a sensitive measure of [K⁺]ᵢₑ₅.

There are several possible mechanisms for a caffeine-induced effect on [K⁺]ᵢₑ₅. First, caffeine may have stimulated the activity in the Na⁺-K⁺ pump indirectly by an elevated catecholamine response (15, 22, 32). Caffeine could also have had a direct effect on the muscle, since it has been shown that high doses of caffeine restore force in K⁺ inhibited muscle in vitro (10). Furthermore, the elevated glucose concentrations in the caffeine trial may per se have improved performance through an effect on the Na⁺-K⁺ pump, as it has been suggested that high glucose concentrations are preventing the deterioration of electrical properties of the muscle fiber membrane (29, 33). The fact that [Na⁺]ᵢₑ₅ tended (P = 0.07) to be higher in CAF gives added support to the notion of caffeine-stimulated increase in Na⁺-K⁺ pump activity. Moreover, the rate of [K⁺]ᵢₑ₅ accumulation was higher in the control trial than in the caffeine trial in the initial phase of the 20-W exercise period (data not shown). Thus caffeine may preactivate or accelerate the Na⁺-K⁺ pump activity and therefore reduce the time lag in pump activity in the first part of an exercise bout. It has been shown that the rate of accumulation in [K⁺]ᵢₑ₅ is highest in the initial part of an intense exercise bout (36, 39), and that the accumulation rate decreases when intense exercise is repeated (36), which may associate with an elevated Na⁺-K⁺ pump activity.

In the present study plasma FFA was markedly elevated in CAF before and during the entire test compared with PLA. Other studies have shown that plasma epinephrine increases ~1 h after caffeine intake in similar doses as in the present study (13, 24, 44), which most likely caused the augmented lipolytic activity. Thus it may be proposed that caffeine may affect performance in an intermittent test by elevating fat oxidation in the recovery bouts and a concomitant glycogen-sparing effect (43). However, it is unlikely that this has had a significant impact on the fatigue resistance during the exercise due to the relatively short duration of the test and limited muscle glycogen usage. In support, it has been shown that the muscle glycogen concentrations are still high after a Yo-Yo IR2 test of similar duration as in this study (31).

The caffeine-induced elevation of catecholamine levels may have had multiple other physiological effects including a proposed accelerated oxygen uptake kinetics and muscle glycolysis (21, 45). Greer et al. (24) showed markedly higher plasma epinephrine levels before four all-out 30-s sprints, but the effect disappeared during exercise. Moreover, neither oxygen uptake nor blood lactate was different during exercise between the caffeine and control trials. In contrast plasma epinephrine was increased during an entire ~80-min simulated rugby game after prior caffeine intake (44). In the present study blood glucose was higher both before the Yo-Yo IR2 test as well as the peak levels reached during the test, indicating increased catecholamine stimulation. However, no difference in blood lactate concentrations during the Yo-Yo IR2 test was observed between the trials, indicating similar glycolytic activity. On the other hand the plasma NH₃ rose to significantly higher values in CAF than PLA, which is in agreement with findings by Greer and coworkers (24) during repeated sprints. Thus the AMP breakdown may be accelerated by caffeine intake, but it does not appear to have affected performance. The ergogenic effect of caffeine ingestion has also been linked to supraspinal or central fatigue, since caffeine apparently increases motorneuron or motor cortical excitability during exercise (28). Thus it cannot be excluded that there have been a central component to the better performance in S1.

In summary, the present study demonstrates that high-intensity intermittent exercise performance is significantly improved by oral caffeine administration. Moreover, for the first time caffeine has been shown to lower muscle interstitial potassium during intense exercise. Thus the performance-enhancing effect of caffeine intake before intense intermittent exercise may be associated with an improved interstitial potassium handling in the exercising muscles.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
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