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Caffeine increases performance in cross-country double poling time trial exercise

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Running title: Caffeine improves double poling capacity

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Abstract

Purpose: Caffeine (CAF) improves performance of both short and long duration in running and cycling where performance relies on power output, and endurance capacity of leg muscles. No studies have so far tested effects of CAF while using the double poling (DP) technique in cross-country skiing (XCS). When DP arm muscles provide the speed generating force, and therefore play an important role to performance outcome. The metabolism of arm muscles differs from that of leg muscles. Thus, results from studies on leg muscles and CAF may not be directly applicable to exercises while DP in XCS. The purpose of our study was therefore to investigate effects of CAF on exercise performance in DP. **Method:** Ten highly trained male cross-country skiers ($\dot{V}O_{2max}$ running, 69.3 ± 1.0 ml·kg^{-1}·min^{-1}) performed a placebo (PLA) and CAF trial using a randomized, double-blinded, cross-over design. Performance was assessed by time to complete an 8 km cross country DP performance test (C-PT). CAF (6 mg·kg^{-1}) or PLA was ingested 75 min before the C-PT. **Results:** CAF ingestion reduced time to complete the 8 km C-PT from $34:26\pm1:25$ to $33:01\pm1:24$ min (p<0.05). The subjects maintained higher speed and heart rate throughout the C-PT, and lactate was higher immediately after the C-PT with CAF exposure compared to PLA. Subjects reported lower rating of perceived exertion at submaximal intensities during CAF compared to PLA, although heart rate was similar. In **conclusion**, CAF intake enhances endurance performance in an 8 km C-PT where arm muscles limit performance. CAF ingestion allowed the participants to exercise with a higher heart rate, and work intensity, possibly by reducing perception of effort or facilitating motor unit recruitment.

Keywords *Exercise; pain; rate of perceived exertion; adenosine receptors; glucose; adrenaline.*

Introduction

Paragraph Number 1 Many studies have demonstrated during the last 40 years that caffeine (CAF) intake (3-9 mg · kg[1]) improves endurance performance in activities such as cycling (25, 26), running (7, 21) and rowing (35). Furthermore, CAF intake can improve exercise performance of short (28) or long duration (12, 27), as well as when exercise performance is measured as time to exhaustion (25) or time to complete a set amount of work (7).

Paragraph Number 2 Multiple mechanisms have been proposed to explain improved endurance performance after CAF ingestion (20, 36). Initially caffeine was observed, and thought to have a carbohydrate sparing effect (15, 25). However this hypothesis cannot explain improved performance of short duration where glycogen content is not likely to be a limiting factor (28, 35). Inhibition of adenosine receptors and increased motor unit recruitment by caffeine are additional mechanisms reported to explain its ergogenic effects (1, 9, 14, 19, 20). Adenosine receptors are expressed in most tissues of the human body, and blockade of these receptors may affect both rate of perceived exertion (RPE) and heart rate (HR) (2, 3, 18). Indeed, lower RPE has been reported at submaximal workloads after caffeine ingestion (2, 3, 13), and higher HR has been reported in time trial studies after caffeine ingestion compared to placebo (3, 7, 26). CAF has also been shown to improve maximal voluntary contraction (MVC) (20). The mechanisms explaining improved MVC is that CAF ingestion may directly affect the muscle (e.g., maintaining electrolyte homeostasis or enhancing sarcoplasmic reticulum Ca^{2+} release) or the CNS (e.g. increasing motor unit recruitment)(20, 31).

Paragraph Number 3 During endurance performance tasks lasting 10-90 min the ergogenic effects of CAF have typically been shown for exercises dependent on endurance capacity of the leg muscles (e.g., cycling, running, and rowing (7, 26). During DP the arm

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muscles provide the speed generating force (37), and endurance capacity of the arm muscles therefore plays a crucial role to performance outcome. The metabolism of arm muscles differs from that of leg muscles with studies observing arm muscles have a higher percentage type 2 fibre compositions, extract less oxygen and rely more on carbohydrate utilization during exercise (10, 22, 37). Because of observed differences between arm and leg muscles, CAF may act differently when an exercise performance is relying on endurance capacity of the arm muscles.

Paragraph Number 4 In cross-country skiing (XCS), DP has become an important technique because of changes in equipment, tracks, and types of competition (4). In races such as Marchialonga and Vasaloppet, DP capacity directly affects performance outcome. Furthermore maximal oxygen uptake in well-trained XCS is lower during DP than in running or diagonal skiing (5, 23). The lower maximum oxygen uptake during DP may be due to a lower active muscle mass indicating that cardiac output *per se* does not limit performance. Or the fact that arm muscles have a lower mitochondria density, and are unable to extract the same amount oxygen as leg muscles (10, 22, 30).

Paragraph Number 5 The effect of CAF on exercise endurance performance has not been investigated while using the DP technique in XCS, where endurance capacity of the arm muscles plays a key role in performance outcome. Thus, the purpose of our study was to determine the effect of CAF on exercise performance limited to the arms while using the double poling technique. We hypothesized that ingestion of CAF will improves performance in DP where arm muscles produce the speed generating force.

Materials and Methods

Subjects and approvals

Paragraph Number 6 Ten healthy highly trained male cross-country skiers participated. Physical characteristics of the subjects were (mean±SE); age: 20.0±1.0 year, height: 181.0±1.0 cm, weight: 73.9±2.3 kg, maximal oxygen uptake during running ($\dot{V}O_{2max\text{-}run}$): 69.3±1.0 mL · kg⁻¹ · min⁻¹ and maximal oxygen uptake during double poling ($\dot{V}O_{2max\text{-}pol}$): 63.2±1.5 mL · kg⁻¹ · min⁻¹. All subjects trained systematically to compete in the Norwegian National Cross-country Skiing Cup. Additional inclusion criteria were that $\dot{V}O_{2max\text{-}run}$ was above 65 ml · kg⁻¹ · min⁻¹. The study was approved by both the Regional Ethics Committee and the Norwegian Medicine Agency. Subjects gave their written consent after being informed about the purpose of the study and risks involved. All testing was performed in the pre-season for the XCS (September-November).

Experimental Procedures

Paragraph Number 7 The study was a randomized double-blinded, placebo-controlled, cross-over design. Treatments included CAF (6 mg · kg body wt⁻¹) and PLA (vehicle only). Caffeine (Coffeinum, Oslo Apotekerproduksjon, Oslo, Norway) was dissolved in a cordial concentrate Fun Light (3 mg/mL) and was prepared by Ullevål Apotek Produksjon (Oslo, Norway). Before the 8 km performance tests (C-PT), the participants underwent a four week training protocol to familiarize the participants with the double poling ergometer and the C-PT. On *day 1* participants performed a $\dot{V}O_{2max\text{-}run}$ test on a treadmill (Woodway, Weil am Rein, Germany) and the highest heart rate was defined as HR$_{max\text{-}run}$. Oxygen consumption and RER were measured with an Oxycon Pro (Jaeger-Toennis Instr. Hochberg, Germany), and air was collected during a mouth V2-mask (Hans Rudolph Instr., USA) in combination with a nose bracket. The $\dot{V}O_{2max\text{-}run}$ test was performed with a standardized warm up consisting of

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four workloads lasting 5 min (8~11 km · h) with 5.3° incline. A one min break was given between each workload were lactate was measured. After the last workload of the warm-up subjects walked five min on 5 km · h^{-1}, before stating the $\dot{V}O_{2max\text{-}run}$ test. Starting speed was 10 km · h^{-1} with an incline on the treadmill of 10.5°. For each half minute speed was increased with 0.5 km · h until subjects were unable to maintain the speed and jumped of the treadmill. All 10 subjects had to meet point one, and at least two of the three other criteria's for that they reached $\dot{V}O_{2max\text{-}run}$: 1) oxygen consumption reached a plateau, meaning $\dot{V}O_2$ increased less than 1 mL · kg^{-1} · min^{-1}, while speed was increased two times 0.5 km · h, 2) RER values were above 1.10, 3) Post blood lactate measurements were above 7.0 mmol/L and 4) RPE ≥19 on the Borg Scale 6-20 (6). $\dot{V}O_{2max\text{-}run}$ was based on average of the two highest measurements. Measurements of oxygen consumption were done each 30 s. Subjects with $\dot{V}O_{2max\text{-}run}$ higher than 65 mL · kg^{-1} · min^{-1} were included. *Day 2* subjects performed 40 min of familiarization DP training on the poling ergometer (Thoraxtrainer Elite) with workloads ranging from 55-85% of their HR$_{max\text{-}run}$. *Day 3* subjects performed a $\dot{V}O_{2max\text{-}pol}$ test on the poling ergometer, the highest heart rate was defined as HF$_{max\text{-}pol}$. Criteria's for that $\dot{V}O_{2max\text{-}pol}$ was reach were the same as for $\dot{V}O_{2max\text{-}run}$. On d*ays 4 and* 5 the participants completed time trials identical to the final C-PTs, but without supplement or blood sampling. Pilot results indicated that performance stabilizes at the third 8 km time-trial (data not shown).

Before each 8 km C-PT test, subjects performed a standardized warm-up protocol lasting 26 min. The warm-up was performed as an incremental test with four, 5-min workloads, equivalent to 40, 50, 60 and 70 % of subjects $\dot{V}O_{2max\text{-}pol}$ with a 2 min break between each workload. Heart rate, VO_2 and RER were measured as means between the 3-4.5 min of each workload. Subjective ratings of perceived exertion (RPE) according to the Borg-scale (from 6 to 20) were determined for each workload (6). Following the warm up

incremental test, a 5 min break was used for blood sampling and preparation for the 8-km C-PT. During the 8 km C-PT subjects self-selected their speed with the goal of using as little time as possible (Time-trial). Encouragement was given during the whole C-PT. Each C-PT was separated by 6 days (wash out period) and subjects were instructed to abstain from hard intensity training (80-100% of HR_{max}) or any strength training 48 h before testing. The tests were performed from September through November, which is a period before the start of the cross-country skiing season.

FIGURE 1 NEAR HERE

The performance tests (C-PT)

Paragraph Number 8 Before the study started a pilot study was performed to determine the variations in subjects performed during an 8 km C-PT. Four highly trained subjects ($\dot{V}O_{2max-pol}$ 57.6±2.3 mL · kg^{-1} · min^{-1}) all familiar with the cross-country DP technique participated. Results showed that each subject needed two 8 km C-PT trials before they had no further improvement in poling performance. The improvements in performance during the 8 km C-PT trails were: test 1 vs. 2, 8%, test 2 vs. 3, 1.6% and test 3 vs. 4, 0.8%. Typical CV% between test 1 vs. 2 was 3.2% (90CL: 2.0 to 9.7; ES=0.33 small), between test 2 vs. 3, 1.1% (90CL: 0.7 to 3.2; ES=0.12 trivial), and test 3 vs. 4, 2.1% (90CL: 1.3 to 6.2; ES=0.24 trivial/small).

Paragraph Number 9 The subjects prepared for each 8 km C-PT as they normally would do prior to a competition. To minimize variation in pre-exercise glycogen stores, diet and exercise diaries were used to standardize food intake and training for each subject. Subjects were asked to refrain from caffeine consumption the last 48 h before each experiment. Four subjects were regular caffeine drinkers (100-250 mg/day). One subject (subject 7) who normally had a high daily intake of caffeine (> 300 mg/day) was allowed to

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consume ¼ of his normal caffeine amount 48 to 24 h before testing, but refrained from caffeine the last 24 h. Their last meal was consumed approximately 1.5 h before arriving at the laboratory on both testing days. Subjects were allowed to choose what this meal consisted of, but were told to eat the same meal as they normally would do before important competitions. This meal should be rich in carbohydrates and proteins. The training performed the last 48 h and food intake the day before was registered before the first CP-T. Subjects were instructed to follow the same training and diet procedure before the second test. After arriving at the laboratory, subject only consumed water prior to and during the testing. During the 8 km C-PT, no intake of water was allowed to mimic real life competitions.

Each subject arrived at the laboratory at the same time of day for each of their C-PT. Upon arrival subjects rested in a supine position for 15 min and the lowest heart rate was recorded as resting heart rate for the day. Next, a catheter was placed (BD Veneflon^{TM} Pro, Helsingborg, Sweden) in the cephalic vein and a 7 ml resting blood sample was drawn in tubes containing EGTA/gluthatione [20 µl 0.2 M glutathione and 0.2 M EGTA per ml blood]. Blood samples were centrifuged (10 min; 2,500 rpm; 4 °C) and three plasma aliquots were stored (Microtube Superspin, VWR International, West Chester, PA, USA) at -80 °C until analyses. Capillary blood was taken from a fingertip for measurement of glucose (HemoCue glucose 201^{+}; Ängelholm, Sweden) and lactate (YSI 1500 SPORT; Yellow Springs Instruments, Yellow Springs, OH USA). The subjects then consumed either CAF or PLA. Thirty min after supplementation, resting heart rate was determined as above, followed by a venous and capillary blood sample. Forty-five min after supplementation, the warm up protocol commenced. Between each submaximal workload there was a 2 min break where blood samples (5 ml) were drawn and prepared as described above. After the venous blood samples, catheters were flushed with saline (Braun Melsungen AG, Germany) and capillary

samples for measurement of glucose and lactate were taken. After each workload subjects reported RPE according to the Borg-scale (6).

During the 8 km C-PT, HR and speed were recorded each km. At 3, 5 and 7 km subjects were asked to report pain in legs and arms on a scale from 1 to 10 (1 as no pain and 10 unbearable/maximal pain). During the C-PT, subjects could see the remaining distance (meters), and subjects were encouraged by a blinded test leader. Venous blood samples (7 ml) were taken 1 and 15 min after completion of the 8 km C-PT described above.

Thorax Trainer – CC-POL

Paragraph Number 10 The cross-country double poling ergometer used in the study was a Thoraxtrainer Elite (Thoraxtrainer, Holbæk, Denmark). Temperature in the test laboratory was between 22-23°C on all test days. Ski poles used during all testing were Swix CT1 (Swix, Lillehammer, Norway) and length standardized to $85 \pm 2\%$ of subject's height. The ski poles were attached to two sleds that moved independently and were connected to a flywheel that provided resistance. A computer displayed work output (W), $km \cdot h^{-1}$, and poling frequency in real time. Resistance in the Thoraxtrainer is generated by air pressure, and the mean barometric air pressure on days for placebo and caffeine trials averaged 966 ± 4 and 970 ± 2.2 mmHg, respectively ($p>0.05$). The Thoraxtrainer Elite was set at level one (easiest) of ten different levels during all testing to optimize technique. For more information about the DP technique and the Thoraxtrainer, see the studies by Bojsen-Moller et al. (2010) and Van Hall et al. (2003).

Plasma caffeine

Paragraph Number 11 Sample preparation of 200 µL plasma and the subsequent measurements of caffeine and theophylline (internal standard; IS) by liquid chromatography mass spectrometry with electrospray ionization (LC-ESI-MSMS) were performed according to a method previously described by Wang *et al (38)*. In brief, 200 µL plasma was added 100

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µL 18 µg/mL IS in MeOH-water (50:50, v/v) and 100 µL MeOH-water (50:50, v/v). After vortex mixing for 30 s, an aliquot (3 mL) of diethyl ether-dichloromethane (3:2, v/v) was added and vortexed for 2 min, followed by centrifugation at 3,000 rpm for 10 min. The upper organic layer was transferred to a 4 mL sample vial and evaporated to dryness at 40 °C under a gentle stream of nitrogen. Residues were then dissolved in 600 µL of mobile phase followed by vortex-mixing for 30 s. A 1 µL aliquot of the reconstituted solution was injected onto the LC-ESI-MSMS system.

Plasma glycerol and FFA

Paragraph Number 12 Glycerol was measured with kit based on colorimetric method (Randox laboratories Ltd; Antrim, UK) and concentration of non-esterified fatty acids (FFA) was measured with kit from DIALAB (Weiner Neudorf, Austria) according to description.

Plasma catecholamines

Paragraph Number 13 Plasma adrenaline and noradrenaline were measured with a Cat Combi Elisa kit (DRG Instruments GmbH, Marburg, Germany) according to description.

Questionnaires

Paragraph Number 14 Pain in arms and legs was evaluated by a 1-10 point scale described by Ritchie & Hopkins (1991). Other questionnaires were used to evaluate motivation, day-form and sleep quality using a scale from 1-100 (34).

Statistical Analysis

Paragraph Number 15 All data in the study are presented as means $\pm$ standard error of the means (SEM), and differences in performance during the 8 km C-PT were evaluated by a paired t-test. A two-way ANOVA for repeated measures was used to elicit differences in HR,

LA, $\dot{V}O_2$, glucose, and RPE during submaximal workloads between the two treatments. If a significant f-ratio was found, a pared t-test was used to test differences between treatments on a workload. All data were tested for normal distribution using Shapiro-Wilk. For analyzing the typical error (CV) of the 8 km C-PT, a spread sheet by Will Hopkins (24) was used. Statistical analyses were performed using GraphPad Prisim 6, and the level of significance was set at $p<0.05$.

Results

Performance Test (8 km C-PT)

Paragraph Number 16 Subjects completed the 8 km C-PT 4 % faster (P<0.003) after ingestion of CAF (33:01±1:24 min) as compared to PLA (34:26±1:25 min) (Fig. 2). Total numbers of poling strokes to complete the C-PT did not differ between treatments (CAF: 1942±126 strokes and PLA: 1932±132 strokes; p<0.46). Moreover, 80 % of the subjects performed the 8 km C-PT faster after CAF ingestion than PLA (Fig. 2). Mean speed was higher from start to finish after CAF ingestion compared to PLA (Fig. 3a). Mean speed for the 8 km C-PT was 14.5±0.6 vs. 13.9±0.6 km · h^{-1} (p<0.003), after CAF and PLA, respectively. Mean heart rate during the C-PT was 179±2 for CAF and 174±2 for PLA (p<0.00001) (Fig. 3b).

FIGURE 2, and 3 NEAR HERE

Plasma caffeine concentrations were low in all subjects on arrival before both tests. Ingestion of 6 mg · kg^{-1} of caffeine increased plasma concentrations to 40.8±1.9 µM prior to the 8 km C-PT and it remained at a similar level throughout the exercise trial (Table 1), whereas ingestion of PLA resulted in no increase in plasma caffeine concentration (Table 1).

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Plasma adrenaline immediately after the C-PT was increased during both CAF and PLA but was higher after CAF than PLA, and remained so at least 15 min after the 8 km C-PT (Table 1). Plasma concentration of noradrenaline increased in CAF and PLA to similar levels immediately after C-PT, but was slightly higher in CAF 15 min after the C-PT. Plasma glycerol and FFA were similar (Table 2) for the two treatments. Blood concentrations of lactate and glucose increased during the 8-km C-PT, and were higher after CAF compared with PLA (Table 2). The concentration of blood lactate and glucose remained elevated 15 min after the C-PT, and remained higher after CAF compared with PLA. The same tendency was observed for glycerol (Table 2). There was no difference in the quantity of sleep before PLA and CAF tests (7.6±1.3 and 7.8±1.4 h).

TABLE 1 AND 2 NEAR HERE

Metabolism at resting and during incremental tests

Paragraph Number 17 Resting heart rate and concentration of adrenaline, noradrenaline, glucose, FFA or glycerol were not increased 35 min after caffeine ingestion (Table 1 and 2). Blood glucose concentration decreased during poling in PLA at 50 % of $\dot{V}O_{2max\text{-}pol}$, and higher intensities. In CAF, blood glucose was higher than PLA at 50 and 70 % of $\dot{V}O_{2max\text{-}pol}$, and only lower than resting values at 70 % of $\dot{V}O_{2max\text{-}pol}$ (Table 2). The heart rate increased similarly in CAF and PLA (Table 3). RER increased with increasing intensity in both PLA and CAF, but was significantly higher during PLA compared to CAF at 70 % of $\dot{V}O_{2max\text{-}pol}$ (Table 3). Plasma concentration of lactate, FFA and glycerol showed no differences at any exercise intensity (Table 2).

Questionnaires

Paragraph Number 18 During the incremental test subjects reported lower RPE after CAF ingestion at 50, 60 and 70 % of $\dot{V}O_{2max-pol}$ (Table 3). Subjects reported "no muscular pain" in neither arms nor legs on arrival (Fig. 3c), but higher pain in arms than in legs during the C-PT. Reported muscular pain in both arms and legs followed a similar pattern in PLA and CAF. Pain increased gradually during the C-PT in arms as well as legs for both treatments. In arms pain was 8.3±0.3 and 8.4±0.3 on scale (almost unbearable) for PLA and CAF at 7 km (Fig. 3c), whereas leg pain was only 6.0±0.8 and 5.7±0.6 (some/quite a lot) at 7 km. The motivation was high before both CAF and PLA trials (84.0±3.9, 84.5±3.4; NS), and subjects experienced similar "day-form" (68.0±2.7; 69.0±4.3; NS). Interestingly, subjects reported better "day-form" after the 8-km C-PT after ingestion of caffeine compared to placebo (78.5±4.2 *vs* 68.2±4.8, p<0.005). The questionnaires revealed that the participants were unable to sense which product they received during the different trials. Diary reports on training and intake of food, liquid and caffeine containing products the last 48 h prior to the C-PT showed that the participants had followed instructions.

TABLE 3 NEAR HERE

Oxygen uptake and metabolism in double poling and running

Paragraph Number 19 Maximum oxygen uptake was higher during running than while DP (69.3±1.0 and 63.2±1.5, respectively; p<0.05). The highest heart rate achieved during the $\dot{V}O_{2max}$ tests was achieved during running compared to when poling (194±2 and 189±1, respectively; p<0.05). Heart rates at the same relative submaximal intensities (60 and 70 % of $\dot{V}O_{2max}$) were similar during running and poling (153±3 and 156±3; NS). Still relative contribution of carbohydrate oxidation measured as RER was much higher during poling compared with running at both 60 % (0.97±0.01 vs. 0.89±0.01; p<0.05) and 70 % (0.98±0.01

vs. 0.91±0.01, respectively; p<0.05) of $\dot{V}O_{2max}$. Lactate concentration was higher during poling compared to running at the same submaximal intensities (70 %: Poling 3.2 ±0.3 vs running 1.3±0.2 mM; p<0.05), but similar concentrations between exercises were reached after finishing the $\dot{V}O_{2max}$ tests (poling: 8.1±0.4 vs running 8.1±0.3 mM).

Discussion

Paragraph Number 20 DP is an important technique for the best skiers in long distance competitions such as Vasaloppet and Marcialonga. The winner of the 90 km long Vasaloppet race in 2013 (Jørgen Aukland) completed the race without any grip wax only using the DP technique. Also in normal XCS DP capacity can determine the final outcome of competitions. Research on the effects of CAF on sports performance has traditionally focused on cycling and running where endurance capacity of the leg muscles determines power output, and performance. In our study maximal oxygen uptake was ~10 % lower while DP compared to that of running. This is in accordance with previous studies (5, 23), showing that even when highly trained XCS DP they are unable to obtain the same maximal oxygen uptake, and HR_{max} as when running. This is most likely related to differences between arm and leg muscles in metabolism and oxygen extraction as a result of lower oxidative capacity and capillarization in the arm muscles (10, 22, 29), or the fact that a smaller muscles mass is being used. Thus, results from studies on leg muscles and CAF may not be directly applicable to exercises while DP during XCS.

Paragraph Number 21 Indeed leg muscles utilize a substantial amount of oxygen during DP (5, 23, 37). However during DP arm muscles contribute as much as 40-50% of total O_2-cost (5, 37), and more importantly represent the main speed generating force (37). Therefore endurance capacity of the arm muscles plays a crucial role in performance

outcome. Results of pain perception during the 8 km C-PT confirmed that arms limit performance in DP as muscular pain was much greater in arms (almost unbearable) than in legs (some pain) (Fig. 3c). Importantly, we show for the first time that CAF increases endurance performance in DP in which the main muscles used to produce power and speed are located in the upper body.

Paragraph Number 22 The results from the present study are of significant interest for performance sports. CV results for reliability of the 8 km C-PT are small (around 1~2%), and show little variation from test to test after the familiarization procedure (Figure 1). The improvement of 4% after ingestion of 6 mg · kg^{-1} CAF observed in the study would most likely effect results in real life XCS competitions. Actually a reduction of 1:25 min to complete the 8 km C-PT is the time difference between the winner and 12th place in the 2011 World Championships in XCS during the 15-km classical relay in Oslo.

Paragraph Number 23 It has been reported that CAF improves performance by 1-5% in cycling (26), running (7) and rowing (8) studies. Our present study is in agreement with these studies. CAF was initially suggested to improve performance because fat oxidation was increased and glycogen was spared (11, 25). The higher lactate concentration after the CP-T with CAF compared to PLA do not support that a glycogen sparing was the reason for the improved performance. Several mechanisms contribute to the ergogenic effects of CAF (20, 36). Many of these effects seem to be related to CAF affecting the CNS. CAF is an antagonist of adenosine receptors with particularly high affinity for A_1 and A_{2A} receptors (16), and our plasma caffeine concentrations of ~ 40 μM (Table 1) would significantly reduce A_1 and A_2 receptor activation (16). Adenosine receptors are expressed in most tissues including brain, heart, skeletal muscles and the vascular system (17). Blockade of adenosine receptors reduce somatic pain (14), improve MVC (39), as well as reducing perceived exertion at submaximal intensities (33, 36). In our present study, caffeine reduced RPE in DP at 50, 60 and 70 % of

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maximum oxygen uptake (Table 3), which is in agreement with findings in running and cycling (2, 3, 13).

Paragraph Number 24 Caffeine-mediated reduction in pain perception or increased MVC may have contributed to the improved performance during the 8 km C-PT. The higher lactate concentration after CAF compared to PLA supports higher effort, but does not alone explain the improved performance. The poling speed was higher in the CAF 8 km C-PT which would require a higher aerobic capacity, and/or power production. Indeed a higher average power per stroke during DP must have been produced after CAF ingestion since the number of DP strokes used to complete the 8 km C-PT was similar in PLA and CAF.

Paragraph Number 25 CAF has been observed to improve MVC and muscular endurance (39). The effect of CAF on MVC seems to be related to improved motor unit recruitment (31), and not as a direct effect on the muscles (39). An improved MVC after CAF ingestion resulting from increased motor unit recruitment (31) would potentially increase DP power production. Facilitation of motor unit recruitment might also reduce RPE as observed at submaximal workloads in the study. Although well-documented effect of caffeine on MVC has only been reported in knee extensors (39), it is possible that the improved performance after CAF ingesting in the study came as a result of improved MVC. However, an improved MVC in arm muscles after caffeine ingestion is not consistently documented (39).

Paragraph Number 26 In the present study pain in the arms becomes intense in the arms (almost unbearable; Figure 3c), and the ability to withstand pain and to maintain an effective DP technique is important for high performance. Our participants exercised at a higher intensity throughout the whole 8 km C-PT, which lead to higher heart rate, and blood lactate accumulation in the CAF trial compared to PLA. Thus, we speculate that reduced pain sensation allows subjects to push themselves harder and exercise at a higher heart rate. The

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higher concentrations of lactate and adrenaline immediately after the 8 km C-PT with CAF ingestion support the idea that reduced pain sensation allowed higher discomfort, heart rate and better effort.

Paragraph Number 27 CAF might have increased heart rate by direct action on adenosine receptors in the heart coupled to $G_{\alpha i}$ or by increasing sympathetic activity (16). However, we did not observe any effect of CAF on heart rate, $\dot{V}O_2$ or adrenaline concentration at rest or during submaximal loads (Table 1 and 3). Increased heart rate after CAF consumption was only observed when speed was higher during the 8 km C-PT. No measurements of $\dot{V}O_2$ were done during the 8 km C-PT, but the average heart rate increase with 5 beats per min (BPM) during the 8 km C-PT, and the speed was improved by 0.6 ± 0.04 $km \cdot h^{-1}$. Results at the submaximal exercise intensities show that an increase of $0.6\ km \cdot h^{-1}$ requires an increase in $\dot{V}O_2$ of $3.2\pm0.5\ ml^{-1} \cdot kg^{-1} \cdot min^{-1}$ and heart rate of 6 BPM. Since heart rate was 5 ± 1 BPM higher during the CAF trial compared with the PLA trial during the 8 km C-PT (Figure 3b), it is tempting to suggest that the ability to maintain a higher heart rate during the CAF time trial increased oxygen uptake. A higher delivery of O_2 to the active muscles in the arms might therefore have allowed for an increased production of power during DP.

Paragraph Number 28 In our present study, carbohydrate oxidation was much higher during arm exercise compared to running at similar relative intensities. It has been shown that blood glucose extraction is higher in arm muscle than in leg muscles exercise (37), and the higher carbohydrate metabolism in arm muscles is explained by lower density of capillaries and mitochondria, and less effective carbohydrate oxidation in arm muscles compared to leg muscles (10, 22, 37). Interestingly, blood glucose decreased during poling at all submaximal intensities in PLA, and blood glucose was higher in CAF compared to PLA at 50 and 70 % of

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$\dot{V}O_{2max-pol}$. Recently it has been shown that intake of caffeine reduces contraction-stimulated glucose uptake in rat skeletal muscles (32). Thus, it is possible that caffeine reduces glucose uptake in arm muscles. Interestingly, at 70 % of $\dot{V}O_{2max-pol}$, RER was lower in CAF compared to PLA, although adrenaline, glycerol and FFA were similar, and glucose concentration higher in CAF. These data suggest that glucose uptake may have been impaired directly by caffeine. Indeed, the higher glucose concentration in CAF may also be due to higher glucose production, and future studies will clarify the effect of caffeine on glucose kinetic during arm exercise.

Paragraph Number 29 *Conclusion:* We are the first to report that ingestion of 6 mg caffeine $\cdot$ kg^{-1} body weight enhances endurance performance in an 8 km double poling time trial, an activity in which arm muscles limits performance. Caffeine ingestion allowed the participants to exercise with a higher heart rate during the time trial, and experienced reduced RPE at submaximal intensities. These results are quite similar to those observed in studies where subjects are mainly limited by endurance capacity of leg muscles e.g. cycling and running. We speculate that CAF-mediated improvement in performance comes as a result of reduced pain sensation allowing subjects to exercise at higher speeds and heart rate.

Acknowledgements

Disclosures

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Reference List

1. Bazzucchi, I., F. Felici, M. Montini, F. Figura, and M. Sacchetti. Caffeine improves neuromuscular function during maximal dynamic exercise. *Muscle Nerve* 43:839-844, 2011.

2. Bell, D. G., I. Jacobs, and J. Zamecnik. Effects of caffeine, ephedrine and their combination on time to exhaustion during high-intensity exercise. *Eur. J. Appl. Physiol Occup. Physiol* 77:427-433, 1998.

3. Bell, D. G. and T. M. McLellan. Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers. *J. Appl. Physiol* 93:1227-1234, 2002.

4. Bjorklund, G., T. Stoggl, and H. C. Holmberg. Biomechanically influenced differences in O2 extraction in diagonal skiing: arm versus leg. *Med. Sci. Sports Exerc.* 42:1899-1908, 2010.

5. Bojsen-Moller, J., T. Losnegard, J. Kemppainen, T. Viljanen, K. K. Kalliokoski, and J. Hallen. Muscle use during double poling evaluated by positron emission tomography. *J. Appl. Physiol* 109:1895-1903, 2010.

6. Borg, G., P. Hassmen, and M. Lagerstrom. Perceived exertion related to heart rate and blood lactate during arm and leg exercise. *Eur. J. Appl. Physiol Occup. Physiol* 56:679-685, 1987.

7. Bridge, C. A. and M. A. Jones. The effect of caffeine ingestion on 8 km run performance in a field setting. *J. Sports Sci.* 24:433-439, 2006.

8. Bruce, C. R., M. E. Anderson, S. F. Fraser, N. K. Stepto, R. Klein, W. G. Hopkins, and J. A. Hawley. Enhancement of 2000-m rowing performance after caffeine ingestion. *Med. Sci. Sports Exerc.* 32:1958-1963, 2000.

9. Burke, L. M. Caffeine and sports performance. *Appl. Physiol Nutr. Metab* 33:1319-1334, 2008.

10. Calbet, J. A., H. C. Holmberg, H. Rosdahl, H. G. van, M. Jensen-Urstad, and B. Saltin. Why do arms extract less oxygen than legs during exercise? *Am. J. Physiol Regul. Integr. Comp Physiol* 289:R1448-R1458, 2005.

11. Costill, D. L., G. P. Dalsky, and W. J. Fink. Effects of caffeine ingestion on metabolism and exercise performance. *Med. Sci. Sports* 10:155-158, 1978.

12. Cox, G. R., B. Desbrow, P. G. Montgomery, M. E. Anderson, C. R. Bruce, T. A. Macrides, D. T. Martin, A. Moquin, A. Roberts, J. A. Hawley, and L. M. Burke. Effect of different protocols of caffeine intake on metabolism and endurance performance. *J. Appl. Physiol* 93:990-999, 2002.

13. Cureton, K. J., G. L. Warren, M. L. Millard-Stafford, J. E. Wingo, J. Trilk, and M. Buyckx. Caffeinated sports drink: ergogenic effects and possible mechanisms. *Int. J. Sport Nutr. Exerc. Metab* 17:35-55, 2007.

14. Doherty, M. and P. M. Smith. Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis. *Scand. J. Med. Sci. Sports* 15:69-78, 2005.

15. Essig, D., D. L. Costill, and P. J. Vanhandel. Muscle Glycogen and Triglyceride Use During Leg Cycling Following Caffeine Ingestion. *Medicine and Science in Sports and Exercise* 12:109, 1980.

16. Fredholm, B. B. Adenosine receptors as drug targets. *Exp. Cell Res.* 316:1284-1288, 2010.

17. Fredholm, B. B., K. Battig, J. Holmen, A. Nehlig, and E. E. Zvartau. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol. Rev.* 51:83-133, 1999.

18. Fredholm, B. B., A. P. IJzerman, K. A. Jacobson, K. N. Klotz, and J. Linden. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53:527-552, 2001.

19. Goldstein, E. R., T. Ziegenfuss, D. Kalman, R. Kreider, B. Campbell, C. Wilborn, L. Taylor, D. Willoughby, J. Stout, B. S. Graves, R. Wildman, J. L. Ivy, M. Spano, A. E. Smith, and J. Antonio. International society of sports nutrition position stand: caffeine and performance. *J. Int. Soc. Sports Nutr.* 7:5, 2010.

20. Graham, T. E. Caffeine and exercise: metabolism, endurance and performance. *Sports Med.* 31:785-807, 2001.

21. Graham, T. E. and L. L. Spriet. Performance and metabolic responses to a high caffeine dose during prolonged exercise. *J. Appl. Physiol* 71:2292-2298, 1991.

22. Helge, J. W. Arm and leg substrate utilization and muscle adaptation after prolonged low-intensity training. *Acta Physiol (Oxf)* 199:519-528, 2010.

23. Holmberg, H. C., S. Lindinger, T. Stoggl, G. Bjorklund, and E. Muller. Contribution of the legs to double-poling performance in elite cross-country skiers. *Med. Sci. Sports Exerc.* 38:1853-1860, 2006.

24. Hopkins, W. G. Measures of reliability in sports medicine and science. *Sports Med.* 30:1-15, 2000.

25. Ivy, J. L., D. L. Costill, W. J. Fink, and R. W. Lower. Influence of caffeine and carbohydrate feedings on endurance performance. *Med. Sci. Sports* 11:6-11, 1979.

26. Ivy, J. L., L. Kammer, Z. Ding, B. Wang, J. R. Bernard, Y. H. Liao, and J. Hwang. Improved cycling time-trial performance after ingestion of a caffeine energy drink. *Int. J. Sport Nutr. Exerc. Metab* 19:61-78, 2009.

27. Jacobson, T. L., M. A. Febbraio, M. J. Arkinstall, and J. A. Hawley. Effect of caffeine co-ingested with carbohydrate or fat on metabolism and performance in endurance-trained men. *Exp. Physiol* 86:137-144, 2001.

28. Jenkins, N. T., J. L. Trilk, A. Singhal, P. J. O'Connor, and K. J. Cureton. Ergogenic effects of low doses of caffeine on cycling performance. *Int. J. Sport Nutr. Exerc. Metab* 18:328-342, 2008.

29. Jensen-Urstad, M. and G. Ahlborg. Is the high lactate release during arm exercise due to a low training status? *Clin. Physiol* 12:487-496, 1992.

30. Jensen-Urstad, M., I. Hallback, and K. Sahlin. High anaerobic energy release during submaximal arm exercise. *Clin. Physiol* 13:81-87, 1993.

31. Kalmar, J. M. The influence of caffeine on voluntary muscle activation. *Med. Sci. Sports Exerc.* 37:2113-2119, 2005.

32. Kolnes, A. J., A. Ingvaldsen, A. Bolling, J. T. Stuenaes, M. Kreft, R. Zorec, P. R. Shepherd, and J. Jensen. Caffeine and theophylline block insulin-stimulated glucose uptake and PKB phosphorylation in rat skeletal muscles. *Acta Physiol (Oxf)* 200:65-74, 2010.

33. Momsen, A. H., M. B. Jensen, C. B. Norager, M. R. Madsen, T. Vestersgaard-Andersen, and J. S. Lindholt. Randomized double-blind placebo-controlled crossover study of caffeine in patients with intermittent claudication. *Br. J. Surg.* 97:1503-1510, 2010.

34. Ritchie, S. E. and W. G. Hopkins. The intensity of exercise in deep-water running. *Int. J. Sports Med.* 12:27-29, 1991.

35. Skinner, T. L., D. G. Jenkins, J. S. Coombes, D. R. Taaffe, and M. D. Leveritt. Dose response of caffeine on 2000-m rowing performance. *Med. Sci. Sports Exerc.* 42:571-576, 2010.

36. Tarnopolsky, M. A. Effect of caffeine on the neuromuscular system--potential as an ergogenic aid. *Appl. Physiol Nutr. Metab* 33:1284-1289, 2008.

37. van, H. G., M. Jensen-Urstad, H. Rosdahl, H. C. Holmberg, B. Saltin, and J. A. Calbet. Leg and arm lactate and substrate kinetics during exercise. *Am. J. Physiol Endocrinol. Metab* 284:E193-E205, 2003.

38. Wang, A., J. Sun., H, Feng., S, Gao., Z, He. Simultanous determination of paracetamol and caffeine in human plasma by LC-ESI-MS. In: Chromatographia: Chromatographia, 2008, pp. 281-285.

39. Warren, G. L., N. D. Park, R. D. Maresca, K. I. McKibans, and M. L. Millard-Stafford. Effect of caffeine ingestion on muscular strength and endurance: a meta-analysis. *Med. Sci. Sports Exerc.* 42:1375-1387, 2010.

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Legends

Figure 1. Experimental design of the 8 km cross country double poling test. (A) Shows tests and training performed during the 6 week period to accustom for the two performance tests in double poling (CP-T). (B) Shows the test procedure on the performance tests. Prior to the CP-T, subjects performed an incremental tests consisting of four intensities for five minutes. Similar protocol to the CP-T was completed at Pretest-1 and Pretest-2, except that caffeine/placebo was not administrated and no blood samples were taken. Abbreviations: $\dot{V}O_{2max-run}$, Training in thorax trainer, $\dot{V}O_{2max-pol}$, Pre-test I, Pre-test II, C-PT I and C-PT II.

Figure 2. Effect of caffeine on time trial performance in a double poling ski ergometer. Average time and individual time for the 8 km C-PT without (open bar) and with caffeine ingestion (filled bar). * $p < 0.05$ compared to placebo.) Subjects 3, 7, 8 and 10 were caffeine users (300-600 mg·day^{-1}). The rest were nonusers (<40 mg·day^{-1}). All subjects except subject 4 and 9 used less time after caffeine ingestion.

Figure 3. Effect of caffeine on mean speed, heart rate and pain in arm and leg muscles during the 8 km C-PT. (A) Mean speed during performance tests in a double poling without (open symbols) and with caffeine ingestion (filled symbols). (B) Mean heart rates during performance tests in a double poling without (open symbols) and with caffeine ingestion (filled symbols). (C) Subjects reported muscular pain in arms (circles) and legs (squares) without (open symbols) and with caffeine (filled symbols) at 3, 5 and 7 km. A scale from 0-10 (no pain to unbearable) was used. Values are presented as means ± SEM. *****Significant difference between treatments ($p<0.05$).

Note: Data on muscle pain in legs and arms before exercise were collected retrospectively 3-4 weeks after the test subjects had performed the two different 8 km C-PT.