Caffeine improves performance in double poling during acute exposure to 2,000 m altitude

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Running title: “Caffeine and exercise performance in altitude”

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Abstract

There is limited research on the physiological effects of caffeine (CAF) ingestion on exercise performance during acute hypoxia. The aim of the present study was therefore to test the effect of placebo (PLA) and CAF (4.5 mg·kg⁻¹) on double poling (DP) performance during acute hypoxia. Methods: Thirteen male sub-elite cross-country skiers (\(\dot{V}O_{2\text{max}}\) 72.6 ± 5.68 mL·kg⁻¹·min⁻¹) were included. Performance was assessed as 1) an 8 km cross-country DP time-trial (C-PT), and 2) time until task failure at a set workload equal to ~90% of DP \(\dot{V}O_{2\text{max}}\). Testing was carried out in a hypobaric chamber, at 800 mBar, (\(P_{O2}\) ~125 mmHg) corresponding to ~2000 m above sea level in a randomized double-blinded, placebo-controlled, cross-over design. Results: CAF improved time to task failure from 6:10 ±1:40 min to 7:22 ±1:30 min (p<0.05), and velocity the first 4 km (p<0.05), but not overall time usage for the 8 km C-PT. During submaximal exercise subjects reported lower pain in arms and rate of perceived exertion (RPE) following CAF ingestion. Throughout C-PTs similar RPE and pain was shown between treatments. However, higher heart rate was observed during the CAF 8 km (187±7 vs. 185±7; p<0.05) and 90 % C-PT (185±7 vs.181±9) associated with increased ventilation, blood lactate, glucose, adrenaline, decreased pH, and bicarbonate. Conclusion: The present study demonstrates for the first time that CAF ingestion improves DP time to task failure although not consistently time-trial performance during acute exposure to altitude. Mechanisms underpinning improvements seem related to reduced pain, RPE and increased HR during CAF C-PTs.

Keywords: Exercise performance, hypoxia, heart rate, rate of perceived exertion, and oxygen consumption
Introduction

During the 1968 Olympic Games in Mexico City at an altitude of 2240m sprinters and jumpers set several world records while long distance runners ran markedly slower compared to sea level results. This launched a scientific interest in understanding mechanisms explaining reduced endurance performance under hypoxic conditions (4; 21; 44).

At sea level the lungs and the pulmonary system normally have no problem fully saturating arterial blood with O\textsubscript{2} (SpO\textsubscript{2}) during rest or high-intensity exercise (3). However, when exposed to hypoxia the reduction in performance and $\dot{V}$O\textsubscript{2max} is highly related to pulmonary limitations in saturating hemoglobin while passing alveolar ducts, due to reduction of partial pressure in the atmosphere (4; 21; 22; 32; 44). This phenomenon, known as the exercise-induced arterial hypoxemia (EIH), is defined as SpO\textsubscript{2} ≤ 92% (44), and despite ventilation ($V_e$) increases to prevent EIH during exercise, a greater reduction of SpO\textsubscript{2} in arterial blood is evident during acute hypoxic exercise compared to sea level conditions (2; 4; 16; 17; 44). The reduction in SpO\textsubscript{2} also triggers a compensatory acceleration of heart rate (HR) during submaximal and maximal exercise to prevent EIH (4; 12; 39; 44). However, a decrease in maximal HR is well established when subjects are exposed to acute hypoxia and may contribute to the observed reduction in $\dot{V}$O\textsubscript{2max} and exercise performance (2; 4; 25; 30; 39; 44). The reason for the reduction in HR\textsubscript{peak} is not entirely understood, but it is believed to be associated with enhanced parasympathetic neural activity due to decreased signals from skeletal muscles (2; 9).

The extensive research related to the effects of caffeine (CAF) ingestion (3–9 mg·kg\textsuperscript{-1}) during sea level testing shows it is beneficial in most sporting conditions for improving endurance performance (10; 14; 41; 42). However, the effects of CAF ingestion on performance during acute hypoxia have so far received little attention. Until now only two studies have addressed the topic at altitudes above 4300m under standardized laboratory conditions.
conditions, whereas the upper limit used in today’s elite sports competitions is ~2,000 m (5; 22). Interestingly, one of the most consistent observations associated with performance improvements after CAF ingestion is increased HR (10; 14; 29; 41; 42). The explanation for higher HR following CAF ingestion is increased intensity during time trials, and/or sympathetic neural activity explained by higher adrenaline and/or inhibition of adenosine receptors (10; 14; 41; 42). Furthermore, studies have also found the increase in HR and performance capacity to be associated with increased \( \bar{V}O_2 \) (1; 27; 35; 41). However, if compromised oxygen saturation limits performance during acute hypoxia, it could be hypothesized that a potential increased HR following CAF ingestion would not necessarily increase \( \bar{V}O_2 \) or improve performance as previously observed during sea-level conditions.

The aim of the present study was therefore to test the effect of CAF (4.5 mg·kg\(^{-1}\)) ingestion on DP performance during acute exposure (2 hours) to hypoxia corresponding to 2000m (800 mBar) in a hypobaric chamber. To investigate the effect of CAF on HR, \( \bar{V}O_2 \), and endurance performance at altitude, an 8 km cross-country skiing double poling (DP) time trial performance test (8 km C-PT) and a time to task failure at a fixed workload (~90% of \( \bar{V}O_2 \)\(_{peak-pol-alt}\); 90 % C-PT) was used.

Materials and Methods

Subjects: thirteen healthy male sub-elite cross-country skiers gave their written consent to participate after being informed of the purposes of the study and risks involved. Their physical characteristics (mean ± SD) were age 21.9 ± 2.7, height 180.0 ± 3.7, body mass 77.4 ± 5.6, \( \bar{V}O_{2max} \) running at sea level (\( \bar{V}O_{2max-run} \)) 72.6 ± 5.7 (mL·kg\(^{-1}\)·min\(^{-1}\)), \( \bar{V}O_{2max} \) DP at sea level (\( \bar{V}O_{2max-pol} \)) 62.9 ± 6.8 and \( \bar{V}O_{2max} \) DP at altitude (\( \bar{V}O_{2max-pol-alt} \)) 53.8 ± 5.3 mL·kg\(^{-1}\)·min\(^{-1}\). Inclusion criteria were: male, \( \bar{V}O_{2max-run} \) above 65 mL·kg\(^{-1}\)·min\(^{-1}\), and training...
seriously to compete in the Norwegian national cross-country skiing cup in the upcoming season.

**Study design:** The study had a randomized double-blind, placebo-controlled, crossover design. The tests and familiarization during the first four weeks of the study were performed at sea-level conditions at the Norwegian School of Sports Sciences (120 m altitude, ~960 mBar). Testing included \( \dot{V}O_{2\text{max}} \) running (wk 1), familiarization DP training (wk 2) and DP \( \dot{V}O_{2\text{max}} \) (wk 3), a test 8 km C-PT (wk 3), and the main 8 km C-PT (wk 4). The remaining five test weeks were carried out during acute (2 h) hypoxia in a hypobaric chamber (Norsk undervannsteknikk A/S, Haugesund, Norge), and included DP \( \dot{V}O_{2\text{max}} \) (wk 5), a pre 8 km C-PT (wk 5), two main 8 km C-PTs (wks 6&7) with and without CAF, and two time to task failure tests at fixed workload approximately 90% of DP \( \dot{V}O_{2\text{max-pol-alt}} \) (wks 8&9) with and without CAF.

**Experimental Procedures:** At sea level subjects the first testing day (Day 1), performed a \( \dot{V}O_{2\text{max-run}} \) test on a treadmill (Woodway, Weil am Rein, Germany) and the highest HR was defined as HR\(_{\max\text{-run}}\). HR was measured during all tests in the study using a HR monitor (Polar RS 800, Finland), with an error of measurement of less than ± 1 % as stated by the manufacturer. Oxygen consumption and RER were measured with the Oxycon Pro metabolic system (Jaeger Hochberg, Germany). The Oxygen Pro is calibrated each month against the Douglas bag method, and the error of measurement of this ergospirometry measurement is reported to be ± 3% (Åstrand et al., 2003). The equipment for measurement of \( \dot{V}O_2 \) was calibrated prior to tests with mixture gasses with known concentrations of O\(_2\) and CO\(_2\) (14.93% O\(_2\) and 5.99% CO\(_2\)) and normal air (approximately 20.95% O\(_2\) and 0.039% CO\(_2\)) both at altitude and at sea level. Volume was calibrated manually using a 3-liter pump (Calibration Syringe, Series 5530, Hans Rudolph Inc., MO, USA). During testing, subjects
used a mouth V2 mask (Hans Rudolph Instr., USA) in combination with a nose bracket. Expired air was sampled through a hose into the mixing chamber (Oxycon Pro) and analyzed with a turbine (Triple V volume transducer). The $\dot{V}O_{2max-run}$ test was performed with a standardized warm-up consisting of four workloads lasting 5 min (8 to 11 km·h$^{-1}$) with a 10.5° uphill incline. A 1 min break was given between each workload during which lactate was measured. After the last workload of the warm-up, subjects walked 5 min at 5 km·h$^{-1}$ before starting the $\dot{V}O_{2max-run}$ test which was performed as a standardized ramp test. The starting speed for the ramp test was 10 km·h$^{-1}$ with a treadmill incline of 10.5°. Each half minute speed was increased by 0.5 km·h$^{-1}$ until subjects were unable to maintain the speed and stepped off the treadmill (voluntary exhaustion). On the basis of the standardized warm-up, a linear regression was done to estimate ending $O_2$ cost. Results showed subjects were performing supramaximal workloads the last 2–2.5 min, and were ending at workloads ~110–115% of reached $\dot{V}O_{2max}$. Furthermore, all 13 subjects had to meet point one, and at least two of the three other criteria to obtain $\dot{V}O_{2max-run}$: 1) oxygen consumption leveled off (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$^{-1}$, 2) RER values were above 1.10, 3) blood lactate was above 7.0 mmol·L post testing and 4) RPE ≥19 on the Borg Scale 6–20 (8). $\dot{V}O_{2max-run}$ was based on the average of the two highest 30s measurements, and the duration of the test was between 5.5–7.5 min. The protocol and criteria for reaching $\dot{V}O_{2max}$ differ from some other protocols used for testing of $\dot{V}O_{2max}$ (36). Indeed, it is debatable, therefore, whether all subjects reached $\dot{V}O_{2max}$. However, the fact that the athletes in the study were highly trained and motivated could partially reduce the issue of whether $\dot{V}O_{2max}$ was reached. Furthermore, the $\dot{V}O_{2max-run}$ test was only used as an inclusion test; only subjects with $\dot{V}O_{2max-run}$ higher than 65 mL·kg$^{-1}$·min$^{-1}$ were included for further participation.
**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$_{\text{max-run}}$.

**Day 3** subjects performed a $\dot{V}$O$_{2\text{max-pol}}$ test on the poling ergometer, with the highest HR defined as HR$_{\text{max-pol}}$. During the $\dot{V}$O$_{2\text{max-pol}}$ test subjects performed a standardized warm-up for 10 min at a velocity equal to 75% of their HR$_{\text{max-run}}$ based on the familiarization training. Thereafter, all subjects started at a velocity of 15 km·h$^{-1}$, and speed was increased by 0.5 km·h$^{-1}$ every 30 s the first 4 min, followed by 3 min where subjects were instructed to maintain as high a velocity as possible for a duration of at least 3 minutes. Criteria for that $\dot{V}$O$_{2\text{max-pol}}$ was reached were the same as described for $\dot{V}$O$_{2\text{max-run}}$.

**Days 4 and 5** participants completed the pre 8 km C-PT and the 8 km C-PT at sea level, but without supplementation since this has previously been investigated by Stadheim et al. (41). Furthermore, Stadheim et al. showed that a minimum of one habituation trial of at least one 8 km C-PT is required to obtain acceptable reliability (CV% approximately 1–2%, (41). The 8 km C-PT started with a standardized warm-up performed as an incremental test with four 5-min workloads, equivalent to loads corresponding to 50, 55, 60 and 65% of subjects’ $\dot{V}$O$_{2\text{max-pol}}$ with a 1 min break between each workload. HR, $\dot{V}$O$_2$ and RER were measured as means between 3 and 4.5 min of each workload. Subjective ratings of perceived exertion (RPE) according to the Borg scale (from 6 to 20), and muscular pain in arms and legs was determined (1–10 point scale) for each workload (8). Following the warm-up, a 5-min break was used for blood sampling and preparation for the 8 km C-PT. During the C-PTs subjects performed the test with the goal of completing the distance in as little time as possible (41). Subjects received the V2 mask and nose bracket ~1.5–2 min before reaching 4 and 8 km for measurement of $\dot{V}$O$_2$. 
Altitude and hypoxic testing started in week 5. On Day 6 subjects performed the same protocol for testing of \( \dot{V}O_{2\text{max-pol-alt}} \) as described for sea level \( \dot{V}O_{2\text{max-pol}} \) testing. In the hypobaric chamber, \( \dot{V}O_2 \) and \( V_E \) were measured using the \( V_{\text{max29}} \) (Sensormedics, USA) which was calibrated against the Oxygen Pro each week.

Day 7 participants completed the pre 8 km C-PT during hypoxic conditions with the same protocol used during sea level testing but without the supplementation.

Days 8 and 9 subjects received either placebo (PLA) or CAF 75 min after acute exposure to hypoxia, meaning 45 min prior to the standardized warm up for the 8 km C-PT. However, compared to during sea level testing, five subjects expressed they “got too little air,” resulting in vomiting reflexes when they received the V2 mask and nose bracket for measurement of \( \dot{V}O_2 \) during pre-8 km C-PT in altitude. For these athletes, \( \dot{V}O_2 \)-measurements were not carried out to optimize test conditions during hypoxic testing.

Days 10 and 11 a time to task failure at a fixed velocity equal of approximately 90% of \( \dot{V}O_{2\text{max-pol-alt}} \) C-PT was performed in hypoxia. The velocity used was estimated based on submaximal DP \( \dot{V}O_2 \) values during the standardized warmup prior to the 8 km C-PT based on a linear regression. Subjects received either PLA or CAF 75 min after acute exposure to hypoxia. Prior to the 90% C-PT the same standardized warm up was performed as prior to the 8 km C-PT. The goal for each subject was to maintain the individual fixed workload for as long as possible. To optimize test conditions for all athletes during the 90% C-PT, \( \dot{V}O_2 \) measurements were only sampled after 3 min.

Hypobaric chamber altitude testing: During all tests in hypoxia, air pressure was reduced to 800 mBar equivalent to ~11.5 psi, or ~590 mmHg, simulating an altitude of ~2,000 m above sea level at 17°C. To ensure maintenance of atmospheric gas concentrations (20.95% \( O_2 \) and 0.039% \( CO_2 \)) during all trials, concentrations were continuously measured for both
F\textsubscript{1}CO\textsubscript{2} with the Vaisala GMT222 Carbon Dioxide Transmitter (Vaisala, Stockholm, Sweden) and F\textsubscript{1}O\textsubscript{2} with the PMA30 M&C O\textsubscript{2} analyzer (Marseille, France). During the first two hours (rest) of acute altitude exposure, approx. \(\sim 1 \text{l} \cdot \text{min}^{-1}\) oxygen was added to maintain atmospheric gas concentrations of air. During physical activity oxygen consumption increased, thus additional oxygen was added to maintain stable F\textsubscript{1}O\textsubscript{2}. On the basis of the pretests, approx. \(\sim 3 \text{l} \cdot \text{min}^{-1}\) of extra oxygen was added to cover the enhanced usage of oxygen during physical activity, but was adjusted (increased or reduced) according to observed F\textsubscript{1}O\textsubscript{2} values for each individual hypoxic trial. Three gas scrubbers containing Sofnolime filters and circulating fans worked as CO\textsubscript{2} traps to try and ensure a stable F\textsubscript{1}CO\textsubscript{2} concentration. However, during the later stages of the C-PTs (\(\sim 5-10\text{min}\)) CO\textsubscript{2} production from the subjects exceeded the capacity of CO\textsubscript{2} removal of the three scrubbers. This resulted in an enhanced F\textsubscript{1}CO\textsubscript{2} concentration of the air inside the chamber with post values of CO\textsubscript{2} between 0.05-0.08\%. Even though CO\textsubscript{2} concentration increased, it never exceeded 0.08\%; these CO\textsubscript{2} values are not considered dangerous for subjects and are unlikely to influence test results. During rest and at sea level testing F\textsubscript{1}CO\textsubscript{2} concentrations were 0.04\% as expected. Encouragement was given during all tests by a blinded test leader.

**Blood samples:** For each main test, the first blood sample was drawn at sea level before subjects went into the hypobaric chamber and test leaders carried out testing at sea level. Blood samples were drawn from the subjects’ median cubital vein using a BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). A 7 ml blood sample was drawn for all blood samples and placed in tubes containing EGTA/glutathione [20 \(\mu\text{l} 0.2 \text{ M glutathione and 0.2 M EGTA per ml blood}\)] for analysis of adrenaline, noradrenaline, and caffeine. Blood samples were immediately placed on ice water and centrifuged at 2,500 rpm for 10 min at 4 °C (Heraeus Megafuge 16R centrifuge, Thermo Electro, Germany). Thereafter, plasma was divided in three Eppendorf tubes (Microtube Superspin, VWR...
International, West Chester, PA, USA) and frozen at -80 °C. For each capillary sample the fingers were punctured by a Saft-T-Pro Plus (Accu-Check, Mannheim, Germany) for measurements of glucose, lactate or bicarbonate. For measurement of blood lactate, capillary blood samples were drawn into a 50 µl capillary tube and a 20 µl pipette was used to drawn blood into the analyzer from the 50 µl capillary tube. The analyzer was calibrated with a 5.0 mmol·l⁻¹ lactate stock solution before each test and between the submaximal workloads and main tests. Values between 4.95 mmol·l⁻¹ and 5.05 mmol·l⁻¹ were accepted. Under normal circumstances the error of measurements are ± 2% for blood lactate values between 0 and 5 mmol·l⁻¹ and ± 3% for values between 5 and 15 mmol·l⁻¹. Blood glucose measurements were taken with HemoCue glucose 201⁺ (Ängelholm, Sweden). For measurements of bicarbonate, a 125 µl capillary tube was filled with capillary blood and then measured using a ABL 80 Flex (Radiometer, Brønshøj, Denmark).

*Plasma caffeine and catecholamines:* Samples of 200 µL plasma were prepared and the subsequent measurements of caffeine and theophylline were taken according to the method previously described in Stadheim et al. (2013). Plasma adrenaline and noradrenaline were measured with a Cat Combi Elisa kit (DRG Instruments GmbH, Marburg, Germany) according to the manufacturer’s instructions.

*Treatments in the study* included PLA (vehicle only) and CAF (4.5 mg·kg⁻¹). CAF (Coffeinum, Oslo Apotekerproduksjon, Oslo, Norway) was dissolved in a cordial concentrate, Fun Light (3 mg/ml), and was prepared by the test leader.

*Thorax Trainer Elite:* The cross-country double poling ergometer used in the study was a Thoraxtrainer Elite (Thoraxtrainer, Holbæk, Denmark). Temperature in the test laboratory was between 16–21°C on all test days. Ski poles used during all testing were Swix CT1 (Swix, Lillehammer, Norway) and length standardized to 85 ± 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·h⁻¹ and poling frequency in real time. Resistance in the Thoraxtrainer is generated by air pressure, and the mean barometric air pressure for PLA and CAF trials averaged 958 ± 4 (sea level), 800 ± 7 mmHg (altitude), 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 double poling technique and the Thoraxtrainer Elite, see Stadheim et al. (2013, 41).

Instructions to test subjects: All subjects were instructed to perform only light training (and no strength training) the last 48 h before each C-PT. To minimize variation in pre-exercise glycogen stores, diet and exercise diaries were used to standardize food intake and training for each subject. The subjects prepared for the C-PTs as they would for a competition and tried to follow the same training and diet regimen before all tests. Before all tests; there was a 7-day washout period between each test. Subjects also refrained from CAF consumption during the last 48 h before each test. Only three subjects in the study had a high intake of CAF products on a daily basis (< 150 mg). For each main test subjects arrived at the laboratory at the same time (± 15 min) and day of the week during all C-PTs.

Questionnaires: Pain in arms and legs was evaluated on a 1–10 point scale as described by Ritchie & Hopkins (1991). Other questionnaires were used to evaluate motivation, current fitness and sleep quality using a scale from 1–100 (37).

Statistical Analysis: All data are presented as means ± standard deviation (SD), and differences in performance during the 8 km and 90% C-PTs were evaluated by a paired t-test. A two-way ANOVA for repeated measures was used to elicit differences in \( \dot{V}O_2 \), HR, LA, HCO₃⁻, glucose, \( V_E \), muscular pain, and RPE during submaximal workloads between the two treatments. If a significant f-ratio was found, a paired t-test was used to test differences between treatments on workloads. All data were tested for normal distribution using the Shapiro-Wilk test. Statistical analyses were performed using SPSS, and the level of
significance was set at $p<0.05$. Performance data were log-transformed to reduce the non-uniformity of error and then back-transformed to obtain the percentage difference in the means between the treatment conditions. Precision of estimation was indicated with a 90% confidence interval (26).

**Results**

*Comparison of sea level DP results to acute altitude:* $\dot{V}O_{2\text{max-pol}}$ was 13.4% lower compared to running. When athletes were exposed to acute altitude, $\dot{V}O_{2\text{max-pol-alt}}$ was further reduced to 14.5% compared to results at sea level, and was associated with a reduction in $HR_{\text{peak}}$ of 2.2%. This was reflected with subjects using 1:39 min longer to complete the 8 km C-PT at altitude compared to sea level ($31.6 \pm 1.2$ min), corresponding to an increase in time to complete the test of 5.2% (Fig 2). The maintenance of higher velocity during the 8 km C-PT at sea level was associated with higher $\dot{V}O_2$ and HR (Table 1, Fig 2). On average $\dot{V}O_2$ was 12.5% and 10.5% higher, respectively, while HR was 2.2 and 2.5% higher, respectively, at time points 4 and 8 km (Table 1, Fig 2). However, subjects reported similar RPE, obtained similar lactate and glucose values after the 8 km C-PT independent of PLA test conditions (Fig 3).

*Performance tests at altitude:* The average time used to complete each km during the 8 km C-PT at altitude showed a progressive reduction in velocity for both treatments from start to finish (Table 2, Fig 2), and a non-significant difference was observed between treatments ($P<0.22$). However, a $0.9 \pm 1.3\%$ (90% confidence interval) improvement was evident in the CAF trial with 9 of the 13 subjects (69%) completing the 8 km C-PT faster (Fig 2, Table 2). Indeed, time used to complete the first half of the test (0–4 km) was improved ($P<0.05$), associated with higher $\dot{V}O_2$, HR and $V_E$ at 4 km during the CAF trial compared to PLA (Table 1, Fig 1). During the second half of the 8 km C-PT (5–8 km) no difference in time usage was
evident between treatments, associated with no difference in $\dot{V}O_2$, but with higher $V_E$ and HR in the CAF trial (Table 1 and 2, Fig 1).

During the 90% C-PT subjects improved time to task failure at the fixed workload following CAF consumption compared to PLA (P<0.02). On average, subjects maintained the workload for 1:12 min longer, resulting in a 20.5 ± 13.8% (± 90% CL) improvement, and 9 out of 13 subjects (69%) improved time to task failure after CAF consumption. During the 90% C-PT no difference in $\dot{V}O_2$, $V_E$ or HR was observed after 3–4 min between treatments (Table 1, Fig 2). However, subjects reached higher $HR_{peak}$ and $V_E$ during CAF testing compared to PLA (Fig 2).

**Blood values:** Blood concentrations of CAF (plasma), lactate (capillary), adrenaline (plasma) and glucose (capillary) were all higher post both CAF C-PTs compared to PLA (Table 1). In contrast blood bicarbonate ($HCO_3^-$) was reduced in both C-PTs, while a significant reduction in pH was only observed after the 8 km C-PT (Table 1).

**Submaximal incremental test:** $\dot{V}O_2$ and HR linearly increased from the first to the last of the four workloads during all standardized warm ups, independent of test conditions (Fig 3). However, higher $\dot{V}O_2$ was observed at sea level during submaximal intensities due to higher velocity at the workload since acute altitude lead to a reduction in $\dot{V}O_{peak-pol-alt}$, meaning the relative workloads and velocity had to be reduced. Despite this, similar HR and $V_E$ was required with lower $\dot{V}O_2$ at acute hypoxia at the same submaximal percentage of DP $\dot{V}O_{peak}$. Furthermore, no difference was observed independent of test conditions for RPE, blood glucose or lactate at submaximal exercise.

When exposed to acute hypoxia, no difference was observed between treatments for $\dot{V}O_2$, $V_E$ or HR during submaximal exercise, but CAF ingestion resulted in elevated levels of blood lactate (Fig 3). Furthermore, CAF ingestion resulted in a significant decrease in $HCO_3^-$. 
values after finishing the last workload of the submaximal incremental test, respectively 25.0 ± 1.0 (PLA) vs 24.1 ± 1.4 (CAF) 8 km C-PT, and 24.5 ± 1.3 (PLA) vs 23.4 ± 1.4 (CAF) 90% C-PT. This observation, however, was not reflected in changes in blood pH between treatments either prior to or after finishing the standardized warm up, respectively 7.41 ± 0.02 (PLA) vs 7.41 ± 0.01 (CAF) 8 km C-PT, and 7.42 ± 0.02 (PLA) vs 7.42 ± 0.02 (CAF) 90% C-PT. No difference in blood glucose was observed between treatments during the incremental testing. RPE was, however, reduced on the last three workloads of the incremental test when subjects consumed CAF dosages compared to that of PLA (Fig 3). CAF ingestion also reduced perceived muscular pain for the arms during all four workloads prior to the 8 km C-PT, while this was only observed for the last two workloads prior to the 90% C-PT. No difference in self-reported muscular pain was observed for the legs between treatments on any tests.

Other results: No differences were observed between groups regarding responses to questionnaires, including “current fitness”, motivation, amount of sleep (hours) or eating pattern before the different treatments. Questionnaires revealed that subjects prior to the 8 km C-PT and 90% C-PT reported: 81 ± 11, 80 ± 12 (PLA), and 81 ± 9, 82 ± 12 (CAF) on current fitness (80 = very high). Ratings of motivation were 74 ± 8, 69 ± 10 (PLA), and 71 ± 15, 73 ± 11 (CAF) (75 = high/very high), (37). The questionnaires revealed that subjects were unable to sense which product they received during the different trials and that subjects had followed instructions given regarding training, food, liquid and CAF consumption the last 48 h prior to each C-PT.

Discussion

The novel finding of the present study is that CAF ingestion improved time to exhaustion by 20.5% during the 90% C-PT for 13 sub-elite subjects. Subjects reduced time...
during the first 4 km of the 8 km C-PT, but the 0.9% reduction in time usage for the whole 8 km C-PT was not significant (p< 0.22). During all CAF C-PTs, higher HR, V\textsubscript{E}, blood lactate, glucose, and adrenaline and lower blood HCO\textsubscript{3}⁻ and pH (8 km C-PT) were observed compared to PLA. Furthermore, subjects reported lower RPE and muscular pain in arms during CAF at submaximal intensities.

To the authors’ knowledge we are the first to investigate DP performance during acute exposure to moderate hypoxia (2,000 m). Results show that during sea level testing, subjects reached 13.4% lower DP \( \dot{V}O_2 \text{max} \) compared to running. These results are comparable to previous studies that have observed that even elite cross-country skiers (XCS) obtain ~10% lower \( \dot{V}O_2 \text{max} \) while DP (11; 41; 42). Therefore, although partial pressure of O\textsubscript{2} was reduced during acute hypoxia exercise, cardiac output (Q) might not limit DP \( \dot{V}O_2 \text{max} \) or endurance performance. Nevertheless, a reduction in altitude DP \( \dot{V}O_2 \text{max} \) (14.5%), performance (5.4%), and HR\textsubscript{peak} (2.2%) similar to previous studies while cycling or running was observed (4; 12; 23; 44). During the 8 km C-PT, reduction in performance was associated with 12.5% and 10.5% lower \( \dot{V}O_2 \), and 2.4% and 1.7% lower HR\textsubscript{peak} and mean at 4 and 8 km respectively. These results indicate DP endurance capacity and performance in acute hypoxia are limited by both supply and extraction, associated with lower HR and \( \dot{V}O_2 \) (21; 32; 44).

The major finding in the study was that CAF improved performance during the 90% C-PT, comparable to Fulco et al. (1994) who found that CAF improved time to exhaustion during acute exposure to hypoxia while cycling at 4,300 m (22). Berglund & Hemminggson have previously reported that CAF improved XCS performance during time trial testing at an altitude of 2,900 m (5). In the present study a non-significant effect of CAF ingestion was observed for the 8 km DP time trial. However, subjects completed the first 4 km faster, and reduced overall time usage with 0.9% with a possible effect with magnitude based statistics.
Indeed, it is well-documented that CAF improves sea level exercise performance, and we have previously found that CAF improves DP performance during the 8-km C-PT (14; 28; 29; 41; 42). The improvements following CAF ingestion are linked to the inhibiting of A1 and A2 adenosine receptors, reducing RPE and pain sensations due to their involvement and effects on nociception (15; 18; 24; 33; 41; 42).

In the present study plasma CAF concentration of ~30 µg/mL would partially inhibit A1 and A2 adenosine receptor activation (18). Results during CAF submaximal exercise show a reduction in both RPE and muscular pain in arms despite increased blood lactate and reduced blood bicarbonate (HCO$_3^-$). However, subjects reported maximal effort during both CAF and PLA C-PTs. Indeed, CAF’s ability to lower sensation of pain and RPE may therefore be beneficial for higher performance during the 90% C-PT since the test requires no pacing strategy, as intensity is predetermined. However, higher velocity during the first 4 km of the 8 km C-PT due to lower pain and RPE could result in higher blood lactate, lower HCO$_3^-$ and pH leading to intracellular perturbations. Early perturbations during the 8-km C-PT could impair overall performance and may explain why the increased velocity was not sustained. The fact that CAF improved the 90% C-PT, but not significantly the 8-km C-PT may indicate that pacing strategy can become inefficient when CAF is ingested at altitude.

Increased exercise duration during the CAF 90 % C-PT would require a higher energy production if work efficiency was not improved. Results from the present study show HR, $V_E$ and $\dot{V}O_2$ increased similarly during the first part of the 90% C-PT, but higher HR was observed at exhaustion in the CAF trial. During the standardized warm up, HR, $V_E$ and $\dot{V}O_2$ also increased in a similar measure for both treatments. These results indicate that CAF does not influence cardiac output or work efficiency during sub-maximal or maximal exercise. Rather, the fact that subjects increased lactate and reduced HCO$_3^-$ post CAF C-PTs indicates a larger anaerobic energy contribution.
Researchers have observed that the acute effects of hypoxia have a minor negative effect on anaerobic capacity (20). An effective way of improving performance following CAF consumption would therefore be to improve the anaerobic energy system by reducing HCO₃⁻ and pH, and by increasing lactate production (6; 31; 34). Numerous studies have demonstrated that metabolic acidosis is an important contributing factor to fatigue during prolonged high-intensity exercise (34). During exercise, hydrogen ions produced are transported to the bloodstream and buffered by blood HCO₃⁻ in an attempt to maintain normal pH in exercising muscles to preserve high-intensity performance (6; 34). The increased reduction in HCO₃⁻ during CAF C-PTs would indicate a larger amount of H⁺ efflux from muscles was buffered by blood HCO₃⁻ possibly preserving favorable intracellular conditions in muscle for high performance. However, improved anaerobic capacity only partly explain improvement during the 90% C-PT. It was therefore interestingly to observe that in contrast to previous acute altitude studies, subjects reached similar HR as in sea level 8 km C-PT testing during the CAF trials.

In the present study lower HRPeak and VO₂ were achieved when comparing sea level and hypoxia 8-km C-PT results. The reduction in HR during acute exposure to hypoxia is believed to be related to enhanced parasympathetic neural activity due to decreased signals from skeletal muscles (2; 9). Fascinatingly, HR was higher and similar to sea level values during the CAF 8 km time trail at altitude, and associated with increased VO₂ at 4 km. Increased HR is actually one of the most common observations related to improved sea level performance after CAF ingestion, and has also been accompanied by higher VO₂ (1; 27; 35; 41). The Fick equation states that a higher HR and similar stroke volume increases VO₂, if the A-VO₂ difference is maintained (7; 38). Yang et al. (2009) observed that CAF ingestion had no effect on activity, VO₂ and HR in mice lacking A₁ and A₂ receptors compared to normal wild mice (45). A₁ receptors are expressed in the human heart where they inhibit adenylyl cyclase (19;
43), and an inhibition following CAF ingestion could increase sympathetic neural activity leading to higher HR and/or maintained contractility qualities of the heart (Q) (18; 42; 45).

In the present study neural activity and Q were not measured. Therefore the effects of the increase in HR on O₂ delivery following CAF ingestion during DP exercise should be interpreted with caution. However, Gonzalez et al. (1998) demonstrated that an increase in HR by atrial pacing increased Q, \( \dot{V}O_2 \text{max} \) and performance in hypoxia (25). During CAF testing, higher HR and \( V_E \) were observed during C-PTs associated with higher \( \dot{V}O_2 \) and velocity in the first 4 km of the CAF 8 km C-PT. The increase in plasma adrenaline and \( V_E \) might also counteract an increase in vagal nervous drive to the heart due to input from pulmonary stretch receptors (23; 39). It is therefore tempting to suggest that the increase in HR increased O₂ delivery and ATP production in working muscles, thus improving performance quality during CAF C-PTs.

The results from the present study are also of interest for sports performance since CAF has been widely used by elite endurance athletes in competitions since its removal from the World Anti Doping Agency list in 2004 (13). The topic is also important because competitions including the Olympics and world championships are sometimes held at moderate altitude (1500-2000m) in sports such as cycling, running and cross-country skiing (XCS). Furthermore, it has been reported that the within-athlete variability in performance times in elite XCS races is approximately 1.1–1.4%, and the smallest worthwhile enhancement is 0.3–0.4% (40). The observed improvement of 0.9 ± 1.3% during the 8 km C-PT, although not significant (p< 0.22), might therefore still have an effect on results in real life competitions.

**Conclusion**

The present study demonstrates for the first time in sport specific exercise and standardized laboratory conditions that CAF might assist in maintaining performance quality at moderate
altitude. Results show that CAF ingestion improved DP time during the 90% C-PT with
20.5%. CAF ingestion also reduced time usage the first 4 km, and though not significant, a
0.9% reduction in time usage was observed for the whole 8 km C-PT. The mechanisms
underpinning improvements seem to be related to reduced pain and RPE, increased HR_{mean},
peak, adrenaline, lactate accumulation and reduced HCO_3-. Furthermore, the study shows that
performance, $\dot{V}O_2$ and HR responses while DP during acute hypoxia are comparable those
reported in studies using exercises where the leg muscles are most active such as when
cycling or running.

**Acknowledgements**

The authors express their appreciation to the subjects for their time and effort. They also wish
to thank Trine Stensrud and Svein Leirstein for their help during the experiments, and Astrid
Bolling for analyses of catecholamines.

**Disclosures**

No conflicts of interest, financial or otherwise, are declared by the author. We disclose all
funding received for this work.


Figure legends

Figure 1: Experimental design for all performance tests (C-PTs). Prior to the C-PTs, subjects performed a standardized warm-up (incremental tests) consisting of four intensities of 5 min duration. A similar protocol to the C-PTs was completed at pretests, except that PLA/CAF was not administrated, and no blood samples were drawn.

Figure 2: A: Time, speed, heart rate, $V_E$ and $VO_2$ displayed as means during the 8 km C-PT at sea level, and 2000 m altitude during PLA and CAF testing. B: Time, speed, heart rate, $V_E$ and $VO_2$ displayed as means during the 90% C-PT at 2000 m altitude during PLA and CAF trials. Values are listed as means ± SD. * Significant difference between PLA and CAF altitude (p<0.05). # Significant difference between PLA sea level and CAF/PLA altitude (p<0.05). Note: $VO_2$ - measurements are missing five subjects.

Figure 3: Physiological and psychological responses during the standardized warm-up during acute hypoxic condition during PLA, CAF and sea level submaximal exercise pre all C-PTs. Data are given as mean ± SD. Data on $\dot{VO}_2$ and $V_E$ are missing from one subject. * Significant different from PLA hypoxic testing (p<0.05). # Significant different from PLA and CAF acute hypoxic testing (p<0.05).
**Table 1:** Physiological and psychological measurements post the 8 km C-PT and task until failure at 90% of $\dot{VO}_{2\text{max-pol-alt}}$ in altitude.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Sea Level Post 8 km C-PT</th>
<th>Altitude 2000 m above sea level (800 mBar) Post 8 km C-PT</th>
<th>Altitude 2000 m above sea level (800 mBar) Post 90% of $\dot{VO}_{2\text{max-pol-alt}}$ C-PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Caffeine (µg/mL) (N=9)</td>
<td>No Data</td>
<td>0.7 ± 0.8</td>
<td>28.1 ± 7.6 *</td>
</tr>
<tr>
<td>Adrenaline (n=13) (nM)</td>
<td>1.1 ± 0.7</td>
<td>1.6 ± 0.9</td>
<td>2.3 ± 1.6 *</td>
</tr>
<tr>
<td>$L_a$ (mM)</td>
<td>6.3 ± 1.7</td>
<td>6.9 ± 1.5</td>
<td>8.2 ± 1.6 *</td>
</tr>
<tr>
<td>HR$_{\text{peak}}$ (beats • min$^{-1}$)</td>
<td>188 ± 10 *</td>
<td>184 ± 7</td>
<td>187 ± 7 *</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>No Data</td>
<td>7.1 ± 1.8</td>
<td>8.2 ± 2.2 *</td>
</tr>
<tr>
<td>$HCO_3^-$ (mM)</td>
<td>No Data</td>
<td>16.5 ± 2.3</td>
<td>13.7 ± 2.0 *</td>
</tr>
<tr>
<td>pH</td>
<td>No Data</td>
<td>7.33 ± 0.05</td>
<td>7.29 ± 0.05 *</td>
</tr>
<tr>
<td>Muscular Pain Arms (1-10)</td>
<td>No Data</td>
<td>7.6 ± 1.4</td>
<td>7.2 ± 1.4</td>
</tr>
<tr>
<td>Muscular Pain Legs (1-10)</td>
<td>No Data</td>
<td>3.9 ± 2.5</td>
<td>3.7 ± 2.8</td>
</tr>
<tr>
<td>RPE (6-20)</td>
<td>19.5 ± 0.5</td>
<td>19.7 ± 0.6</td>
<td>19.4 ± 0.9</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. Abbreviations: Blood lactate ($L_a$), Heart rate peak (HR$_{\text{peak}}$), Bicarbonate ($HCO_3^-$). (1) = Missing values from one subject. (2) = Missing values from two subjects. * Significant different from placebo between treatments (p<0.05).
**Table 2:** Time used for each km during the 8 km C-PT during PLA and CAF trials.

<table>
<thead>
<tr>
<th></th>
<th>Placebo Time (min) (mean ± SD)</th>
<th>Caffeine Time (min) (mean ± SD)</th>
<th>% difference (±90% CL)</th>
<th>Effect size (±90% CL)</th>
<th>Qualitative inference</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 km</td>
<td>3.92 ± 0.29</td>
<td>3.86 ± 0.28</td>
<td>-1.6 ±1.7</td>
<td>0.22 ±0.23</td>
<td>Possible</td>
<td>0.11</td>
</tr>
<tr>
<td>2 km</td>
<td>4.06 ± 0.33</td>
<td>3.96 ± 0.33*</td>
<td>-2.4 ±1.5</td>
<td>0.28 ±0.18</td>
<td>Likely</td>
<td>0.02*</td>
</tr>
<tr>
<td>3 km</td>
<td>4.11 ± 0.34</td>
<td>4.05 ± 0.34</td>
<td>-1.7 ±1.7</td>
<td>0.20 ±0.21</td>
<td>Likely</td>
<td>0.12</td>
</tr>
<tr>
<td>4 km</td>
<td>4.18 ± 0.41</td>
<td>4.16 ± 0.33</td>
<td>-0.4 ±1.9</td>
<td>0.04 ±0.15</td>
<td>Unclear</td>
<td>0.62*</td>
</tr>
<tr>
<td><strong>AVG 0-4 km</strong></td>
<td><strong>16.27 ± 1.31</strong></td>
<td><strong>16.02 ± 1.24</strong></td>
<td>-1.5 ±1.3</td>
<td>0.18 ±0.15</td>
<td>Likely</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>5 km</td>
<td>4.23 ± 0.44</td>
<td>4.17 ± 0.37</td>
<td>-1.3 ±2.2</td>
<td>0.12 ±0.20</td>
<td>Possible</td>
<td>0.27</td>
</tr>
<tr>
<td>6 km</td>
<td>4.27 ± 0.44</td>
<td>4.25 ± 0.42</td>
<td>-0.4 ±2.1</td>
<td>0.04 ±0.20</td>
<td>Unclear</td>
<td>0.69</td>
</tr>
<tr>
<td>7 km</td>
<td>4.27 ± 0.43</td>
<td>4.28 ± 0.44</td>
<td>0.4 ±2.2</td>
<td>0.04 ±0.22</td>
<td>Unclear</td>
<td>0.76</td>
</tr>
<tr>
<td>8 km</td>
<td>4.21 ± 0.42</td>
<td>4.2 ± 0.43</td>
<td>-0.4 ±1.8</td>
<td>0.04 ±0.17</td>
<td>Unclear</td>
<td>0.73*</td>
</tr>
<tr>
<td><strong>AVG 5-8 km</strong></td>
<td><strong>17.0 ± 1.7</strong></td>
<td><strong>16.9 ± 1.7</strong></td>
<td>-0.4 ±1.7</td>
<td>0.04 ±0.16</td>
<td>Unclear</td>
<td>0.76</td>
</tr>
<tr>
<td>8 km CC-PT</td>
<td>33.25 ± 2.95</td>
<td>32.94 ± 2.86</td>
<td>-0.9 ±1.3</td>
<td>0.10 ±0.14</td>
<td>Possible</td>
<td>0.22*</td>
</tr>
<tr>
<td>90% of (\dot{V}O_2\text{max-pol-alt})</td>
<td>6.17 ± 1.67</td>
<td>7.36 ± 1.55*</td>
<td>20.5 ±13.8</td>
<td>0.71 ±0.44</td>
<td>Very Likely</td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. Paired t-test was used to compare time on each kilometer (PLA vs. CAF). * Significant difference between treatments (p<0.05)