

HIGHLIGHTED TOPIC | Hypoxia 2015

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

H. K. Stadheim,¹ E. M. Nossum,¹ R. Olsen,² M. Spencer,¹ and J. Jensen¹

¹Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway; and ²Department of Chemical and Biological Working Environment, National Institute of Occupational Health, Oslo, Norway

Submitted 16 June 2015; accepted in final form 6 October 2015

Stadheim HK, Nossum EM, Olsen R, Spencer M, Jensen J. Caffeine improves performance in double poling during acute exposure to 2,000-m altitude. *J Appl Physiol* 119: 1501–1509, 2015. First published October 29, 2015; doi:10.1152/jappphysiol.00509.2015.— 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) on double poling (DP) performance during acute hypoxia. Thirteen male subelite cross-country skiers ($\dot{V}O_{2\max}$ 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 $\dot{V}O_{2\max}$. Testing was carried out in a hypobaric chamber, at 800 mbar (P_{iO_2} : ~125 mmHg) corresponding to ~2,000 m above sea level in a randomized double-blinded, placebo-controlled, cross-over design. CAF improved time to task failure from 6.10 ± 1.40 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. 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 heart rate during CAF C-PTs.

exercise performance; hypoxia; heart rate; rate of perceived exertion; and oxygen consumption

DURING THE 1968 OLYMPIC GAMES in Mexico City at an altitude of 2,240 m sprinters and jumpers set several world records while long distance runners ran markedly slower compared with 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 O₂ (Sp_{O₂}) during rest or high-intensity exercise (3). However, when exposed to hypoxia, the reduction in performance and $\dot{V}O_{2\max}$ 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 Sp_{O₂} ≤ 92% (44), and despite ventilation (\dot{V}_E) increases to prevent EIH during exercise, a greater reduction of Sp_{O₂} in arterial blood is evident during acute hypoxic exercise compared with sea-level conditions (2, 4, 16, 17, 44). The reduction in Sp_{O₂} 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 $\dot{V}O_{2\max}$ and exercise performance (2, 4, 25, 30, 39, 44). The reason for the reduction in HR_{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) 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 4,300 m under standardized laboratory 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 $\dot{V}O_2$ (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 $\dot{V}O_2$ 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) ingestion on DP performance during acute exposure (2 h) to hypoxia corresponding to 2,000 m (800 mbar) in a hypobaric chamber. To investigate the effect of CAF on HR, $\dot{V}O_2$, 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 $\dot{V}O_{2\text{PEAK-DOL-ALT}}$; 90% C-PT) was used.

Address for reprint requests and other correspondence: H. K. Stadheim, Dept. of Physical Performance, Norwegian School of Sport Sciences, P.O. Box 4014 Ullevål Stadion, 0806 Oslo, Norway (e-mail: stadheim@hotmail.no).

MATERIALS AND METHODS

Subjects. Thirteen healthy male subelite cross-country skiers gave their written consent to participate after being informed of the purposes of the study and risks involved. Their physical characteristics (means \pm SD) were age 21.9 ± 2.7 , height 180.0 ± 3.7 , body mass 77.4 ± 5.6 , $\dot{V}O_{2\max}$ running at sea level ($\dot{V}O_{2\max\text{-run}}$) 72.6 ± 5.7 ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), $\dot{V}O_{2\max}$ DP at sea level ($\dot{V}O_{2\max\text{-pol}}$) 62.9 ± 6.8 and $\dot{V}O_{2\max}$ DP at altitude ($\dot{V}O_{2\max\text{-pol-alt}}$) 53.8 ± 5.3 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Inclusion criteria were male, $\dot{V}O_{2\max\text{-run}}$ above 65 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, 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, cross-over design and was evaluated and approved by the Regional Ethics Committee of Southern Norway. The tests and familiarization during the first 4 wk of the study were performed at sea-level conditions at the Norwegian School of Sports Sciences (120-m altitude, ~ 960 mbar). Testing included $\dot{V}O_{2\max}$ running (*week 1*), familiarization DP training (*week 2*) and DP $\dot{V}O_{2\max}$ (*week 3*), a test 8-km C-PT (*week 3*), and the main 8-km C-PT (*week 4*). The remaining 5 test wk were carried out during acute (2 h) hypoxia in a hypobaric chamber (Norsk Indervannsteknikk, Haugesund, Norway) and included DP $\dot{V}O_{2\max}$ (*week 5*), a pre 8-km C-PT (*week 5*), two main 8-km C-PTs (*weeks 6 and 7*) with and without CAF, and two time to task failure tests at fixed workload $\sim 90\%$ of DP $\dot{V}O_{2\max\text{-pol-alt}}$ (*weeks 8 and 9*) with and without CAF.

Experimental procedures. At sea level subjects the first testing day (*day 1*) performed a $\dot{V}O_{2\max\text{-run}}$ test on a treadmill (Woodway, Weil am Rhein, 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), with an error of measurement of less than $\pm 1\%$ as stated by the manufacturer. Oxygen consumption and respiratory exchange ratio (RER) were measured with the Oxycon Pro metabolic system (Jaeger Hochberg). The Oxycon Pro is calibrated each month against the Douglas bag method, and the error of measurement of this ergospirometry measurement is reported to be $\pm 3\%$. The equipment for measurement of $\dot{V}O_2$ was calibrated before 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 ($\sim 20.95\%$ 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 Instruments). During testing, subjects used a mouth V2 mask (Hans Rudolph Instruments) 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 $\dot{V}O_{2\max\text{-run}}$ test was performed with a standardized warm-up consisting of four workloads lasting 5 min (8 to 11 km/h) 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 before starting the $\dot{V}O_{2\max\text{-run}}$ test, which was performed as a standardized ramp test. The starting speed for the ramp test was 10 km/h with a treadmill incline of 10.5° . Each half minute speed was increased by 0.5 km/h 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 $\dot{V}O_{2\max}$. Furthermore, all 13 subjects had to meet point one and at least two of the three other criteria to obtain $\dot{V}O_{2\max\text{-run}}$: 1) oxygen consumption leveled off (plateau), meaning $\dot{V}O_2$ increased less than 1 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, while speed was increased two times 0.5 km/h; 2) RER values were >1.10 ; 3) blood lactate was above 7.0 mmol/l posttesting; and 4) rate of perceived exertion (RPE) ≥ 19 on the Borg Scale 6–20 (8). $\dot{V}O_{2\max\text{-run}}$ was based on the average of the two highest 30-s measurements, and the duration of the test was between 5.5 and 7.5 min. The protocol and criteria for reaching $\dot{V}O_{2\max}$ differ from some other protocols used for testing of $\dot{V}O_{2\max}$ (36). Indeed, it

is debatable, therefore, whether all subjects reached $\dot{V}O_{2\max}$. However, the fact that the athletes in the study were highly trained and motivated could partially reduce the issue of whether $\dot{V}O_{2\max}$ was reached. Furthermore, the $\dot{V}O_{2\max\text{-run}}$ test was only used as an inclusion test; only subjects with $\dot{V}O_{2\max\text{-run}}$ higher than 65 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ 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 to 85% of their $HR_{\max\text{-run}}$.

Day 3 subjects performed a $\dot{V}O_{2\max\text{-pol}}$ test on the poling ergometer, with the highest HR defined as $HR_{\max\text{-pol}}$. During the $\dot{V}O_{2\max\text{-pol}}$ test subjects performed a standardized warm-up for 10 min at a velocity equal to 75% of their $HR_{\max\text{-run}}$ based on the familiarization training. Thereafter, all subjects started at a velocity of 15 km/h, and speed was increased by 0.5 km/h 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 min. Criteria for that $\dot{V}O_{2\max\text{-pol}}$ was reached were the same as described for $\dot{V}O_{2\max\text{-run}}$.

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 [coefficient of variation (%) ~ 1 – 2%]. 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' $\dot{V}O_{2\max\text{-pol}}$ 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 RPE according to the Borg scale (from 6 to 20), and muscular pain in arms and legs were 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 $\dot{V}O_{2\max\text{-pol-alt}}$ as described for sea-level $\dot{V}O_{2\max\text{-pol}}$ testing. In the hypobaric chamber, $\dot{V}O_2$ and $\dot{V}E$ were measured using the $V_{\max 29}$ (SensorMedics), which was calibrated against the Oxycon 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 before the standardized warm-up for the 8-km C-PT. However, compared with 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 $\sim 90\%$ of $\dot{V}O_{2\max\text{-pol-alt}}$ C-PT was performed in hypoxia. The velocity used was estimated based on submaximal DP $\dot{V}O_2$ values during the standardized warm-up before the 8-km C-PT based on a linear regression. Subjects received either PLA or CAF 75 min after acute exposure to hypoxia. Before the 90% C-PT, the same standardized warm-up was performed as before 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 $\sim 2,000$ 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 Fi_{CO_2} with the Vaisala GMT222 Carbon Dioxide Transmitter (Vaisala, Stockholm, Sweden) and Fi_{CO_2} with the PMA30 M&C O_2 analyzer (Marseille, France). During the first 2 h (rest) of acute altitude exposure, an ~ 1 l/min oxygen was added to maintain atmospheric gas concentrations of air. During physical activity oxygen consumption increased, thus additional oxygen was added to maintain stable Fi_{O_2} . On the basis of the pretests, ~ 3 l/min of extra oxygen was added to cover the enhanced usage of oxygen during physical activity but was adjusted (increased or reduced) according to observed Fi_{O_2} values for each individual hypoxic trail. Three gas scrubbers containing Sofnolime filters and circulating fans worked as CO_2 traps to try and ensure a stable Fi_{CO_2} concentration. However, during the later stages of the C-PTs (~ 5 – 10 min) CO_2 production from the subjects exceeded the capacity of CO_2 removal of the three scrubbers. This resulted in an enhanced Fi_{CO_2} concentration of the air inside the chamber with postvalues of CO_2 between 0.05 and $\sim 0.08\%$. Even though CO_2 concentration increased, it never exceeded 0.08%; these CO_2 values are not considered dangerous for subjects and are unlikely to influence test results. During rest and at sea-level testing, Fi_{CO_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, Franklin Lakes, NJ). A 7-ml blood sample was drawn for all blood samples and placed in tubes containing EGTA/glutathione (20 μl 0.2 M glutathione and 0.2 M EGTA/ml blood) for analysis of adrenaline, noradrenaline, and CAF. 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). Thereafter, plasma was divided in three Eppendorf tubes (Microtube Superspin; VWR International, West Chester, PA) 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 draw blood into the analyzer from the 50- μl capillary tube. The analyzer was calibrated with a 5.0 mmol/l lactate stock solution before each test and between the submaximal workloads and main tests. Values between 4.95 and 5.05 mmol/l were accepted. Under normal circumstances the error of measurements are $\pm 2\%$ for blood lactate values between 0 and 5 mmol/l and $\pm 3\%$ for values between 5 and 15 mmol/l. 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 CAF 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. (41). Plasma epinephrine and norepinephrine were measured with a Cat Combi Elisa kit (DRG Instruments, Marburg, Germany) according to the manufacturer's instructions.

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

Thoraxtrainer Elite. The cross-country DP ergometer used in the study was a Thoraxtrainer Elite (Thoraxtrainer, Holbæk, Denmark). Temperature in the test laboratory was between 16 and 21°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/h, 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) and 800 ± 7 mmHg (altitude), respectively ($P > 0.05$). The Thoraxtrainer Elite was set at level 1 (easiest) of 10 different levels during all testing to optimize technique. For more information about the DP technique and the Thoraxtrainer Elite [see Stadheim et al. (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 preexercise 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 and Hopkins (37). 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 \pm 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, lactate, HCO_3^- , glucose, \dot{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 nonuniformity 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. $\dot{V}_{\text{O}_{2\text{max-pol}}}$ was 13.4% lower compared with running. When athletes were exposed to acute altitude, $\dot{V}_{\text{O}_{2\text{max-pol-alt}}}$ was further reduced to 14.5% compared with results at sea level and was associated with a reduction in HR_{peak} of 2.2%. This was reflected with subjects using 1.39 min longer to complete the 8-km C-PT at altitude compared with sea level (31.6 ± 1.2 min), corresponding to an increase in time to complete the test of 5.2% (see 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; see 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; see Fig. 2). However, subjects reported similar RPE, obtained similar lactate and glucose values after the 8-km C-PT independent of PLA test conditions (see Fig. 3).

Performance tests at altitude. The average time used to complete each kilometer during the 8-km C-PT at altitude showed a progressive reduction in velocity for both treatments from start to finish (Table 2; see Fig. 2), and a nonsignificant 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 (Table 2; see Fig. 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 \dot{V}_{E}

Table 1. Physiological and psychological measurements post the 8-km C-PT and task until failure at 90% of $\dot{V}O_{2max-pol-alt}$ in altitude

Measure	Sea Level Post 8-km C-PT	Altitude 2,000 m Above Sea Level (800 mBar) Post-8-km C-PT				Altitude 2,000 m Above Sea Level (800 mBar) Post 90% of alt $\dot{V}O_{2max-pol-alt}$ C-PT			
	Placebo	Placebo	Caffeine	Qualitative inference	P values	Placebo	Caffeine	Qualitative inference	P values
Plasma caffeine, $\mu\text{g/ml}$ ($n = 9$)	No data	0.7 ± 0.8	$28.1 \pm 7.6^*$	Very likely	0.01	1.5 ± 2.7	$30.3 \pm 2.5^*$	Very likely	0.01
Epinephrine, nM ($n = 13$)	1.1 ± 0.7	1.6 ± 0.9	$2.3 \pm 1.6^*$	Very likely	0.16	1.6 ± 0.8	$2.5 \pm 1.5^*$	Very likely	0.16
$\text{La}^-_{\text{Post}}$, mM	6.3 ± 1.7	6.9 ± 1.5	$8.2 \pm 1.6^*$	Very likely	0.03	8.3 ± 2.1	$9.8 \pm 2.1^*$	Very likely	0.01
HR_{peak} , $\text{beats}/\text{min}^{-1}$	$188 \pm 10^*$	184 ± 7	$187 \pm 7^*$	Likely	0.03	$181 \pm 9^{\ddagger}$	$185 \pm 7^{\ddagger}$	Very likely	0.05
Glucose, mM	No data	7.1 ± 1.8	$8.2 \pm 2.2^*$	Most likely	0.01	6.1 ± 0.7	$7.0 \pm 0.9^*$	Most likely	0.01
HCO_3^- , mM	No data	16.5 ± 2.3	$13.7 \pm 2.0^*$	Most likely	0.01	14.5 ± 1.9	$12.9 \pm 1.7^*$	Most likely	0.01
pH	No data	7.33 ± 0.05	$7.29 \pm 0.05^*$	Most likely	0.01	7.28 ± 0.04	7.27 ± 0.04	Unclear	0.50
Muscular pain arms (1–10)	No Data	7.6 ± 1.4	7.2 ± 1.4	Likely	0.38	9.1 ± 1.4	8.7 ± 1.8	Likely	0.11
Muscular pain legs (1–10)	No Data	3.9 ± 2.5	3.7 ± 2.8	Unclear	0.69	5.5 ± 2.6	5.5 ± 2.8	Unclear	0.74
RPE (6–20)	19.5 ± 0.5	19.7 ± 0.6	19.4 ± 0.9	Likely	0.08	18.8 ± 0.7	18.8 ± 0.5	Unclear	0.99

Data are given as means \pm SD. La^- , blood lactate; HR_{peak} , heart rate peak; HCO_3^- , bicarbonate; RPE, rate of perceived exertion. * $P < 0.05$, significant different from placebo between treatments. \ddagger Missing values from 1 subject. \ddagger Missing values from 2 subjects.

at 4 km during the CAF trial compared with 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 $\dot{V}E$ and HR in the CAF trial (Tables 1 and 2; Fig. 1).

During the 90% C-PT subjects improved time to task failure at the fixed workload following CAF consumption compared with PLA ($P < 0.02$). On average, subjects maintained the workload for 1.12 min longer, resulting in a $20.5 \pm 13.8\%$ ($\pm 90\%$ 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 $\dot{V}O_2$, $\dot{V}E$, or HR was observed after 3–4 min between treatments (Table 1; Fig. 2). However, subjects reached higher HR_{peak} and $\dot{V}E$ during CAF testing compared with 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 with 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 $\dot{V}O_{2\text{peak-pol-alt}}$, meaning the relative workloads and velocity had to be reduced. Despite this, similar HR and $\dot{V}E$ were required with lower $\dot{V}O_2$ at acute hypoxia at the same submaximal percentage of DP $\dot{V}O_{2\text{peak}}$. 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 among treatments for $\dot{V}O_2$, $\dot{V}E$, 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

Table 2. Time used for each kilometer during the 8-km C-PT during PLA and CAF trials

	Placebo Time, min	Caffeine Time, min	%Difference, $\pm 90\%$ CL	Effect Size, $\pm 90\%$ CL	Qualitative Inference	P Values
0–4 km						
1 km	3.92 ± 0.29	3.86 ± 0.28	-1.6 ± 1.7	0.22 ± 0.23	Possible	0.11
2 km	4.06 ± 0.33	$3.96 \pm 0.33^*$	-2.4 ± 1.5	0.28 ± 0.18	Likely	0.02
3 km	4.11 ± 0.34	4.05 ± 0.34	-1.7 ± 1.7	0.20 ± 0.21	Likely	0.12
4 km	4.18 ± 0.41	4.16 ± 0.33	-0.4 ± 1.9	0.04 ± 0.15	Unclear	0.62
Average 0–4 km	16.27 ± 1.31	$16.02 \pm 1.24^*$	-1.5 ± 1.3	0.18 ± 0.15	Likely	0.05
5–8 km						
5 km	4.23 ± 0.44	4.17 ± 0.37	-1.3 ± 2.2	0.12 ± 0.20	Possible	0.27
6 km	4.27 ± 0.44	4.25 ± 0.42	-0.4 ± 2.1	0.04 ± 0.20	Unclear	0.69
7 km	4.27 ± 0.43	4.28 ± 0.44	0.4 ± 2.2	0.04 ± 0.22	Unclear	0.76
8 km	4.21 ± 0.42	4.2 ± 0.43	-0.4 ± 1.8	0.04 ± 0.17	Unclear	0.73
Average 5–8 km	17.0 ± 1.7	16.9 ± 1.7	-0.4 ± 1.7	0.04 ± 0.16	Unclear	0.76
8-km CC-PT	33.25 ± 2.95	32.94 ± 2.86	-0.9 ± 1.3	0.10 ± 0.14	Possible	0.22
90% of $\dot{V}O_{2max-pol-alt}$	6.17 ± 1.67	$7.36 \pm 1.55^*$	20.5 ± 13.8	0.71 ± 0.44	Very likely	0.02

Data are given as means \pm SD. Paired t -test was used to compare time on each kilometer (placebo vs. caffeine). CL, confidence level. *Significant difference between treatments ($P < 0.05$).

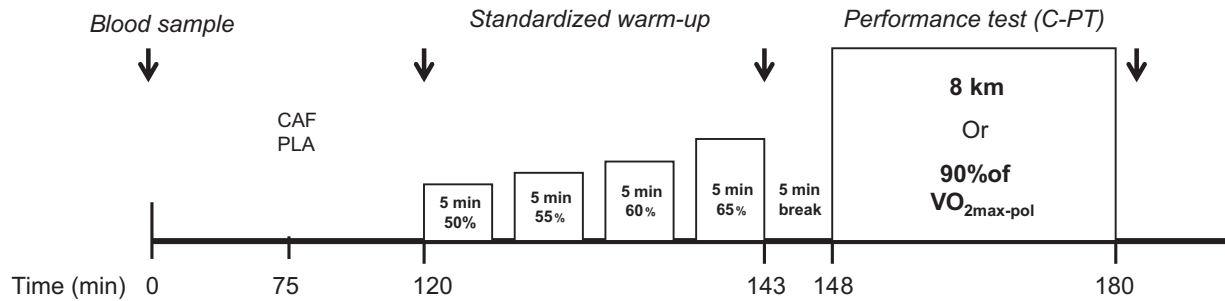


Fig. 1. Experimental design for all performance tests (C-PTs). Before the C-PTs, subjects performed a standardized warm-up (incremental tests) consisting of 4 intensities of a 5-min duration. A similar protocol to the C-PTs was completed at pretests, except that placebo (PLA)/caffeine (CAF) was not administered, and no blood samples were drawn.

between treatments either before 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 with that of PLA (Fig. 3). CAF ingestion also reduced perceived muscular pain for the arms during all four workloads before the 8-km C-PT, while this was only observed for the last two workloads before 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 before the 8-km C-PT and 90% C-PT reported: 81 ± 11 , 80 ± 12 (PLA), and 81 ± 9 , and 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 before 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 subelite 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, $\dot{V}E$, blood lactate, glucose, and epinephrine and lower blood HCO_3^- and pH (8-km C-PT) were observed compared with 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 $\dot{V}O_{2max}$ compared with running. These results are comparable to previous studies that have observed that even elite cross-country skiers obtain $\sim 10\%$ lower $\dot{V}O_{2max}$ while DP (11, 41, 42). Therefore, although the partial pressure of O_2 was reduced during acute hypoxia

exercise, cardiac output (Q) might not limit DP $\dot{V}O_{2max}$ or endurance performance. Nevertheless, a reduction in altitude DP $\dot{V}O_{2max}$ (14.5%), performance (5.4%), and HR_{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 $\dot{V}O_{2max}$ and 2.4 and 1.7% lower HR_{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. (22) who found that CAF improved time to exhaustion during acute exposure to hypoxia while cycling at 4,300 m. Berglund and Hemmingsson (5) have previously reported that CAF improved cross-country skiing performance during time trial testing at an altitude of 2,900 m. In the present study a nonsignificant 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 A_1 and A_2 adenosine receptors, reducing RPE and pain sensations due to their involvement and effects on nociception (10a, 15, 18, 24, 33, 41, 42).

In the present study plasma CAF concentration of ~ 30 $\mu\text{g/ml}$ would partially inhibit A_1 and A_2 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 and 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

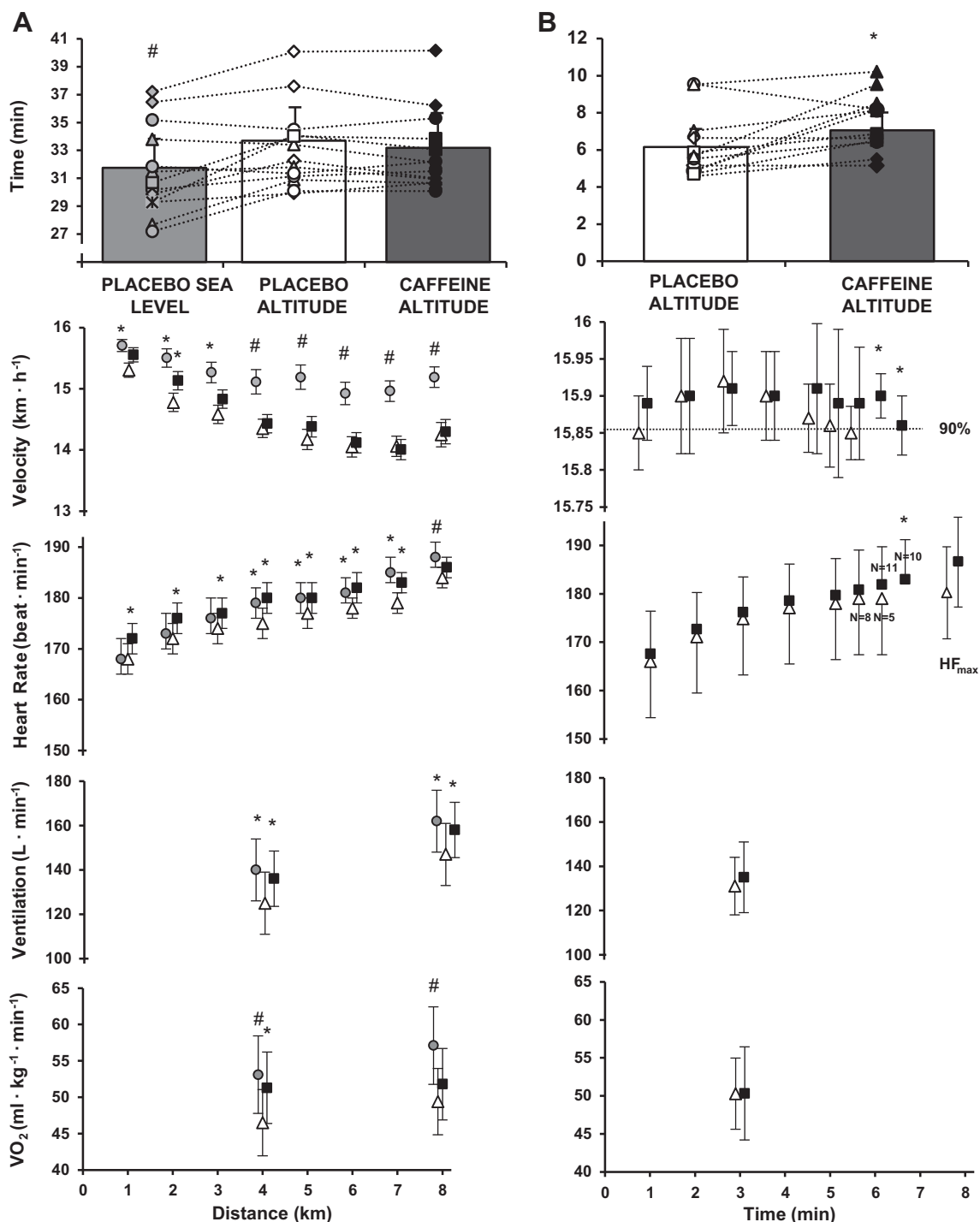


Fig. 2. *A*: time, speed, heart rate, \dot{V}_E , and $\dot{V}O_2$ displayed as means during the 8-km C-PT at sea level and 2,000-m altitude during PLA and CAF testing. *B*: time, speed, heart rate, \dot{V}_E , and $\dot{V}O_2$ displayed as means during the 90% C-PT at 2,000 m altitude during PLA and CAF trials. Values are listed as means \pm 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: $\dot{V}O_2$ measurements are missing 5 subjects.

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, \dot{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, \dot{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 submaximal or maximal exercise. Rather, the fact that subjects increased lactate and reduced HCO_3^- post-CAF C-PTs indicates a larger anaerobic energy contribution.

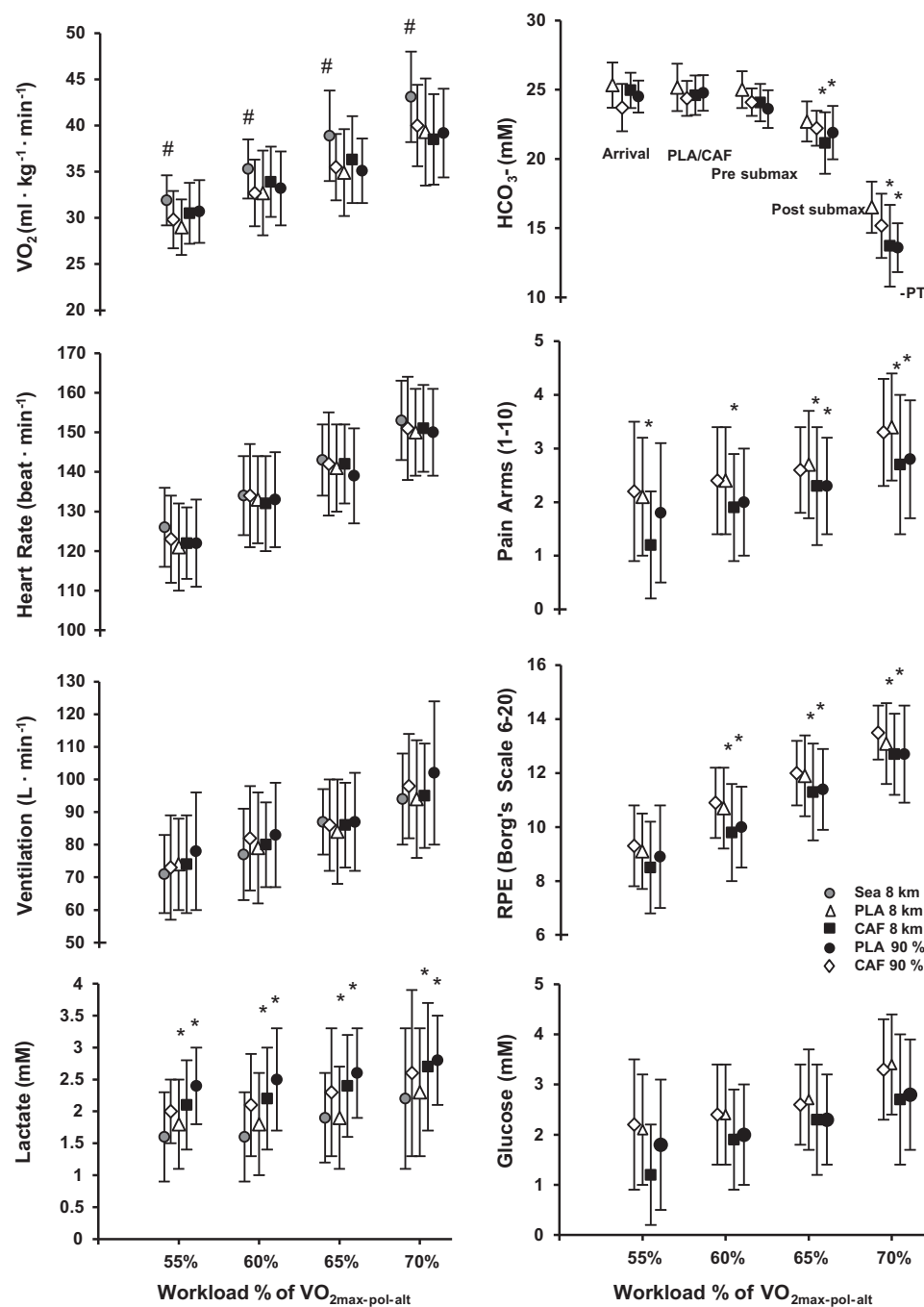


Fig. 3. Physiological and psychological responses during the standardized warm-up during acute hypoxic condition during PLA, CAF, and sea-level submaximal exercise before all C-PTs. Data are given as means \pm SD. Data on $\dot{V}O_2$ and $\dot{V}E$ are missing from 1 subject. *Significant difference from PLA hypoxic testing ($P < 0.05$). #Significant difference from PLA and CAF acute hypoxic testing ($P < 0.05$).

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 HCO_3^- 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 HCO_3^- in an attempt to maintain normal pH in exercising muscles to preserve high-intensity performance (6, 34). The increased reduction in HCO_3^- during CAF C-PTs would

indicate a larger amount of H^+ efflux from muscles was buffered by blood HCO_3^- 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 HR_{peak} and $\dot{V}O_2$ 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). Fascinat-

ingly, HR was higher and similar to sea-level values during the CAF 8-km time trail at altitude and associated with increased $\dot{V}O_2$ 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 $\dot{V}O_2$ (1, 27, 35, 41). The Fick equation states that a higher HR and similar stroke volume increases $\dot{V}O_2$, if the A- $\dot{V}O_2$ difference is maintained (7, 38). Yang et al. (45) observed that CAF ingestion had no effect on activity, $\dot{V}O_2$, and HR in mice lacking A₁ and A₂ receptors compared with normal wild mice. 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. (25) demonstrated that an increase in HR by atrial pacing increased Q, $\dot{V}O_{2\max}$ and performance in hypoxia. During CAF testing, higher HR and $\dot{V}E$ 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 $\dot{V}E$ 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 (1500–2,000 m) in sports such as cycling, running, and cross-country skiing. Furthermore, it has been reported that the within-athlete variability in performance times in elite cross-country skiing races is ~1.1–1.4%, and the smallest worthwhile enhancement is 0.3–0.4% (40). The observed improvement of $0.9 \pm 1.3\%$ 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 although 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, epinephrine, and lactate accumulation; and reduced HCO₃⁻. Furthermore, the study shows that performance and $\dot{V}O_2$ 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.

ACKNOWLEDGMENTS

We express appreciation to the subjects for time and effort. We also thank Trine Stensrud and Svein Leirstein for help during the experiments and Astrid Bolling for analyses of catecholamines.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: H.K.S., E.M.N., and J.J. conception and design of research; H.K.S., E.M.N., M.S., and J.J. performed experiments; H.K.S., R.O., M.S., and J.J. analyzed data; H.K.S., E.M.N., R.O., M.S., and J.J. interpreted results of experiments; H.K.S. prepared figures; H.K.S., E.M.N., R.O., M.S., and J.J. drafted manuscript; H.K.S., E.M.N., R.O., M.S., and J.J. edited and revised manuscript; H.K.S., E.M.N., R.O., M.S., and J.J. approved final version of manuscript.

REFERENCES

- Alves MN, Ferrari-Auarek WM, Pinto KM, Sa KR, Viveiros JP, Pereira HA, Ribeiro AM, Rodrigues LO. Effects of caffeine and tryptophan on rectal temperature, metabolism, total exercise time, rate of perceived exertion and heart rate. *Braz J Med Biol Res* 28: 705–709, 1995.
- Bartsch P, Gibbs JS. Effect of altitude on the heart and the lungs. *Circulation* 116: 2191–2202, 2007.
- Bassett DR Jr., Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 32: 70–84, 2000.
- Benoit H, Busso T, Castells J, Geysant A, Denis C. Decrease in peak heart rate with acute hypoxia in relation to sea level VO_{2max}. *Eur J Appl Physiol* 90: 514–519, 2003.
- Berglund B, Hemmingsson P. Effects of caffeine ingestion on exercise performance at low and high altitudes in cross-country skiers. *Int J Sports Med* 3: 234–236, 1982.
- Bishop D, Edge J, Davis C, Goodman C. Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. *Med Sci Sports Exerc* 36: 807–813, 2004.
- Blomqvist CG, Saltin B. Cardiovascular adaptations to physical training. *Annu Rev Physiol* 45: 169–189, 1983.
- Borg G, Hassmen P, Lagerstrom M. Perceived exertion related to heart rate and blood lactate during arm and leg exercise. *Eur J Appl Physiol Occup Physiol* 56: 679–685, 1987.
- Boushel R, Calbet JA, Radegran G, Sondergaard H, Wagner PD, Saltin B. Parasympathetic neural activity accounts for the lowering of exercise heart rate at high altitude. *Circulation* 104: 1785–1791, 2001.
- Bridge CA, Jones MA. The effect of caffeine ingestion on 8 km run performance in a field setting. *J Sports Sci* 24: 433–439, 2006.
- 10a Burnstock G, Sawynok J. *Pharmacology of Pain-Adenosine Triphosphate and Adenosine Receptors and Pain*. Seattle, WA: IASP, 2010.
- Calbet JA, Holmberg HC, Rosdahl H, van HG, Jensen-Urstad M, Saltin B. Why do arms extract less oxygen than legs during exercise? *Am J Physiol Regul Integr Comp Physiol* 289: R1448–R1458, 2005.
- Chapman RF, Emery M, Stager JM. Degree of arterial desaturation in normoxia influences VO_{2max} decline in mild hypoxia. *Med Sci Sports Exerc* 31: 658–663, 1999.
- Del CJ, Munoz G, Munoz-Guerra J. Prevalence of caffeine use in elite athletes following its removal from the World Anti-Doping Agency list of banned substances. *Appl Physiol Nutr Metab* 36: 555–561, 2011.
- Desbrow B, Biddulph C, Devlin B, Grant GD, Anoopkumar-Dukie S, Leveritt MD. The effects of different doses of caffeine on endurance cycling time trial performance. *J Sports Sci* 30: 115–120, 2012.
- Doherty M, Smith PM. 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.
- Fan JL, Burgess KR, Basnyat R, Thomas KN, Peebles KC, Lucas SJ, Lucas RA, Donnelly J, Cotter JD, Ainslie PN. Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO₂. *J Physiol* 588: 539–549, 2010.
- Fan JL, Burgess KR, Thomas KN, Peebles KC, Lucas SJ, Lucas RA, Cotter JD, Ainslie PN. Influence of indomethacin on the ventilatory and cerebrovascular responsiveness to hypoxia. *Eur J Appl Physiol* 111: 601–610, 2011.

18. Fredholm BB. Adenosine receptors as drug targets. *Exp Cell Res* 316: 1284–1288, 2010.
19. Fredholm BB, Abbracchio MP, Burnstock G, Dubyak GR, Harden TK, Jacobson KA, Schwabe U, Williams M. Towards a revised nomenclature for P1 and P2 receptors. *Trends Pharmacol Sci* 18: 79–82, 1997.
20. Friedmann B, Frese F, Menold E, Bartsch P. Effects of acute moderate hypoxia on anaerobic capacity in endurance-trained runners. *Eur J Appl Physiol* 101: 67–73, 2007.
21. Fulco CS, Rock PB, Cymerman A. Maximal and submaximal exercise performance at altitude. *Aviat Space Environ Med* 69: 793–801, 1998.
22. Fulco CS, Rock PB, Trad LA, Rose MS, Forte VA Jr, Young PM, Cymerman A. Effect of caffeine on submaximal exercise performance at altitude. *Aviat Space Environ Med* 65: 539–545, 1994.
23. Gavin TP, Derchak PA, Stager JM. Ventilation's role in the decline in VO₂max and SaO₂ in acute hypoxic exercise. *Med Sci Sports Exerc* 30: 195–199, 1998.
25. Gonzalez NC, Clancy RL, Moue Y, Richalet JP. Increasing maximal heart rate increases maximal O₂ uptake in rats acclimatized to simulated altitude. *J Appl Physiol (1985)* 84: 164–168, 1998.
26. Hopkins WG. *Analysis of a Post-Only Crossover Trial* (Online Excel spreadsheet). <http://newstats.org/xPostOnlyCrossover.xls> [2006].
27. Ivy JL, Costill DL, Fink WJ, Lower RW. Influence of caffeine and carbohydrate feedings on endurance performance. *Med Sci Sports* 11: 6–11, 1979.
28. Ivy JL, Costill DL, Fink WJ, Lower RW. Influence of caffeine and carbohydrate feedings on endurance performance. *Med Sci Sports* 11: 6–11, 1979.
29. Ivy JL, Kammer L, Ding Z, Wang B, Bernard JR, Liao YH, Hwang J. Improved cycling time-trial performance after ingestion of a caffeine energy drink. *Int J Sport Nutr Exerc Metab* 19: 61–78, 2009.
30. Lundby C, Araoz M, van Hall G. Peak heart rate decreases with increasing severity of acute hypoxia. *High Alt Med Biol* 2: 369–376, 2001.
31. Matson LG, Tran ZV. Effects of sodium bicarbonate ingestion on anaerobic performance: a meta-analytic review. *Int J Sport Nutr* 3: 2–28, 1993.
32. Mollard P, Woorons X, Letournel M, Lamberto C, Favret F, Pichon A, Beaudry M, Richalet JP. Determinant factors of the decrease in aerobic performance in moderate acute hypoxia in women endurance athletes. *Respir Physiol Neurobiol* 159: 178–186, 2007.
33. Momsen AH, Jensen MB, Norager CB, Madsen MR, Vestersgaard-Andersen T, Lindholt JS. Randomized double-blind placebo-controlled crossover study of caffeine in patients with intermittent claudication. *Br J Surg* 97: 1503–1510, 2010.
34. Morris DM, Shafer RS, Fairbrother KR, Woodall MW. Effects of lactate consumption on blood bicarbonate levels and performance during high-intensity exercise. *Int J Sport Nutr Exerc Metab* 21: 311–317, 2011.
35. Olcina GJ, Munoz D, Timon R, Caballero MJ, Maynar JI, Cordova A, Maynar M. Effect of caffeine on oxidative stress during maximum incremental exercise. *J Sports Sci Med* 5: 621–628, 2006.
36. Poole DC, Wilkerson DP, Jones AM. Validity of criteria for establishing maximal O₂ uptake during ramp exercise tests. *Eur J Appl Physiol* 102: 403–410, 2008.
37. Ritchie SE, Hopkins WG. The intensity of exercise in deep-water running. *Int J Sports Med* 12: 27–29, 1991.
38. Saltin B. Physiological adaptation to physical conditioning. Old problems revisited. *Acta Med Scand Suppl* 711: 11–24, 1986.
39. Siebenmann C, Rasmussen P, Sorensen H, Bonne TC, Zaar M, Achmann-Andersen NJ, Nordsborg NB, Secher NH, Lundby C. Hypoxia increases exercise heart rate despite combined inhibition of beta-adrenergic and muscarinic receptors. *Am J Physiol Heart Circ Physiol* 308: H1540–H1546, 2015.
40. Spencer M, Losnegard T, Hallen J, Hopkins WG. Variability and predictability of performance times of elite cross-country skiers. *Int J Sports Physiol Perform* 9: 5–11, 2014.
41. Stadheim HK, Kvamme B, Olsen R, Drevon CA, Ivy JL, Jensen J. Caffeine increases performance in cross-country double-pole time trial exercise. *Med Sci Sports Exerc* 45: 2175–2183, 2013.
42. Stadheim HK, Spencer M, Olsen R, Jensen J. Caffeine and performance over consecutive days of simulated competition. *Med Sci Sports Exerc* 46: 1787–1796, 2014.
43. Wang Y, Yang JN, Arner A, Boels PJ, Fredholm BB. Adenosine A₁ receptors and vascular reactivity. *Acta Physiol (Oxf)* 199: 211–220, 2010.
44. Wehrli JP, Hallen J. Linear decrease in VO₂max and performance with increasing altitude in endurance athletes. *Eur J Appl Physiol* 96: 404–412, 2006.
45. Yang JN, Chen JF, Fredholm BB. Physiological roles of A₁ and A_{2A} adenosine receptors in regulating heart rate, body temperature, and locomotion as revealed using knockout mice and caffeine. *Am J Physiol Heart Circ Physiol* 296: H1141–H1149, 2009.