Stadheim, H. K., Spencer, M., Olsen, R., Jensen, J. (2014). Caffeine and performance over consecutive days of simulated competition. *Medicine & Science in Sports & Exercise, 46*, 1787-1796.

Caffeine and performance over consecutive days of simulated competition

Hans Kristian Stadheim[1*], Matthew Spencer[1], Raymond Olsen[2], Jørgen Jensen[1].

[1] Department of Physical Performance, Norwegian School of Sport Sciences, P.O.Box 4014 Ullevål Stadion, 0806, Norway

[2] Department of Chemical and Biological Working Environment, National Institute of Occupational Health, P.O.Box 8149 Dep, N-0033 Oslo, Norway

Running title: *Caffeine and performance on consecutive days*

*Corresponding author: Hans Kristian Stadheim, [1]Department of Physical Performance, Norwegian School of Sport Sciences, P.O.Box 4014 Ullevål Stadion, 0806, Norway

Fax (+47) 23264220 Phone (+47) 90569720

E-mail: stadheim@hotmail.no

H.K Stadheim declares no conflicts of interests. M. Spencer declares no conflicts of interests. R. Olsen declares no conflicts of interests, and J. Jensen declares no conflicts of interests.

Disclosure of funding received for this work from any of the following organizations: National Institutes of Health (NIH); Welcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).

Abstract

Purpose: Performance improvements after caffeine (CAF) ingestion are well documented when using a one day protocol. In numerous competitions such as the Tour de France, Tour de Ski, World Championships and NCAA Championships, athletes compete several days in a row. To date no studies have investigated the effects of CAF when competing consecutive days in a row. **Aim:** Investigate the effects of placebo (PLA) and two different CAF doses (3 and 4.5 mg · kg^{-1} body mass) on performance in a 10-min all-out cross-country double poling ergometer test (C-PT) two days in a row. **Method**: 8 highly trained male cross-country skiers ($\dot{V}O_{2max\text{-}run}$ 78.5±1.6 ml·kg^{1}·min^{-1}) participated in the study which was a randomized double-blinded, placebo-controlled, cross-over-design. Performance was assessed as distance covered during a 10-min all-out C-PT. Oral ingestion of CAF or PLA was consumed 75 min before the all-out C-PT. **Results**: Poling distance was improved after CAF ingestions compared to PLA both days. The improvements on day one were 4.0% (90% confidence limits: ± 3.3) and 4.0% ± 2.9 for both CAF doses respectively ($P<0.05$), while improvements on day two were 5.0 ± 3.6 and 5.1% ± 2.8 for CAF3 and CAF4.5 compared to PLA. Improved performance was associated with increased heart rate, adrenaline, blood lactate and $\dot{V}O_2$ consumption after CAF ingestion. Furthermore, performance was elevated despite higher creatine kinase and muscular pain at arrival on day two for both CAF doses. **Conclusion:** Both CAF doses improved performance in the 10-min all-out C-PT compared with PLA over two consecutive days. Therefore, CAF seems useful for athletes competing over consecutive days, despite higher muscle damage occurring after enhanced performance the first day.

Keywords: Exercise *performance, oxygen consumption, heart rate, creatine kinase and muscular pain*

Introduction

Paragraph Number 1 The ergogenic effects of caffeine (CAF) have been researched since the early 1900's and several studies the last 40 years have observed that CAF ingestion (3-9 mg · kg^1) can have a positive effect on exercise performance when using a one day protocol. This has been observed in cycling [25], running [7], cross-country skiing (XCS) [33] and rowing [31]. CAF intake can also improve exercise performance of both short [24] and long duration [9, 23] events, regardless of whether exercise performance is measured as time to exhaustion [22] or time to complete a set amount of work [33].

Paragraph Number 2 The observed improvements after CAF ingestion normally varies between 1-5 % during time trials lasting 10-60 min [19, 25, 33]. Due to variation in performance improvements after CAF ingestion, exercise physiologists have studied the CAF and dose response relationship. Results from these studies have observed that optimized effects after CAF ingestion is highly individual, but seems to occur with doses between 3-6 mg · kg^1 [11, 19, 20]. Higher doses (9-12 mg · kg^1) do not seem to result in additional improvements, but rather lead to stronger side effects such as headaches or nausea [19].

Paragraph Number 3 The main theory explaining improved performance after CAF ingestion is inhibition of adenosine receptors [1, 19, 20], reduction in muscle pain and rate of perceived exertion (RPE) [12, 33]. Still inhibition of adenosine receptors could also affect facilitation of motor unit recruitment, heart rate or have a direct effect on the muscle [16, 17, 38]. Indeed, lower RPE has been reported at submaximal workloads after CAF ingestion [10, 33], and similar RPE has been observed when performing a higher work intensity after CAF administration. The higher work intensity during performance tests are very often associated with higher heart rate [7, 25, 33] and/or blood lactate accumulation [33]. CAF has also been observed to improve maximal voluntary contraction (39). It seems therefore that several

mechanisms contribute to performance improvements after CAF administration, and that CAF is an effective stimulant drug to improve exercise intensity and performance [7, 11, 22, 24, 31, 33].

Paragraph Number 4 There is a potential risk that the improved exercise intensity after CAF consumption could lead to larger muscular damage, possibly impairing performance the following day during competitions like the Olympics, World Championships (running, swimming, rowing), Tour de France (cycling) or Tour De Ski (XCS). Increased exercise intensity have been reported to increase muscular damage [30, 36] and muscle soreness [26] due to tissue inflammation from muscular and cell damage. So far, no studies have examined the potential of CAF consumption to improve the performance over consecutive days of competition, or if different doses could result in a difference response.

Paragraph Number 5 The aim of the present study was therefore to test the effect of placebo (PLA) and two different CAF doses (3 and 4.5 mg $\cdot kg^{-1}$) on a 10-min all-out cross-country double poling test (C-PT) when using a two day test protocol. The duration of the test is similar to some of the races in the: World Cup, Tour de Ski, World championships or Olympics in XCS competitions.

We hypothesized that ingestion of CAF would improve performance in double poling (DP) on day one as observed in previous studies. However, due to a higher exercise intensity day one, subjects would be more fatigued day two leading to impaired performance in the CAF groups compared to PLA. Furthermore we wanted to observe if the two different CAF doses potentially gave different responses the second day of testing on performance or muscular damage.

Materials and Methods

Paragraph Number 6 *Subjects:* Eight healthy male elite cross-country skiers (3 seniors, and 5 juniors), gave their written consent to participate in the study after being informed of the purposes of the study and risks involved. Their physical characteristics (mean±SE) were age 20.0 ± 1.0 (yr.), height 180.4 ± 1.7 (cm), weight 70.6 ± 2.9 (kg), $\dot{V}O_{2max}$ running ($\dot{V}O_{2max\text{-}run}$) 78.5 ± 1.6 (ml · kg^{-1} · min^{-1}) and $\dot{V}O_{2peak}$ double poling ($\dot{V}O_{2peak\text{-}pol}$) 70.5 ± 1.6 (ml · kg^{-1} · min^{-1}). Inclusion criteria were that all subjects had to be: male, have a $\dot{V}O_{2max\text{-}run}$ above 70 ml · kg^{-1} · min^{-1}, and that they would train seriously to compete in the Norwegian National Cross-country Skiing Cup the upcoming season. The study was approved by the Regional Ethics Committee.

Paragraph Number 7 *Experimental Procedures:* The study had a randomized double-blinded, placebo-controlled, cross-over-design. Before the performance tests (C-PT), the participants underwent a four week training protocol to familiarize with the double poling ergometer (Thoraxtrainer Elite) and the 10 min all-out test (Figure 1). On *day 1* participants performed a $\dot{V}O_{2max\text{-}run}$ test on a treadmill (Woodway, Weil am Rein, Germany) and the highest heart rate was defined as $HR_{max\text{-}run}$. Oxygen consumption and RER were measured with a Oxycon Pro metabolic system (Jaeger Hochberg, Germany) and air was collected using a mouth V2-mask (Hans Rudolph Instr., USA) in combination with a nose bracket. The $\dot{V}O_{2max\text{-}run}$ test was performed with a standardized warm-up consisting of four workloads lasting 5 min (8 to 11 km · h^{-1}) with a 5.3° uphill incline. A one min break was given between each workload where lactate was measured. After the last workload of the warm-up, subjects walked five min at 5 km · h^{-1}, before starting the $\dot{V}O_{2max\text{-}run}$ test. Starting speed was 10 km · h^{-1} with a treadmill incline of 10.5°. Each half minute speed was increased with 0.5 km · h until

subjects were unable to maintain the speed and stepped off the treadmill. All 8 subjects had to meet criteria one, and at least two of the three other criteria's to obtain $\dot{V}O_{2max\text{-}run}$: 1) oxygen consumption reached a plateau, meaning $\dot{V}O_2$ increased less than 1 mL · kg^{-1} · min^{-1}, while speed was increased two times 0.5 km · h, 2) RER values were above 1.10, 3) Post blood lactate measurements were above 7.0 mM and 4) RPE ≥19 on the Borg Scale 6-20 [5]. $\dot{V}O_{2max\text{-}run}$ was based on the average of the two highest measurements. Subjects with $\dot{V}O_{2max\text{-}run}$ higher than 70 ml · kg^{-1} · min^{-1} were included. *Day 2* subjects performed 40 min of familiarization DP training on the poling ergometer (Thoraxtrainer Elite) with workloads ranging from 55-85% of their $HR_{max\text{-}run}$. *Day 3* subjects performed a $\dot{V}O_{2peak\text{-}pol}$ test on the poling ergometer, with the highest heart rate defined as $HR_{max\text{-}pol}$. Criteria for that $\dot{V}O_{2peak\text{-}pol}$ was reached were the same as for $\dot{V}O_{2max\text{-}run.}$ On *days 4 and* 5 the participants completed pre C-PTs identical to the final C-PTs, but without supplement or blood sampling. Using similar test protocols, our previous study suggested that a minimum of two familiarization trials of the 10-min all-out period of the C-PTs were required to obtain acceptable reliability (CV% approximately 1-2% [33].

Paragraph Number 8 On remaining test days subjects received PLA or one of the two CAF doses two days in a row, 45 min prior to the standardized warm-up and C-PT. The warm-up was performed as an incremental test with four, 5-min workloads, equivalent to 50, 55, 60 and 65% of subjects $\dot{V}O_{2peak\text{-}pol}$ with a one min break between each workload. Heart rate, $\dot{V}O_2$ and RER were measured as means between the 3-4.5 min of each workload. Subjective ratings of perceived exertion (RPE) according to the Borg-scale (from 6 to 20) were determined for each workload [5]. Following the warm-up, a 5-min break was used for blood sampling and preparation for the C-PT. During the C-PT, the first 15 min of the test consisted of two standardized workloads equivalent to 75% (10 min) and 80% (5 min) of $\dot{V}O_{2max\text{-}run}$

$O_{2peak\ -pol}$. For the remaining 10 min all-out period of the C-PT, subjects self-selected their speed with the goal of performing the largest workload possible (Fig 1). Performance was measured as distance covered during the 10-min all-out C-PT. Encouragement was given during the whole 10 min all-out test by a blinded test leader and the subjects could see remaining time. During the C-PT, HR, $\dot{V}O_2$, RPE and speed were recorded after: 4, 10, 15 min (standardized workloads), 17.5, 20, 22.5 and 25 minutes (10-min all-out).

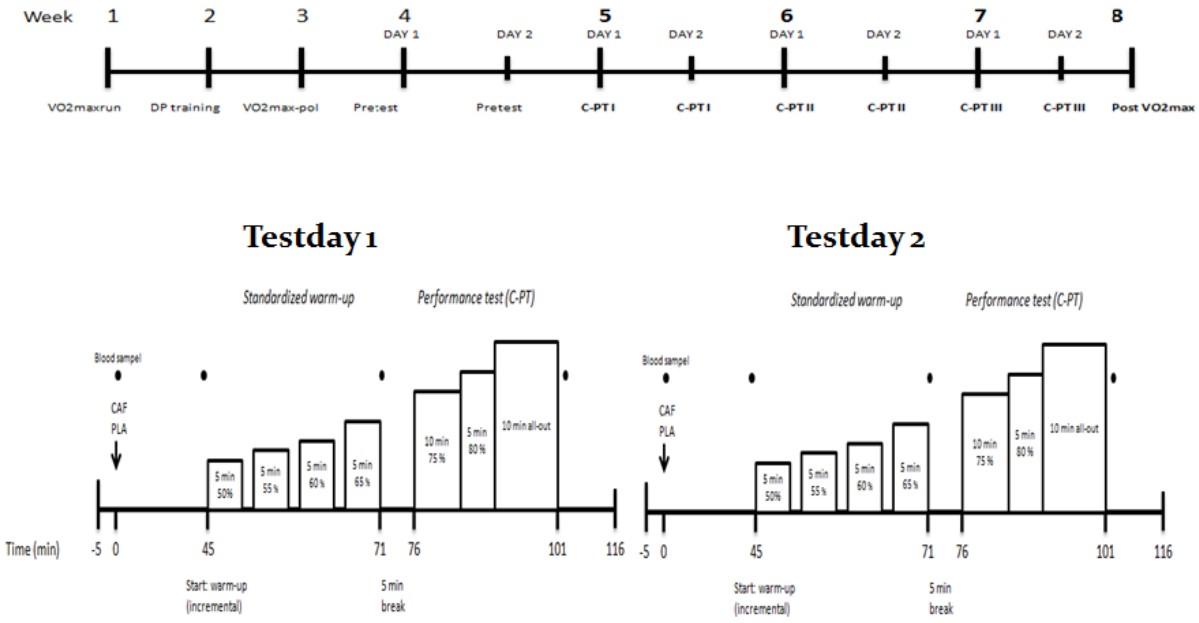

Figure 1: Experimental design. Top line shows tests and training performed during the 8 weeks to familiarize for the three, two-day performance tests in double poling (CP-T). The bottom figure shows the test procedure for all performance tests. Prior to the C-PT, subjects performed a standardized warm-up (incremental tests) consisting of four intensities all lasting five minutes. A similar protocol to the C-PT was completed at Pretest-1 and Pretest-2, except that caffeine/placebo was not administrated, and no blood samples were taken.

Abbreviations: $\dot{V}O_{2max\text{-}run}$, Training in thorax trainer, $\dot{V}O_{2peak\text{-}pol}$, Pre-test I, Pre-test II, C-PT I, C-PT II and C-PT III.

Paragraph Number 9 After finishing the C-PT day one, the last blood samples were drawn, and all subjects had to perform a low intensity jog for 10 min on a treadmill with a workload equivalent of 50 % of $\dot{V}O_{2max\text{-}run}$. During the 10-min jog subjects were also given an energy drink to ensure refilling of glycogen stores. The 500 ml sports drink contained: Water (0.4 dl), funlight cordial concentrate (0.1 dl), 53 g carbohydrate (26.5 g maltodextrin, AppiChem GmbH, Damstadt, Germany and 26.5 g Glucose, Prolab VWR, Levven, Belgium), 26.5 g protein (Arla Foods, Videnbaek, Denmark) and 0.2 g sodium chloride. The overall goal of the cool down was to optimize restitution so that subjects were able to perform their best during the C-PT the next day (test day two). In addition, before leaving the laboratory day one, subjects had to finish a questionnaire about what product they believed they had received, their dayform and motivation. They were also given the option to eat a small carbohydrate rich meal consisting of 0.3 dl chocolate milk, 1 banana, 4-6 small chocolate chip cookies and a sweet bun. Subjects themselves choose the amount they wanted to eat. Most subjects ingested the whole meal, and repeated it for all trials; i.e. the same amount was eaten for all trials compared to how much they ate after the first test.

Paragraph Number 10 *Performance tests (C-PT):* All subjects were informed to only perform light training (and no strength training) the last 48 hours before each C-PT. The subjects prepared to the C-PT as prior to a competition and followed the same training and diet regime before all tests with an interval of six days between each two day testing. To minimize variation in pre-exercise glycogen stores, diet and exercise diaries were used to standardize food intake and training for each subject. After the first test subjects were instructed to perform the same training and food consumption 48h and 24 h prior to the remaining two-day trials. Copies of training and nutritional diaries were provided to each subject so they could replicate this for the remaining trials. Also subjects refrained from caffeine consumption the last 48 h before each test day. No subject in the study had a high

intake of caffeine products on a daily basis (< 150 mg) based on a self-reported caffeine intake questionnaire.

Paragraph Number 11 The subjects arrived to the laboratory at the same time on all tests (±15 min). Each two day trial was separated by a 6 day washout period. After arrival, subjects rested in a supine position (in a bed) before resting heart rate was measured over a 10-min period. The first blood sample was drawn from the subject's median cubital vein using a BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). A 7 ml blood sample was drawn in tubes containing EGTA/gluthatione [20 µl 0.2 M glutathione and 0.2 M EGTA per ml blood] for analysis of adrenaline, creatine kinase (CK) and caffeine. Blood samples were immediately placed in ice water and centrifuged at 2500 rpm for 10 min at 4 ºC (Heraeus Megafuge 16R centrifuge, Thermo Electro, Deutschland). Plasma was divided in three Eppendorf tubes (Microtube Superspin, VWR International, West Chester, PA, USA) and frozen at -80°C.

Paragraph Number 12 Capillary blood was taken from a fingertip for measurement of glucose (HemoCue glucose 201^{+}; Ängelholm, Sweden) and lactate (YSI 1500 SPORT; Yellow Springs Instruments, Yellow Springs, OH USA). The subjects then consumed either CAF or PLA drinks. Treatments included two CAF doses (3 and 4.5 mg · kg^{-1}) and PLA (vehicle only). Doses selected in the study were chosen due to that they would be below the limit that falls under the Norwegian paragraph for clinical testing of medicine on humans. Furthermore, both doses are commonly used by XCS athletes during competitions. Higher doses does not seem to give additional effects, and few studies have tested the effects of 4.5 mg· kg^{-1} CAF on performance. Caffeine (Coffeinum, Oslo Apotekerproduksjon, Oslo, Norway) was dissolved in a cordial concentrate Fun Light (3 mg/ml) and was prepared at the laboratory. Resting measurement of heart rate, (over 10 min) was then performed 30 min after consumption of CAF or PLA followed by new venous and capillary blood samples. After the

blood sampling, subjects prepared for the test and started the standardized warm-up (incremental testing) 45 min after ingestion of CAF or PLA.

Paragraph Number 13 *Thorax Trainer – CC-POL:* The cross-country DP ergometer used in the study was a Thoraxtrainer Elite (Thoraxtrainer, Holbæk, Denmark). Temperature in the test laboratory was between 21-23°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), speed ($km \cdot h^{-1}$) and poling frequency in real time. Resistance in the Thoraxtrainer is generated by air pressure, and the mean barometric air pressure for PLA and CAF trials averaged 958±4, 960±7 and 968±2 mmHg, respectively ($p>0.05$). The Thoraxtrainer Elite was set at level one (easiest) of ten different levels during all testing to optimize technique. For more information about the DP technique and the Thoraxtrainer Elite, see studies by Bojsen-Moller et.al.[4] and Van Hall et al. [35].

Paragraph Number 14 *Plasma caffeine:* Sample preparation of 200 µL plasma and the subsequent measurements of caffeine and theophylline were done according to the method previously described in the Stadheim *et al.* (2013) study [33]. *Plasma catecholamines:* Plasma adrenaline was measured with a Cat Combi Elisa kit (DRG Instruments GmbH, Marburg, Germany) according to description. *Plasma creatine kinase* was measured according to the manufacturer instructions. Plasma creatine kinase was measured using a Maxmat S.A (ZAC du millenaire, Montpellier, France), and analysis was done using the colorimetric enzymatic method-kinetic type [13].

Paragraph Number 15 *Questionnaires:* Pain in arms and legs was evaluated by a 1-10 point scale described by Ritchie & Hopkins [28]. Other questionnaire scales were used to

evaluate motivation and day-form 1-100 [28]. Sleep quality was evaluated by each subject using a 1-10 VAS scale.

Paragraph Number 16 *Statistical Analysis:* All data in the study are presented as means ± standard error of the means (SEM), and differences in performance during the 10-min C-PT were evaluated by ANOVA. ANOVA was also used to assess treatment/day interaction. A two-way ANOVA for repeated measures was used to elicit differences in HR, LA, $\dot{V}O_2$, glucose, and RPE during submaximal workloads between the two treatments. If a significant f-ratio was found, a paired t-test was used to test differences between treatments on a workload. All data were tested for normal distribution using the Shapiro-Wilk test. Statistical analyses were performed using GraphPad Prisim 6, 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 90% confidence limits [21].

Results

Paragraph Number 17 *Performance Test.* Of all subjects participating in the study, 7 of the 8 test subjects improved performance as a result of CAF ingestion both testing days. Total distances covered in meters during the all-out test for days one and two are presented in figure 2. On the first testing day, subjects improved performance after ingestion of CAF3 and CAF4.5 by 4.0% (90%CL: ± 3.3%) and 4.0% ± 2.9 compared to PLA. The following day, improvements were 5.0% ± 3.6 and 5.1% ± 2.8 respectively, compared to PLA. Improved performance came both days as a result of subjects increasing work output leading to higher mean speed and greater distance covered. Total numbers of poling strokes to complete the all-out test did not differ between treatments on any of the two testing days (PLA: 618 ± 42, 638 ± 32, CAF3: 625 ± 32, 621 ± 22 and CAF4.5: 619 ± 26, 623 ± 29). Mean speed was 15.5 ±

0.2 and 15.4 ± 0.4 km · h^{-1} respectively for PLA day one and two. After CAF ingestion mean speed increased to 16.2 ± 0.4 km · h^{-1} for both CAF3 and CAF4.5 day one, while for day two, the same average speed was observed for CAF4.5 while a small decrease was observed for CAF3 to 16.1 ± 0.4 km·h^{-1}.

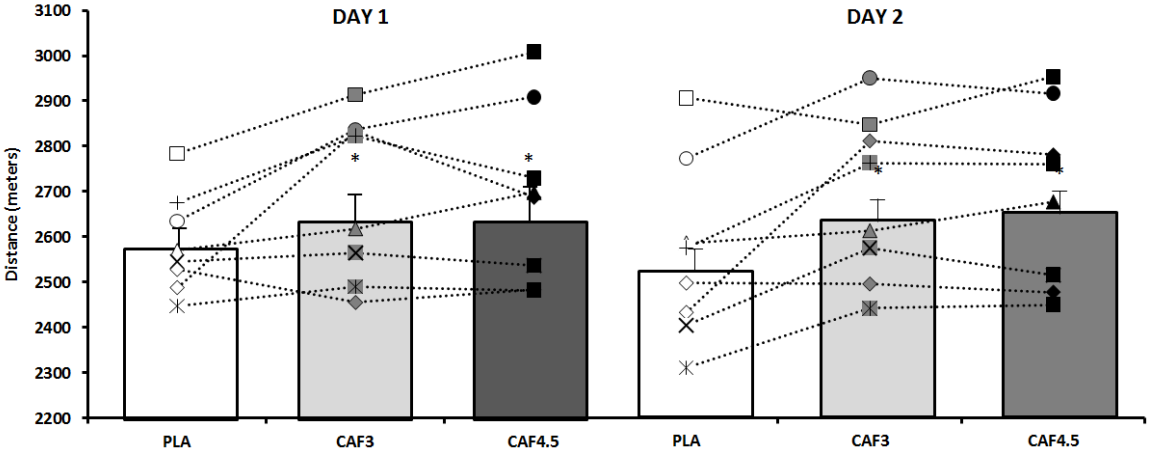

Figure 2: Mean and individual distances covered during the two days of the 10-min all-out C-PT after consuming PLA, CAF3 or CAF4.5.

Values are mean ± SEM. * Significantly different from placebo ($p < 0.05$)

Paragraph Number 18 *Heart rate and* $\dot{V}O_2$*:* Mean heart rate (HR) was higher when subjects performed the all-out test after CAF consumption (Fig 3). Mean HR (bpm) during day one and two was 180 ± 3 and 180 ± 4 for PLA. During CAF trials, higher average heart rates were observed for both CAF3 (184 ± 3) and CAF4.5 (185 ± 3) (Fig 3) day one. The same trend was observed the second testing day with both CAF treatments having an average heart rate of 184 ± 3 (bpm). Oxygen uptake was progressively increased during CAF 10 min all-out tests, although only significantly different on the first testing day for both CAF3 and CAF4.5 compared to PLA (difference of 4.2% ± 3.8 and 4.4% ± 3.8 respectively). On the second testing day, only a tendency was observed for increased $\dot{V}O_2$ ($p=0.12$) during CAF

trials (difference 1.2% ± 5.0 and 1.4% ± 5.8%). Unexpectedly all subjects were able to reach new $\dot{V}O_{2peak\text{-}pol}$ values during CAF trials compared to PLA trials, and 7 of 8 subjects set new $HR_{max\text{-}pol}$ values (Fig 3).

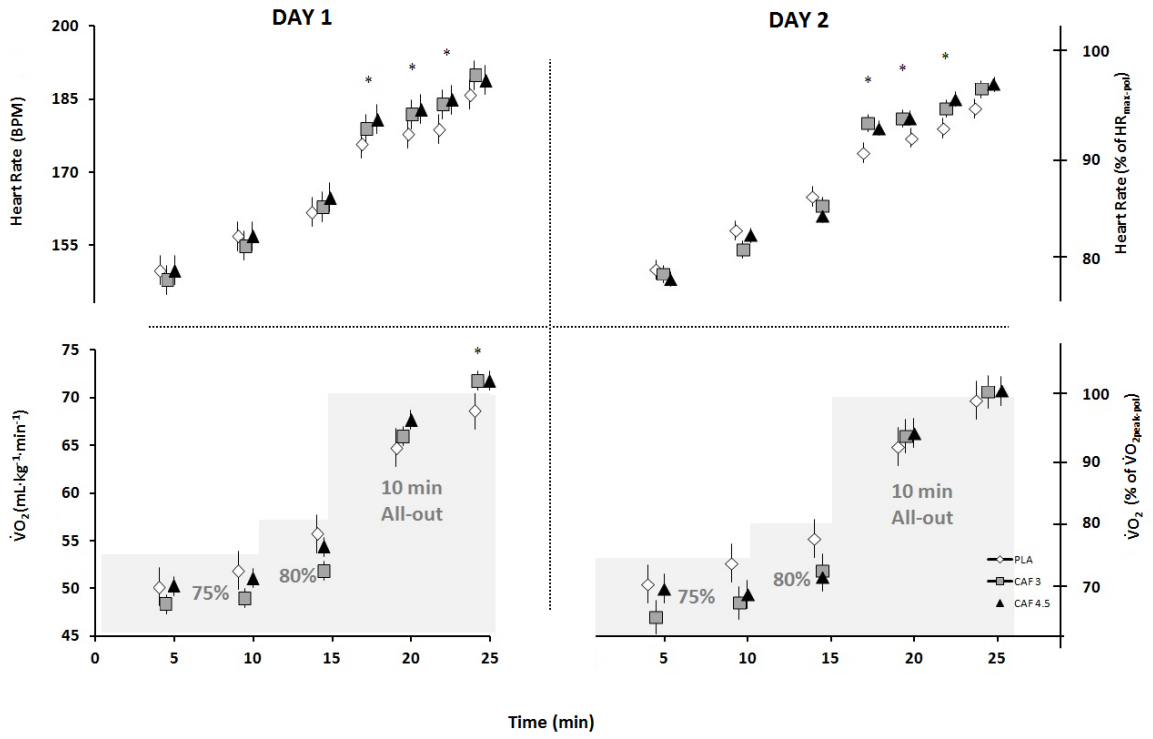

Figure 3: Heart rate and $\dot{V}O_2$-response after consuming PLA, CAF3 and CAF4.5 during the whole performance test 15 min + 10-min all-out C-PT. Values are mean ± SEM. * Significantly different from placebo ($p < 0.05$)

Paragraph Number 19 *Blood values:* Blood concentrations of lactate and glucose were higher after finishing the 10 min all-out CP-T during after CAF consumption compared to PLA. Similar results were observed for adrenaline values on the first testing day (Table 1). No difference in CK was observed upon arrival or after finishing the 10-min all-out C-PT day one between treatments. However, higher CK was observed day two in both CAF trials compared to PLA upon arrival and after finishing the performance test.

Table 1: Mean speed, blood lactate, glucose, muscular arm pain, plasma concentrations of caffeine, adrenaline and creatine kinase before starting and after finishing the 15 min + 10-min all-out C-PT.

		DAY 1			DAY 2		
Time point	**Variable**	**PLA**	**CAF 3 mg**	**CAF 4.5 mg**	**PLA**	**CAF 3 mg**	**CAF 4.5 mg**
C-PT	Speed (mean)	15.5±0.2	16.2±0.4*	16.2±0.4*	15.4±0.4	16.1±0.4*	16.2±0.4*
0 min (start)	Pre Caffeine	0.5±0.2	19.7±0.8*	28.6±1.3*	0.6±0.3	18.1±1.8*	30.5±0.8*
0 min (start)	Pre LA-	1.1±0.2	1.4±0.6	1.6±0.1	1.3±0.1	1.4±0.2	1.5±0.2
25 min (Finish)	Post LA-	6.2±0.2	7.7±0.2*	7.5±0.3*	6.4±0.6	7.0±0.3*	7.2±0.4*
0 min (start)	Pre Adrenalin	0.62±0.07	0.45±0.27	0.42±0.02*	0.48±0.08	0.35±0.32	0.36±0.22
25 min (Finish)	Post Adrenalin	1.39±0.17	3.02±0.02*	2.90±0.04*	1.90±0.36	2.38±0.24	2.22±0.51
0 min (Arrival)	Pre Creatin Kinase	236.4±37.8	253.5±26.2	246.1±26.6	253.8±29.4	365.1±65.2*#	319.4±46.7*#
25 min (Finish)	Post Creatin Kinase	301.8±39.4	317.0±36.4	317.9±26.2	323.3±28.5	450.6±71.4*#	398.9±53.6*#
0 min (Arrival)	Pre Muscular pain arms (1-100)	11.3±4.6	11.9±5.4	13.4±4.7	11.3±3.6	18.1±5.7*#	18.8±5.0*
25 min (Finish)	Post Muscular pain arms (1-100)	78.1±7.1	73.1±6.1	80.0±3.3	79.4±7.6	75.6±5.5	76.9±3.7
0 min (start)	Pre GLU	5.4±0.2	5.4±0.1	5.6±0.2	6.2±1.1	5.3±0.2	5.3±0.1
25 min (Finish)	Post GLU	7.2±0.6	8.3±0.3*	8.5±0.5*	7.0±0.5	8.1±0.3*	7.8±0.3*

Values are mean ± SEM. * Significantly different from placebo ($p < 0.05$) # Significantly different from value day one ($p < 0.05$).

Paragraph Number 20 *Resting measurements and metabolism during standardized warm up (incremental tests):* No significant difference in resting heart rate (54-58), blood lactate or glucose among treatments prior to the standardized warm-up protocol (incremental test) were observed. During the standardized warm-up, no systematic difference was observed for either: HR, $\dot{V}O_2$, RER, blood lactate, glucose or VE between treatments (Fig 4). However, lower RPE and higher lactate at the last workload (65% of $\dot{V}O_{2peak\text{-}pol}$) was observed the first testing day after consuming 3 or 4.5 mg $\cdot$ kg^1 CAF compared to PLA. There were no observed difference for these parameters the second testing day.

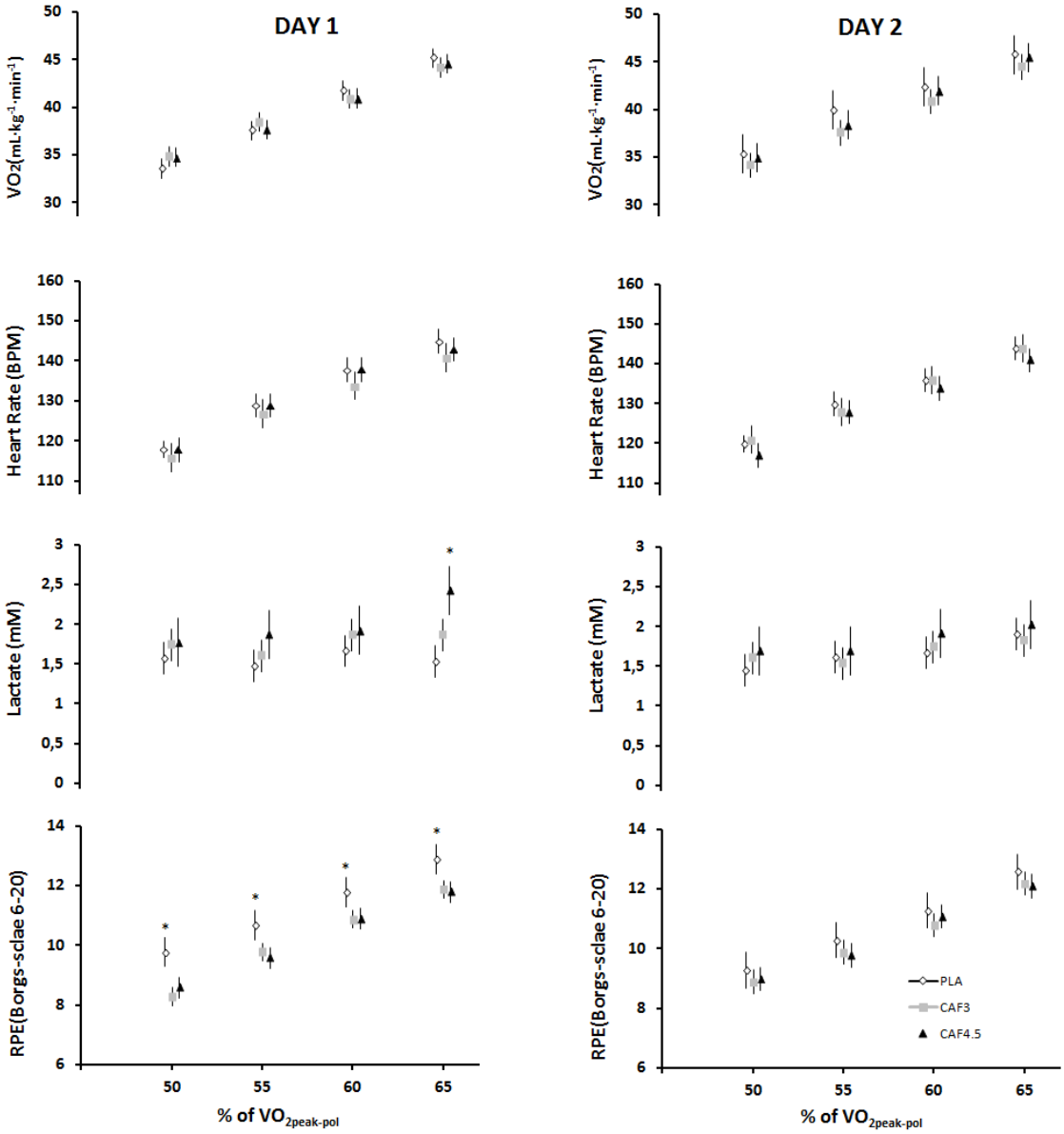

Figure 4: $\dot{V}O_2$-uptake, HR, lactate and RPE as a function of increasing workload during the standardized warm up (submaximal incremental exercise) after consuming PLA, CAF3 and CAF4.5. Values are mean ± SEM. * Significantly different from placebo ($p < 0.05$)

Paragraph Number 21 *Questionnaires*. Motivation was high before all trials of PLA (78 ± 5, 82 ± 5 = "very high"), CAF3 (87 ± 4, 80 ± 4 = "very high"), and CAF4.5 (80 ± 6, 82 ± 5 = "very high"). Subjects also reported similar day-form prior to and after finishing all tests (72-77 ± 5 = "very well"). Furthermore, no difference in muscular pain or RPE was detected

during the C-PT. However higher muscular pain was observed upon arrival in the CAF groups the second day compared to PLA (Table 1). Questionnaires revealed that subjects were unable to sense which product they received during the different trials. Diary reports on training and intake of food, liquid and caffeine containing products the last 48 h prior to the C-PT showed that subjects had followed instructions given. No difference in quality (5.2-6.3 = "good sleep") or sleep amount (7.2-8.4 hours) before PLA, CAF3 or CAF4.5 was observed prior to either testing days.

Paragraph Number 22 *Standardized workloads*: The first 15 min of the performance test showed no differences in mean: HR (162-166 bpm), $\dot{V}O_2$-uptake (51.9-54.4 mL · kg^{-1} · min^{-1}), RPE (14.8-15.3) or muscular pain in arms (48.8-58.1 = "moderate pain") regardless of product consumption on any of the two testing days. Furthermore, no difference in HR (89-96), blood glucose or lactate were observed between treatments before subjects started the performance test on any one of the two testing days (Table 1).

Paragraph Number 23 *Comparing oxygen uptake when double poling and running:* Maximal oxygen uptake was higher in running compared to double poling. The highest heart rate achieved during the different $\dot{V}O_{2max}$ tests was higher when running compared to double poling. No differences were observed in $\dot{V}O_{2max\text{-}run}$ (78.5 ± 1.6, 77.0 ± 1.6) or $\dot{V}O_{2peak\text{-}pol}$ (70.5 ± 1.2, 69.6 ± 2.0) for test subjects from pre (September) to post (October) measurements.

Discussion

Paragraph Number 24 In the present study we show for the first time that CAF ingestion of 3 or 4.5 mg · kg^1 improved performance compared to PLA during a 10-min all-out DP performance test when performed consecutive days in a row. Despite higher CK and muscular pain associated with the increased performance after CAF ingestion on day one, both CAF doses led to improved performance the second consecutive day of testing.

Paragraph Number 25 DP performance was improved day one by 4.0% during the simulated DP XCS competition for both CAF treatments compared to PLA. Improved performance came as a result of subjects increasing work intensity, which was associated with higher $\dot{V}O_2$, HR, adrenaline and blood lactate accumulation after CAF consumption compared to PLA. Results from the present study show that ingestion of 3 mg · kg^1 CAF leads to similar exercise improvements as 4.5 mg· kg^1. Furthermore, results from the present study agree with previous studies testing ergogenic effects of CAF using a one day protocol [19], also when using the DP technique in XCS [33].

Paragraph Number 26 Higher exercise intensity and performance, as observed in the present study after CAF ingestion day one, is often associated with higher muscular soreness due to a larger muscular damage [6, 27, 30, 36]. CK is a marker of muscular damage during exercise, and is observed to be higher during both ultra-distance marathon running and strength training [6]. Interestingly, we observed that CK was higher on arrival the second day after CAF testing day one compared to PLA. Also, it has recently been reported that CAF ingestion resulted in increased oxidative stress markers (IL-6 and IL-10) after a 15 km running competition compared to PLA [34]. It was therefore somewhat unexpected that subjects improved performance the second testing day as observed day one. On average, performance was improved by 5.0 and 5.1% on day two respectively after ingesting 3 and 4.5 mg · kg^1 CAF compared to PLA, but no difference was observed between performance day one and two. No difference in CK was observed prior or after finishing the C-PT day one. Higher CK values upon arrival day two after CAF testing day one therefore presumably came as a result of the improved exercise intensity. Higher muscular pain in the arms was also reported in the CAF groups compared to PLA before starting the C-PT day two (table 1). Higher muscular pain is reported to be accompanied with strength loss and a reduced range of

motion the following day [26]. However results in the present study show performance the second day was not affected by higher CK or muscular soreness.

Paragraph Number 27 Based on available literature, the actions of CAF ingestion improving performance seems to be multi-functional [19, 20]. When DP, the ability to withstand the increasing pain in the arms is important for high performance [33]. In the present study, subjects reported lower RPE day one during the standardized warm up. This was however, not observed the second consecutive day, maybe due to higher muscular damage resulting in higher muscular pain from the improved exercise intensity day one after CAF ingestion. During all 10-min all-out tests, subjects chose a similar level of exertion and muscular pain during both CAF and PLA trials. However after CAF ingestion discomfort was reduced, when RPE is expressed as per given work output.

Paragraph Number 28 Adenosine receptors are plentiful in many areas of the heart, brain and muscles [20], and inhibition is observed to reduce both somatic pain as well as RPE during steady state exercise [14, 18]. If work economy was not improved the increased exercise intensity after CAF ingestion would require a higher energy production due to break down and use of adenosine triphosphate. This means that even if CAF ingestion allowed higher discomfort due to inhibition of adenosine receptors, it would not explain why subjects were able to produce more energy to maintain the increased exercise intensity.

Paragraph Number 29 Our plasma caffeine concentrations of ~ 18 (3 mg) and ~ 30 (4.5 mg) μM in the study would reduce both A_1 and A_2 adenosine receptor activation [14]. A_1 receptors inhibit adenylyl cyclase [15, 37]**,** and a blockage of A_1 receptors in the heart could increase the response to sympathetic activity, and potentially remove a "safety break" in the heart resulting in improved contractility and/or pumping capacity [14]. Higher HR after CAF ingestion is one of the most commonly observed effects during high intensity performance

tests lasting 30-60 minutes [7, 11, 19, 22, 33]. Furthermore higher HR during performance tests after CAF ingestion could be associated with subjects consuming larger amounts of oxygen if the refilling (diastole) and pumping (systole) of each heart beat is unchanged (stroke volume) [3, 29]. When using the Fick equation a higher HR, and the same stroke volume (cardiac output), should lead to higher oxygen consumption, if the arterio-venous difference is unchanged [3, 29]. Ivy *et al. [22]* observed that subjects after CAF ingestion were able to produce a higher average power which was associated with both higher HR and oxygen consumption compared to PLA during a cycling performance test. In the present study higher HR rate was associated with the improved work intensity during the 10-min all-out C-PT after CAF consumption both days (Fig 3). $\dot{V}O_2$ was also higher day one after subjects ingested CAF doses compared to PLA, but only a tendency was observed for this the second day ($p < 0.12$). Results from submaximal exercise show no difference in HR or $\dot{V}O_2$ between treatments while doing the same workload, and additionally that they increase in a linear fashion ($R^2 = 0.97$). The higher adrenaline values after CAF performances would also strengthen a theory that CAF ingestion could improve contractility qualities of the heart, hence increasing HR, $\dot{V}O_2$, oxygen delivery to exercising muscles, production of adenosine triphosphate, and maintenance of higher exercise intensity. Impressingly all subjects (8/8) set new $\dot{V}O_{2peak\text{-}pol}$, and 7 of 8 subjects new $HR_{max\text{-}pol}$ during CAF 10-min all-out C-PTs. Post-testing in week 8 of $\dot{V}O_{2peak\text{-}pol}$, and measurements of $\dot{V}O_{2peak}$ during the PLA 10-min all-out C-PTs showed no difference from pre measurements. Furthermore similar results were observed for $HR_{max\text{-}pol.}$

Paragraph Number 30 Indeed, a higher average power per stroke while DP had to be produced after CAF ingestion both days since the number of strokes used to complete the 10-min all-out C-PT was similar between treatments all days [38]. Studies have observed that CAF ingestion can improve strength-power performance when using arm muscles. In DP, the

arm muscles represent the speed generation force and are therefore, of high relevance for performance outcome [33, 35]. In a study by Beck *et al*. (2006) subjects improved number of repetitions until exhaustion at 80% of individual 1RM bench-press [2]. However an improved muscular strength or contractility qualities of exercising muscles has so far only been observed when using the knee extensors [38], and a well-documented effect of CAF on strength in arm muscles has so far not been reported [38]. However it is still possible that CAF ingestion improved contractility qualities of exercising muscles due to improved or more efficient muscle recruitment. This was, however, not measured in the study, and based on our results work economy or efficiency during submaximal exercise was not improved by CAF ingestion.

Paragraph Number 31 Results from the present study are of great interest for sports performance since CAF was removed from the World Anti Doping Agency list of prohibited substances in 2004, and is now legal to use [8]. The clear improvements of day one and day two for CAF3 and CAF4.5 would most likely affect results in elite XCS competitions. For example, it has been reported that the within-athlete variability in performance times in elite XCS races for the best skiers is approximately 1.1-1.4% for both sprint and distance races, and the smallest worthwhile enhancement is as small as 0.3-0.4% [32]. Knowledge of effects of caffeine on sports performance when performing two consecutive days in a row is also of high relevance since many sports have competitions lasting several days.

Conclusion

Paragraph Number 32 Ingestion of either 3 and 4.5 mg · kg^1 caffeine improved performance for eight elite cross-country skiers compared to PLA during a 10-min all-out performance test in a cross-country poling ergometer over two consecutive days. The improvement in performance was 4.0% for both CAF doses the first day, and 5.0 and 5.1% day two

respectively for CAF3 and CAF4.5. Furthermore, 7 of 8 test subjects improved performance after ingesting CAF compared to PLA. Results show the improvement in performance came as a result of subjects increasing average speed, which was associated with higher heart rate, $\dot{V}O_2$, lactate and adrenaline during the 10-min all-out test following CAF ingestion. Interestingly, performance with intake of CAF was the same both competing days, although subjects reported higher muscular pain in the arms, and had higher CK values on arrival the second day in the CAF groups. Based on our results CAF may indeed assist in maintaining performance quality for athletes competing consecutive days in real life competitions.

Acknowledgements

Thanks to all test subjects for their time and effort. The authors would also like to thank Astrid Bolling for assisting with measurements of adrenalin, Hege Østgaard for measurements of Creatin Kinase, and Kristoffer Jensen and Per Inge Rustad for help with taking blood samples during the different exercise trials.

The results of the present study do not constitute endorsement by ACSM. Founding was done by the Department of Physical Performance at the Norwegian School of Sport Sciences.

Disclosures

No conflicts of interest, financial or otherwise are declared by the author. We disclosure funding received for this work from any of the following organizations: American College of Sports Medicine, National Institutes of Health (NIH); Welcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).

Reference List

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

2. Beck TW, Housh TJ, Schmidt RJ, Johnson GO, Housh DJ, Coburn JW, Malek MH. The acute effects of a caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities. J.Strength.Cond.Res. 2006; 20:506-510

3. Blomqvist CG, Saltin B. Cardiovascular adaptations to physical training. Annu.Rev.Physiol 1983; 45:169-189

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

5. 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 1987; 56:679-685

6. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. Clin.Chem.Lab Med. 2010; 48:757-767

7. Bridge CA, Jones MA. The effect of caffeine ingestion on 8 km run performance in a field setting. J.Sports Sci. 2006; 24:433-439

8. Chester N, Wojek N. Caffeine consumption amongst British athletes following changes to the 2004 WADA prohibited list. Int.J.Sports Med. 2008; 29:524-528

9. Cox GR, Desbrow B, Montgomery PG, Anderson ME, Bruce CR, Macrides TA, Martin DT, Moquin A, Roberts A, Hawley JA, Burke LM. Effect of different protocols of caffeine intake on metabolism and endurance performance. J.Appl.Physiol 2002; 93:990-999

10. Cureton KJ, Warren GL, Millard-Stafford ML, Wingo JE, Trilk J, Buyckx M. Caffeinated sports drink: ergogenic effects and possible mechanisms. Int.J.Sport Nutr.Exerc.Metab 2007; 17:35-55

11. 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. 2012; 30:115-120

12. 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 2005; 15:69-78

13. Fossati P, Prencipe L, Berti G. Enzymic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin.Chem. 1983; 29:1494-1496

14. Fredholm BB. Adenosine receptors as drug targets. Exp.Cell Res. 2010; 316:1284-1288

15. 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. 1997; 18:79-82

16. Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol.Rev. 1999; 51:83-133

17. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol.Rev. 2001; 53:527-552

18. Ganio MS, Klau JF, Casa DJ, Armstrong LE, Maresh CM. Effect of caffeine on sport-specific endurance performance: a systematic review. J.Strength.Cond.Res. 2009; 23:315-324

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

20. Graham TE. Caffeine and exercise: metabolism, endurance and performance. Sports Med. 2001; 31:785-807

21. Hopkins, W. G. Analysis of a post-only crossover trial (Excel spreadsheet). 2006. newstats.org/xPostOnlyCrossover.xls. (GENERIC)
Ref Type: Internet Communication

22. Ivy JL, Costill DL, Fink WJ, Lower RW. Influence of caffeine and carbohydrate feedings on endurance performance. Med.Sci.Sports 1979; 11:6-11

23. Jacobson TL, Febbraio MA, Arkinstall MJ, Hawley JA. Effect of caffeine co-ingested with carbohydrate or fat on metabolism and performance in endurance-trained men. Exp.Physiol 2001; 86:137-144

24. Jenkins NT, Trilk JL, Singhal A, O'Connor PJ, Cureton KJ. Ergogenic effects of low doses of caffeine on cycling performance. Int.J.Sport Nutr.Exerc.Metab 2008; 18:328-342

25. McNaughton LR, Lovell RJ, Siegler J, Midgley AW, Moore L, Bentley DJ. The effects of caffeine ingestion on time trial cycling performance. Int.J.Sports Physiol Perform. 2008; 3:157-163

26. Miles MP, Clarkson PM. Exercise-induced muscle pain, soreness, and cramps. J.Sports Med.Phys.Fitness 1994; 34:203-216

27. Munoz MD, Olcina G, Timon R, Robles MC, Caballero MJ, Maynar M. Effect of different exercise intensities on oxidative stress markers and antioxidant response in trained cyclists. J.Sports Med.Phys.Fitness 2010; 50:93-98

28. Ritchie SE, Hopkins WG. The intensity of exercise in deep-water running. Int.J.Sports Med. 1991; 12:27-29

29. Saltin B. Physiological adaptation to physical conditioning. Old problems revisited. Acta Med.Scand.Suppl 1986; 711:11-24

30. Shing CM, Peake JM, Ahern SM, Strobel NA, Wilson G, Jenkins DG, Coombes JS. The effect of consecutive days of exercise on markers of oxidative stress. Appl.Physiol Nutr.Metab 2007; 32:677-685

31. Skinner TL, Jenkins DG, Coombes JS, Taaffe DR, Leveritt MD. Dose response of caffeine on 2000-m rowing performance. Med.Sci.Sports Exerc. 2010; 42:571-576

32. Spencer M, Losnegard T, Hallen J, Hopkins WG. Variability and Predictability of Performance Times of Elite Cross-Country Skiers. Int.J.Sports Physiol Perform. 2013;

33. Stadheim HK, Kvamme B, Olsen R, Drevon CA, Ivy JL, Jensen J. Caffeine Increases Performance in Cross-country Double-Poling Time Trial Exercise. Med.Sci.Sports Exerc. 2013; 45:2175-2183

34. Tauler P, Martinez S, Moreno C, Monjo M, Martinez P, Aguilo A. Effects of caffeine on the inflammatory response induced by a 15-km run competition. Med.Sci.Sports Exerc. 2013; 45:1269-1276

35. van Hall G, Jensen-Urstad M, Rosdahl H, Holmberg HC, Saltin B, Calbet JA. Leg and arm lactate and substrate kinetics during exercise. Am.J.Physiol Endocrinol.Metab 2003; 284:E193-E205

36. Viguie CA, Frei B, Shigenaga MK, Ames BN, Packer L, Brooks GA. Antioxidant status and indexes of oxidative stress during consecutive days of exercise. J.Appl.Physiol 1993; 75:566-572

37. Wang Y, Yang JN, Arner A, Boels PJ, Fredholm BB. Adenosine A(1) receptors and vascular reactivity. Acta Physiol (Oxf) 2010; 199:211-220

38. Warren GL, Park ND, Maresca RD, McKibans KI, Millard-Stafford ML. Effect of caffeine ingestion on muscular strength and endurance: a meta-analysis. Med.Sci.Sports Exerc. 2010; 42:1375-1387