Caffeine and Performance over Consecutive Days of Simulated Competition

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ABSTRACT

STADHEIM, H. K., M. SPENCER, R. OLSEN, and J. JENSEN. Caffeine and Performance over Consecutive Days of Simulated Competition. *Med. Sci. Sports Exerc.*, Vol. 46, No. 9, pp. 1787–1796, 2014. **Purpose**: Performance improvements after caffeine (CAF) ingestion are well documented when using a 1-d protocol. In numerous competitions such as the Tour de France, Tour de Ski, world championships, and National College Athletic Association championships, athletes compete for several days in a row. To date, no studies have investigated the effects of CAF when competing for consecutive days in a row. This study aimed to investigate the effects of placebo (PLA) and two different CAF doses (3 and 4.5 $mg \cdot kg^{-1}$ body mass) on performance in a 10-min all-out, cross-country, double poling ergometer test (C-PT) 2 d in a row. **Method**: Eight highly trained male cross-country skiers ($\dot{V}O_{2max\text{-}run}$, 78.5 ± 1.6 $mL \cdot kg^{-1} \cdot min^{-1}$) participated in the study, which was a randomized, double-blind, PLA-controlled, crossover 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 with that after PLA on both days. The improvements on day 1 were 4.0% (90% confidence limits, ±3.3) and 4.0% ± 2.9% for both CAF doses, respectively ($P < 0.05$), whereas improvements on day 2 were 5.0% ± 3.6% and 5.1% ± 2.8% for CAF3 and CAF4.5, respectively, compared with those for PLA. Improved performance was associated with increased HR, adrenaline concentration, blood lactate concentration, and $\dot{V}O_2$ consumption after CAF ingestion. Furthermore, performance was elevated despite higher creatine kinase concentration and muscular pain at arrival on day 2 for both CAF doses. **Conclusions**: 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 on the first day. **Key Words:** EXERCISE PERFORMANCE, OXYGEN CONSUMPTION, HEART RATE, CREATINE KINASE, MUSCULAR PAIN

The ergogenic effects of caffeine (CAF) have been researched since the early 1900s, and several studies during the last 40 yr have observed that CAF ingestion (3–9 $mg \cdot kg^{-1}$) can have a positive effect on exercise performance when using a 1-d 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).

The observed improvements after CAF ingestion normally varies between 1% and 5% during time trials lasting 10–60 min (19,25,33). Because of variations in performance improvements after CAF ingestion, exercise physiologists have studied the CAF and dose response relation. Results from these studies have observed that optimized effects after CAF ingestion are highly individual but seem to occur with doses between 3 and 6 $mg \cdot kg^{-1}$ (11,19,20). Higher doses (9–12 $mg \cdot kg^{-1}$) do not seem to result in additional improvements but rather lead to stronger side effects such as headaches or nausea (19).

The main theory explaining improved performance after CAF ingestion is inhibition of adenosine receptors (1,19,20) and reduction in muscle pain and RPE (12,33). Still, inhibition of adenosine receptors could also affect facilitation of motor unit recruitment and HR 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 HR (7,25,33) and/or blood lactate accumulation (33). CAF has also been observed to improve maximal voluntary contraction (38). 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).

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

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during competitions like the Olympics, world championships (running, swimming, rowing), Tour de France (cycling), or Tour de Ski (XCS). Increased exercise intensity has 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.

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 2-d 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 1, as observed in previous studies. However, because of a higher exercise intensity on day 1, subjects would be more fatigued on day 2, leading to impaired performance in the CAF groups compared with that in PLA. Furthermore, we wanted to observe if the two different CAF doses potentially gave different responses on performance or muscular damage on the second day of testing.

MATERIALS AND METHODS

Subjects. Eight healthy male elite cross-country skiers (three seniors and five juniors) gave their written consent to participate in the study after being informed of the purposes of the study and the 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), $\dot{V}O_{2max}$ when running ($\dot{V}O_{2max\text{-}run}$) (78.5 ± 1.6 $mL \cdot kg^{-1} \cdot min^{-1}$), and $\dot{V}O_{2peak}$ when DP ($\dot{V}O_{2peak\text{-}pol}$) (70.5 ± 1.6 $mL \cdot kg^{-1} \cdot min^{-1}$). Inclusion criteria were that all subjects had to be male, have a $\dot{V}O_{2max\text{-}run}$ greater than 70 $mL \cdot kg^{-1} \cdot min^{-1}$, and that they would train seriously to compete in the Norwegian National Cross-Country Skiing Cup in the upcoming season. The study was approved by the regional ethics committee.

Experimental procedures. The study had a randomized, double-blind, PLA-controlled, crossover design. Before the performance tests (C-PT), the participants underwent a 4-wk training protocol to familiarize with the DP ergometer (ThoraxTrainer Elite) and the 10-min all-out test (Fig. 1). On day 1, participants performed a $\dot{V}O_{2max\text{-}run}$ test on a treadmill (Woodway, Weil am Rhein, Germany) and the highest HR was defined as $HR_{max\text{-}run}$. Oxygen consumption and RER were measured with an Oxycon Pro metabolic system (Jaeger, Hochberg, Germany), and air was collected using a mouth V2 mask (Hans Rudolph, Inc.) 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 (8–11 $km \cdot h^{-1}$) with a 5.3° uphill incline. A 1-min break was given between each workload where lactate concentration was measured. After the last workload of the warm-up, subjects walked for 5 min at 5 $km \cdot h^{-1}$ before starting the $\dot{V}O_{2max\text{-}run}$ test. The starting speed was 10 $km \cdot h^{-1}$, with a treadmill incline of 10.5°. Each 0.5-min speed was increased with 0.5 $km \cdot h^{-1}$ until subjects were unable to maintain the speed and stepped off the treadmill. All eight subjects had to meet criterion 1, and at least two of the three other criteria to obtain $\dot{V}O_{2max\text{-}run}$: 1) oxygen consumption reached a plateau, meaning $\dot{V}O_2$ increased less than 1 $mL \cdot kg^{-1} \cdot min^{-1}$ whereas speed was increased two times 0.5 $km \cdot h^{-1}$, 2) RER values were greater than 1.10, 3) postmeasurements of blood lactate concentration were

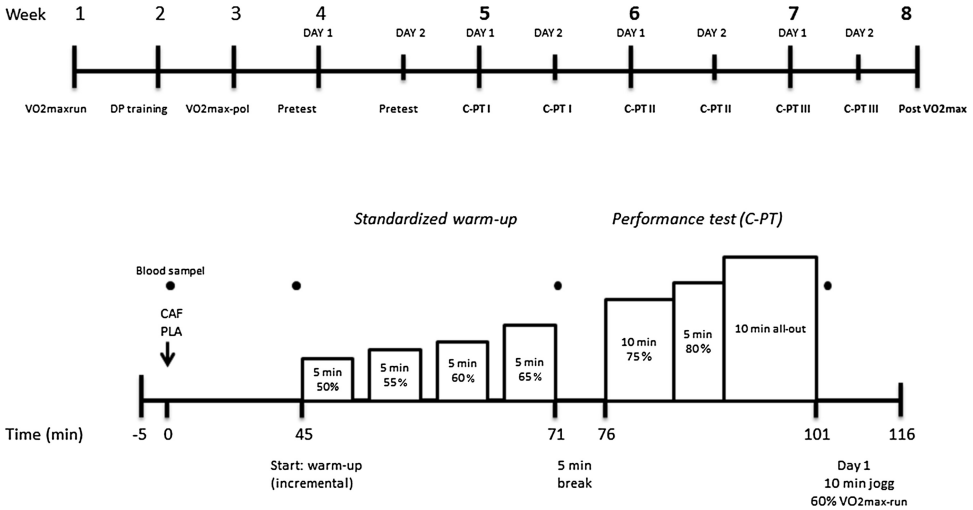

FIGURE 1—Experimental design. ***Top line*** **shows tests and training performed during the 8 wk to familiarize for the three 2-d performance tests in DP (C-PT). The bottom figure shows the test procedure for all performance tests. Before the C-PT, subjects performed a standardized warm-up (incremental tests) consisting of four intensities all lasting 5 min. A similar protocol to the C-PT was completed at pretest 1 and pretest 2, except that CAF/PLA was not administrated and no blood samples were taken. $\dot{V}O_{2max\text{-}run}$, Training in ThoraxTrainer, $\dot{V}O_{2peak\text{-}pol}$, pretest 1, pretest 2, C-PT 1, C-PT 2, and C-PT 3.**

greater than 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 $mL \cdot kg^{-1} \cdot min^{-1}$ were included. Day 2 subjects performed 40 min of familiarization DP training on the poling ergometer (ThoraxTrainer Elite), with workloads ranging from 55% to 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 HR defined as $HR_{max\text{-}pol}$. Criteria for that $\dot{V}O_{2peak\text{-}pol}$ were reached were the same as those for $\dot{V}O_{2max\text{-}run}$. On days 4 and 5, the participants completed pre–C-PT identical to the final C-PT 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-PT were required to obtain acceptable reliability (coefficient of variation (CV)%, approximately 1%–2%) (33).

On remaining test days, subjects received PLA or one of the two CAF doses 2 d in a row, 45 min before 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 1-min break between each workload. HR, $\dot{V}O_2$, and RER were measured as means between the 3–4.5 min of each workload. Subjective RPE according to the Borg scale (6–20) were determined for each workload (5). After 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 $\dot{V}O_{2peak\text{-}pol}$. 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 the remaining time. During the C-PT, HR, $\dot{V}O_2$, RPE, and speed were recorded after 4, 10, and 15 min (standardized workloads) and 17.5, 20, 22.5, and 25 min (10-min all-out).

After finishing the C-PT on day 1, 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 to 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), Fun Light cordial concentrate (0.1 dL), 53 g CHO (26.5 g maltodextrin; AppliChem GmbH, Darmstadt, Germany, and 26.5 g glucose; Prolab VWR, Leuven, Belgium), 26.5 g protein (Arla Foods, Videbaek, Denmark), and 0.2 g sodium chloride. The overall goal of the cooldown was to optimize restitution so that subjects were able to perform their best during the C-PT the next day (test day 2). In addition, before leaving the laboratory on day 1, subjects had to finish a questionnaire about what product they believed they had received, their day form, and motivation. They were also given the option to eat a small CHO-rich meal consisting of 0.3 dL chocolate milk, one banana, 4–6 small chocolate chip cookies, and a sweet bun. Subjects themselves chose 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 with how much they ate after the first test.

Performance tests (C-PT). All subjects were informed to only perform light training (and no strength training) on the last 48 h before each C-PT. The subjects prepared for the C-PT as before a competition and followed the same training and diet regimen before all tests, with an interval of 6 d between each 2-d testing. To minimize variation in preexercise 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 48 h and 24 h before the remaining 2-d trials. Copies of training and nutritional diaries were provided to each subject so that they could replicate this for the remaining trials. In addition, subjects refrained from CAF consumption during the last 48 h before each test day. No subject in the study had a high intake of CAF products on a daily basis (<150 mg), based on a self-reported CAF intake questionnaire.

The subjects arrived at the laboratory at the same time for all tests (±15 min). Each 2-d trial was separated by a 6-d washout period. After arrival, subjects rested in a supine position (in a bed) before resting HR 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). A 7-mL blood sample was drawn in tubes containing ethylene glycol tetraacetic acid/glutathione (20 μL, 0.2 M glutathione and 0.2 M ethylene glycol tetraacetic acid per milliliter of blood) for analysis of adrenaline, creatine kinase (CK), and CAF. Blood samples were immediately placed in ice water and centrifuged at 2500 rpm for 10 min at 4°C (Heraeus Megafuge 16R centrifuge; Thermo Electro, Deutschland, Germany). Plasma was divided into three Eppendorf tubes (Microtube SuperSpin; VWR International, West Chester, PA) and frozen at −80°C.

Capillary blood was taken from a fingertip for measurement of glucose concentration (HemoCue Glucose 201+; HemoCue, Ängelholm, Sweden) and lactate concentration (YSI 1500 SPORT; Yellow Springs Instruments Life Sciences, Yellow Springs, OH). The subjects then consumed either CAF or PLA drinks. Treatments included two CAF doses (3 and 4.5 $mg \cdot kg^{-1}$) and PLA (vehicle only). Doses selected in the study were chosen because they would be less than 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 do not seem to give additional effects, and few studies have tested the effects of CAF 4.5 $mg \cdot kg^{-1}$ on performance. CAF (Coffeinum; Oslo Apotekproduksjon, Oslo, Norway) was dissolved in a cordial concentrate Fun Light (3 $mg \cdot mL^{-1}$) and was prepared at the laboratory. Measurement of resting HR (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.

ThoraxTrainer Elite. The cross-country DP ergometer used in the study was a ThoraxTrainer Elite (ThoraxTrainer, Holbæk, Denmark). The temperature in the test laboratory was between 21°C and 23°C on all test days. The ski pole used during all testing was Swix CT1 (Swix, Lillehammer, Norway), and length was 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 mm Hg, 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 studies by Bojsen-Moller et al. (4) and van Hall et al. (35).

Plasma CAF. Sample preparation of 200-μL plasma and the subsequent measurements of CAF and theophylline were done according to the method previously described in the study of Stadheim et al. (33). For plasma catecholamines, plasma adrenaline concentration was measured with a Cat Combi enzyme-linked immunosorbent assay kit (DRG Instruments GmbH, Marburg, Germany) according to description. Plasma CK concentration was measured according to the manufacturer's instructions. Plasma CK concentration was measured using a Maxmat SA (ZAC du Millenaire, Montpellier, France), and analysis was done using the colorimetric enzymatic method-kinetic type (13).

Questionnaires. Pain in arms and legs was evaluated by a 1–10 point scale described by Ritchie and Hopkins (28). Other questionnaire scales were used to evaluate motivation and day form from 1 to 100 (28). Sleep quality was evaluated by each subject using a 1–10 visual analog scale.

Statistical analysis. All data in the study are presented as 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 concentration, 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 Prism 6, 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 90% confidence limits (21).

RESULTS

Performance test. Of all subjects participating in the study, seven of the eight test subjects improved performance as a result of CAF ingestion on both testing days. Total distances covered in meters during the all-out test for days 1 and 2 are presented in Figure 2. On the first testing day, subjects improved performance after ingestion of CAF3 and CAF4.5 by 4.0% (90% confidence limit, ±3.3%) and 4.0% ± 2.9% compared with that after ingestion of PLA. The following day, improvements were 5.0% ± 3.6% and 5.1% ± 2.8%, respectively, compared with those in PLA. Improved performance came on both days as a result of subjects' increasing work output, leading to higher mean speed and greater distance covered. The total numbers of poling strokes to complete the all-out test did not differ between treatments

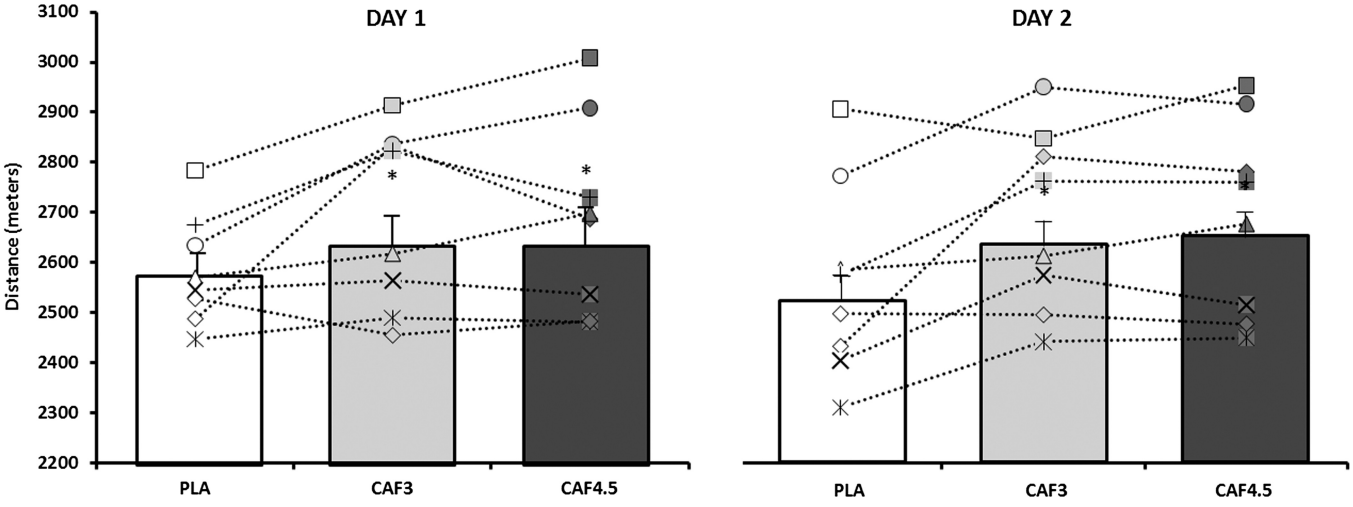

FIGURE 2—Mean and individual distances covered during the 2 d of the 10-min all-out C-PT after consuming PLA, CAF3, or CAF4.5. Values are mean ± SEM. *Significantly different from PLA ($P < 0.05$).

on any of the two testing days (PLA, 618 ± 42 and 638 ± 32; CAF3, 625 ± 32 and 621 ± 22; and CAF4.5, 619 ± 26 and 623 ± 29). Mean speed was 15.5 ± 0.2 and 15.4 ± 0.4 $km \cdot h^{-1}$ for PLA days 1 and 2, respectively. After CAF ingestion, mean speed increased to 16.2 ± 0.4 $km \cdot h^{-1}$ for both CAF3 and CAF4.5 on day 1 whereas for day 2, the same average speed was observed for CAF4.5 and a small decrease to 16.1 ± 0.4 $km \cdot h^{-1}$ was observed for CAF3.

HR and $\dot{V}O_2$. Mean HR was higher when subjects performed the all-out test after CAF consumption (Fig. 3). Mean HR (bpm) during days 1 and 2 were 180 ± 3 and 180 ± 4 bpm for PLA. During CAF trials, higher average HR were observed for both CAF3 (184 ± 3 bpm) and CAF4.5 (185 ± 3 bpm) (Fig. 3) on day 1. The same trend was observed on the second testing day, with both CAF treatments having an average HR 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 with that for 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 with PLA trials, and seven of eight subjects set new $HR_{max\text{-}pol}$ values (Fig. 3).

Blood values. Blood concentrations of lactate and glucose were higher after finishing the 10-min all-out C-PT after CAF consumption compared with those after PLA consumption. Similar results were observed for adrenaline values on the first testing day (Table 1). No difference in CK concentration was observed upon arrival or after finishing the 10-min all-out C-PT day 1 between treatments. However, a higher CK concentration was observed on day 2 in both CAF trials compared with that in PLA upon arrival and after finishing the performance test.

Resting measurements and metabolism during standardized warm-up (incremental tests). No significant difference in resting HR (54–58 bpm), blood lactate concentration, or glucose concentration among treatments before 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 concentration, glucose concentration, or $\dot{V}E$ between treatments (Fig. 4). However, lower RPE and higher lactate concentration at the last workload (65% of $\dot{V}O_{2peak\text{-}pol}$) were observed on the first testing day after consuming CAF 3 or 4.5 $mg \cdot kg^{-1}$ compared with those after consuming PLA. There were no observed difference for these parameters on the second testing day.

Questionnaires. Motivation was high before all trials of PLA (78 ± 5, 82 ± 5 = "very high"), CAF3 (87 ± 4,

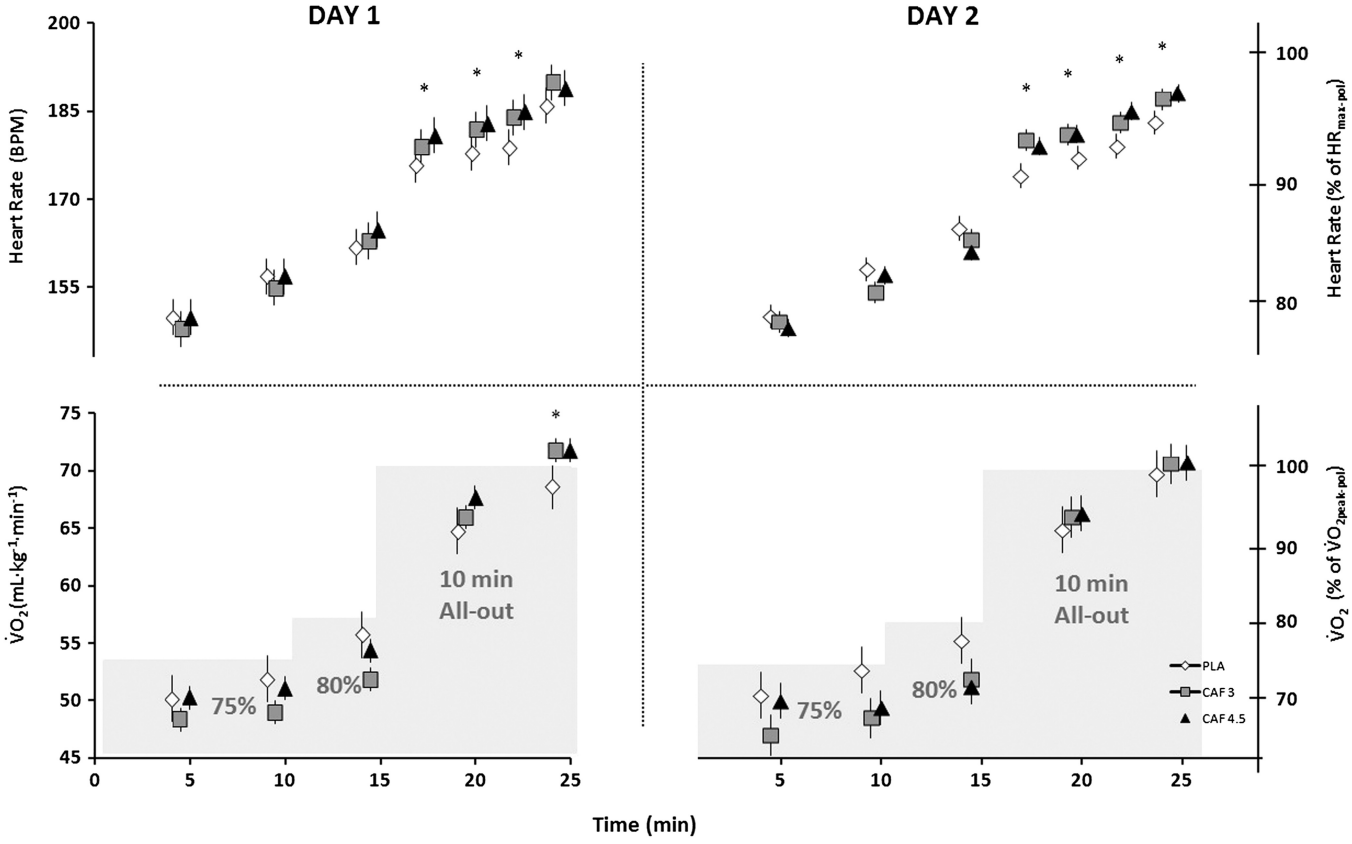

FIGURE 3—HR and $\dot{V}O_2$ response after consuming PLA, CAF3, and CAF4.5 during the whole performance test 15-min and 10-min all-out C-PT. Values are mean ± SEM. *Significantly different from those in PLA ($P < 0.05$).

TABLE 1. Mean speed, blood lactate concentration, glucose concentration, muscular arm pain, and plasma concentrations of CAF, adrenaline, and CK before starting and after finishing the 15-min and 10-min all-out C-PT.

		Day 1			Day 2		
Time Point	**Variable**	**PLA**	**CAF3**	**CAF4.5**	**PLA**	**CAF3**	**CAF4.5**
C-PT finished	Mean speed ($km \cdot h^{-1}$)	15.5 ± 0.2	16.2 ± 0.4*	16.2 ± 0.4*	15.4 ± 0.4	16.1 ± 0.4*	16.2 ± 0.4*
Arrival	CAF (μM)	Not measured	Not measured	Not measured	0.6 ± 0.2	0.7 ± 0.1*	0.9 ± 0.3*
C-PT finished	CAF (μM)	0.5 ± 0.2	19.7 ± 0.8*	28.6 ± 1.3*	0.6 ± 0.3	18.1 ± 1.8*	30.5 ± 0.8*
Pre-start C-PT	Lactate concentration (mM)	1.1 ± 0.2	1.4 ± 0.6	1.6 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.5 ± 0.2
C-PT finished	Lactate concentration (mM)	6.2 ± 0.2	7.7 ± 0.2*	7.5 ± 0.3*	6.4 ± 0.6	7.0 ± 0.3*	7.2 ± 0.4*
Pre-start C-PT	Adrenaline concentration (nM)	0.62 ± 0.07	0.45 ± 0.27	0.42 ± 0.02*	0.48 ± 0.08	0.35 ± 0.32	0.36 ± 0.22
C-PT finished	Adrenaline concentration (nM)	1.39 ± 0.17	3.02 ± 0.02*	2.90 ± 0.04*	1.90 ± 0.36	2.38 ± 0.24	2.22 ± 0.51
Arrival	CK concentration (U/L)	236.4 ± 37.8	253.5 ± 26.2	246.1 ± 26.6	253.8 ± 29.4	365.1 ± 65.2*,**	319.4 ± 46.7*,**
C-PT finished	CK concentration (U/L)	301.8 ± 39.4	317.0 ± 36.4	317.9 ± 26.2	323.3 ± 28.5	450.6 ± 71.4*,**	398.9 ± 53.6*,**
Arrival	Muscular pain in 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*
C-PT finished	Muscular pain in 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
Pre-start C-PT	Glucose concentration (mM)	5.4 ± 0.2	5.4 ± 0.1	5.6 ± 0.2	6.2 ± 1.1	5.3 ± 0.2	5.3 ± 0.1
C-PT finished	Glucose concentration (mM)	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 that in PLA ($P < 0.05$).
**Significantly different from value on day 1 ($P < 0.05$).

80 ± 4 = "very high"), and CAF4.5 (80 ± 6, 82 ± 5 = "very high"). Subjects also reported similar day forms before 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 on the second day compared with that in 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 CAF-containing products during the last 48 h before the C-PT showed that subjects had followed the instructions given. No difference in quality (5.2–6.3 = "good sleep") or sleep amount (7.2–8.4 h) before PLA, CAF3, or CAF4.5 was observed before either testing days.

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 $mL \cdot kg^{-1} \cdot min^{-1}$), 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 bpm), blood glucose concentration, or lactate concentration were observed between treatments before subjects started the performance test on any one of the two testing days (Table 1).

Comparing oxygen uptake when DP and running. Maximal oxygen uptake was higher when running compared to that while DP. The highest HR achieved during the different $\dot{V}O_{2max}$ tests was higher when running compared with that when DP. 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 premeasurements (September) to postmeasurements (October).

DISCUSSION

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

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

Higher exercise intensity and performance, as observed in the present study after CAF ingestion on day 1, 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 ultradistance marathon running and strength training (6). Interestingly, we observed that CK concentration was higher on arrival on the second day after CAF testing on day 1 compared with that in PLA. Also, it has recently been reported that CAF ingestion resulted in increased oxidative stress markers (interleukin 6 and 10) after a 15-km running competition compared with that in PLA (34). It was therefore somewhat unexpected that subjects had improved performance on the second testing day as observed on day 1. On average, performance was improved by 5.0% and 5.1% on day 2 after ingesting CAF 3 and 4.5 $mg \cdot kg^{-1}$, respectively, compared with that after ingesting PLA, but no difference was observed between performance on days 1 and 2. No difference in CK concentration was observed before or after finishing the C-PT day 1. Higher CK values upon arrival on day 2 after CAF testing on day 1 therefore presumably came as a result of the improved exercise intensity.

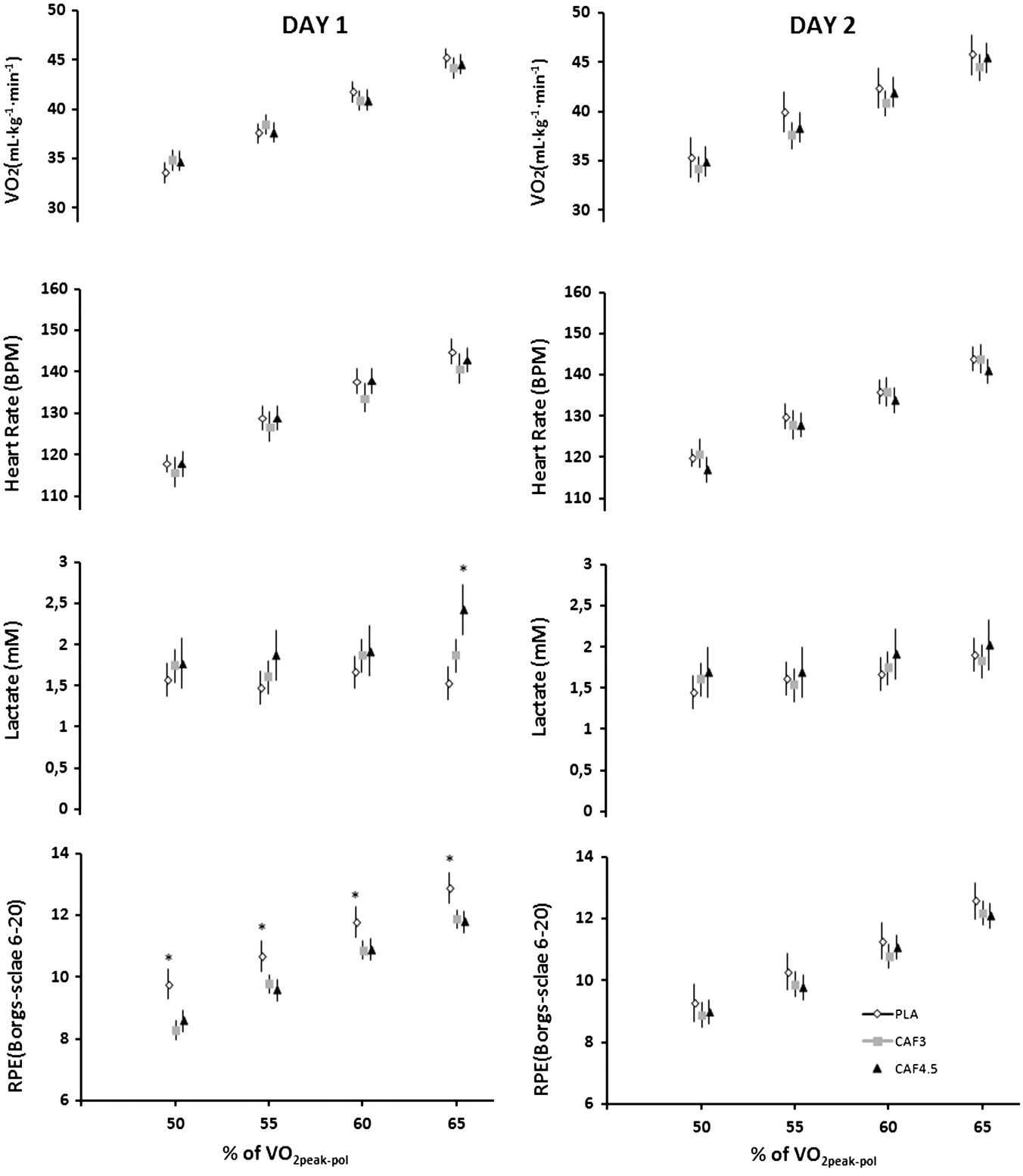

FIGURE 4—$\dot{V}O_2$ uptake, HR, lactate concentration, and RPE as a function of increasing workload during the standardized warm-up (submaximal incremental exercise) after consuming PLA, CAF3, and CAF4.5. Values are mean ± SEM. *Significantly different from PLA ($P < 0.05$).

Higher muscular pain in the arms was also reported in the CAF groups compared with that in PLA before starting C-PT day 2 (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 that the performance on the second

day was not affected by higher CK concentration or muscular soreness.

On the basis of available literature, the actions of CAF ingestion improving performance seems to be multifunctional (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 on day 1 during the standardized warm-up. This was, however, not observed on the second consecutive day, maybe because of higher muscular damage, resulting in higher muscular pain from the improved exercise intensity on day 1 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.

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 because of the breakdown and use of adenosine triphosphate. This means that even if CAF ingestion allowed higher discomfort because of inhibition of adenosine receptors, it would not explain why subjects were able to produce more energy to maintain the increased exercise intensity.

Our plasma CAF concentrations of approximately 18 μM (3 mg) and approximately 30 μM (4.5 mg) 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 min (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 heartbeat 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 arteriovenous difference is unchanged (3,29). Ivy et al. (22) observed that after CAF ingestion, subjects were able to produce a higher average power, which was associated with both higher HR and oxygen consumption compared with that after PLA ingestion during a cycling performance test. In the present study, higher HR was associated with improved work intensity during the 10-min all-out C-PT after CAF consumption on both days (Fig. 3).$\dot{V}O_2$ was also higher on day 1 after subjects ingested CAF doses compared with that after PLA ingestion, but only a tendency was observed for 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, in addition, 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. Impressively, all subjects (8/8) set new $\dot{V}O_{2peak\text{-}pol}$, and seven of eight subjects, new $HR_{max\text{-}pol}$ during CAF 10-min all-out C-PT. Posttesting 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-PT showed no difference from premeasurements. Furthermore, similar results were observed for $HR_{max\text{-}pol}$.

Indeed, a higher average power per stroke while DP had to be produced after CAF ingestion on both days because the number of strokes used to complete the 10-min all-out C-PT was similar between treatments on 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. (2), subjects improved the number of repetitions until exhaustion at 80% of individual one-repetition maximum bench press. 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 of the arm muscles has so far not been reported (38). However, it is still possible that CAF ingestion improved contractility qualities of exercising muscles because of improved or more efficient muscle recruitment. This was, however, not measured in the study, and on the basis of our results, work economy or efficiency during submaximal exercise was not improved by CAF ingestion.

Results from the present study are of great interest for sports performance because 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 on day 1 and day 2 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 CAF on sports performance when performing for two consecutive days in a row is also of high relevance because many sports have competitions lasting several days.

CONCLUSIONS

Ingestion of either CAF 3 and 4.5 $mg \cdot kg^{-1}$ improved performance for eight elite cross-country skiers compared with PLA during a 10-min all-out performance test in C-PT over two consecutive days. The improvement in performance

was 4.0% for both CAF doses on the first day and 5.0% and 5.1% on day 2 for CAF3 and CAF4.5, respectively. Furthermore, seven of eight test subjects improved performance after ingesting CAF compared with that after ingesting PLA. Results show the improvement in performance came as a result of subjects' increasing average speed, which was associated with higher HR, $\dot{V}O_2$, lactate concentration, and adrenaline concentration during the 10-min all-out test after CAF ingestion. Interestingly, performance with intake of CAF was the same for both competing days, although subjects reported higher muscular pain in the arms and had higher CK values on arrival on the second day in the CAF groups. On the basis of our results, CAF may indeed assist in maintaining performance quality for athletes competing for consecutive days in real-life competitions.

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