Caffeine increases exercise performance, maximal oxygen uptake and oxygen deficit in elite male endurance athletes

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Abstract

Caffeine increases endurance performance but the physiological mechanisms improving high intensity endurance capacity are not well characterised. **The aim** of the present study was to test the hypothesis that caffeine increases maximal oxygen uptake (VO_{2max}), and to characterise the physiological mechanisms underpinning improved high intensity endurance capacity. **Method**: 23 elite endurance trained male athletes were tested twice with and twice without caffeine (four tests) in a randomized, double-blinded and placebo-controlled study with cross-over-design. Caffeine (4.5 $mg \cdot kg^{-1}$) or placebo was consumed 45 min before standardized warm-up. Time-to-exhaustion during an incremental test (running 10.5º incline, start speed 10.0 $km \cdot h^{-1}$, and 0.5 $km \cdot h^{-1}$ increase in speed every 30 s) determined performance. Oxygen uptake was measured continuously to determine VO_{2max} and O_2-deficit was calculated. **Results**: Caffeine increased time-to-exhaustion from 355±41 to 375±41 s (Δ19.4±16.5 s; $p<0.001$). Importantly, caffeine increased VO_{2max} from 75.8±5.6 to 76.7±6.0 $ml \cdot kg^{-1} \cdot min^{-1}$ (Δ 0.9±1.7 $ml \cdot kg^{-1} \cdot min^{-1}$; $p<0.003$). Caffeine increased maximal heart rate (HR_{peak}) and ventilation (VE_{peak}). Caffeine increased O_2-deficit from 63.1±18.2 to 69.5±17.5 $ml \cdot kg^{-1}$ ($p<0.02$) and blood lactate compared to placebo. The increase in time-to-exhaustion after caffeine ingestion was reduced to 11.7 s after adjustment for the increase in VO_{2max}. Caffeine did not significantly increase VO_{2max} after adjustment for VE_{peak} and HR_{peak}. Adjustment for O_2-deficit and lactate explained 6.2 s of the caffeine-induced increase in time-to-exhaustion. The increase in VO_{2max}, ventilation, heart rate, O_2-deficit and lactate explained 63% of the increased performance after caffeine intake.

Conclusion: Caffeine increased VO_{2max} in elite athletes, which contributed to improvement in high intensity endurance performance. Increases in O_2-deficit and lactate, also contributed to the caffeine-induced improvement in endurance performance.

Keywords: Exercise performance, oxygen consumption, heart rate, oxygen deficit and lactate

Introduction

Caffeine ingestion improves endurance performance of both short and longer duration (1-5), and whether the performance is measured as time-to-exhaustion (1;6) or time trial (2;7;8). Importantly, caffeine reduces rate of perceived exertion (RPE) at standard loads (9;10). Caffeine also increases anaerobic capacity and power (11;12) and lactate accumulation is higher after maximal effort exercise such as during time trials or time-to-exhaustion exercise (2;4;9;13).

The higher performance at time trials after caffeine intake requires higher power production and is associated with higher heart rate (HR) and ventilation (VE) (2-4;9;14). The higher workload following caffeine ingestion also elevates cardiac output and increases oxygen uptake (8;9;13;15-18). It seems likely that caffeine improves performance, at least partly, via inhibition of adenosine receptors (4;19). However, adenosine receptors are expressed in most tissues including brain, heart, muscles, blood vessels, and lungs (20). Therefore, caffeine-induced inhibition of adenosine receptors can theoretically affect several physiological mechanisms contributing to improved endurance performance.

Maximal oxygen uptake (VO_{2max}) represents the integrated capacity of the pulmonary, cardiovascular and muscle systems to take up, transport and utilize oxygen (21-23) and VO_{2max} is a major determinant of endurance capacity. Although test protocols for reaching VO_{2max} has been a topic of controversy since introduced in the 1920s (24;25) there is broad agreement that VO_{2max} determines endurance capacity. It is generally accepted that VO_{2max} is reached during an incremental protocol of 4-8 min duration after warm-up (23;26-29). Importantly, the same protocol can also be used to test performance and measure maximal ventilation (VE_{max}), maximal heart rate (HR_{max},) and O_2-deficit (23).

Maximal oxygen uptake seems under most circumstances to be limited by the capacity to transport oxygen to the working muscles (21;24;30). At sea level, cardiac output (Qc) and

the blood volume (or total hemoglobin mass) restrict VO_{2max} in most people (30). Indirectly, this is also supported by the fact that elite endurance athletes have high Qc and total hemoglobin mass (30-32). However, there are indications that arterial O_2-desaturation occurs in elite athletes during maximal aerobic exercise supporting a pulmonary limitation of maximal oxygen uptake (21;33-37). In support of this idea, it has been shown that breathing O_2 enriched air (26% vs 21% O_2) prevented O_2-desaturation and increased VO_{2max} in highly endurance trained athletes (36). Caffeine increases maximal ventilation and heart rate (17), which raises the possibility that caffeine may also increase maximal oxygen uptake in elite athletes.

Recently, we observed that professional cross-country skiers obtained higher maximal oxygen uptake during a 10-min double-poling time trial after intake of caffeine compared to maximal oxygen uptake during an incremental test without caffeine intake (9). The higher maximal oxygen uptake after caffeine ingestion was associated with both higher VE_{max} and HR_{max} (9). However, caffeine is not believed to increase maximal oxygen uptake (3;17). Furthermore, maximal oxygen uptake during double poling was found to be ~10 % lower than during running (9). Therefore, it remains unknown whether caffeine increases maximal oxygen uptake.

The present study was designed to test the hypothesis that caffeine increases VO_{2max} in elite endurance athletes during running. The incremental protocol used to determine maximal oxygen uptake, also was used to assess time to exhaustion (performance). VE_{max}, HR_{max}, O_2-deficit, and blood lactate, in addition to $VO_{2max,}$ were determine in order to assess their influence over any observed caffeine-induced improvement in endurance performance.

Materials and Methods

Subjects: Twenty-three healthy male endurance trained athletes (cross-country skiing, running and triathlon), gave their written consent to participate in the study after being informed of the purposes of the study and risks involved. The study was reviewed by the Regional Ethics Commit (REK sør-øst B; 2011/2554) concluding that approval from REK was not required in order to perform the study as described. The study was conducted according to the Declaration of Helsinki. Physical characteristics (mean ± SD) of the participants were; age 24.0 ± 1.0 (years), height 182.1 ± 1.3 (cm), weight 73.0 ± 1.6 (kg), VO_{2max} running (VO_{2max}) 75.9 ± 5.8 $ml \cdot kg^{-1} \cdot min^{-1}$ at the pre-test. Inclusion criteria were that all subjects were male, with a VO_{2max} above 65 $ml \cdot kg^{-1} \cdot min^{-1}$, and training competitively to qualify for national or international endurance competitions the upcoming season.

Experimental Procedures: The study was conducted using a randomized double-blinded, placebo-controlled, cross-over-design. Before the main VO_{2max} performance testing started, each participant performed a pre-test for familiarization with the testing procedure, and to verify all subjects had VO_{2max} above 65 $ml \cdot kg^{-1} \cdot min^{-1}$. A schematic overview of the study is shown in Figure 1. The study had one dropout because of illness.

Pre-test: During the pre-test all subjects performed a standardized incremental treadmill test consisting of four workloads at 7, 8, 9 and 10 $km \cdot h^{-1}$ with each lasting five minutes. All workloads were performed with 10.5° uphill incline on the treadmill (Woodway, Weil am Rein, Germany), and a 1-minute break was given between each workload. Oxygen uptake at the four workloads were then used to estimate the individual oxygen cost for calculation of O_2-deficit during the VO_{2max} performance tests as previously described by Medbø et al. (38). Linear regression was also used to calculate individual speeds equal to 55, 60, 65 and 70 % of VO_{2max} performed as a standardized warm-up (incremental test) before each main VO_{2max} performance test. When the standardized warm-up was finished, all

74 subjects walked 5 min at 5 km·h^{-1}, before starting the pre-VO_{2max} test. Starting velocity during
75 all testing was 10 km·h^{-1} with an uphill incline of 10.5° on the treadmill. The VO_{2max}
76 performance tests was performed as an incremental test where velocity was increased by 0.5
77 km·h^{-1} every 30 s until subjects were unable to maintain the speed and stepped/jumped off the
78 treadmill. The highest HR and VE during the test were defined as HR_{peak} and VE_{peak}. Criteria
79 for having reached VO_{2max} during all testing were: 1) voluntary exhaustion, 2) oxygen
80 consumption plateaued, meaning VO_2 increased less than 1 ml·kg^{-1}·min^{-1} when there were
81 consecutive increases in treadmill speed of 0.5 km·h^{-1}, 3) respiratory exchange ratio (RER) >
82 1.10 and 4) blood lactate > 7.0 mM. The VO_{2max} was calculated as the average of the two
83 highest 30 s measurements.

84 **VO_{2max} performance tests**: To test the effect of caffeine on $VO_{2max,}$ each subject
85 completed four tests over a two-week period. During both weeks, one test was performed with
86 caffeine and the other with placebo in a randomized order. Before all main tests, resting HR
87 and lung function (described below) were measured at arrival, and 30 minutes after
88 consuming either placebo or caffeine. After finishing lung function testing subjects were
89 given a 10-minute break before starting the standardized warm-up. The warm-up consisted of
90 four workloads (55, 60, 65 and 70 % of VO_{2max}) each lasting 5 minutes, with a 1-minute break
91 in between when blood glucose and lactate was measured. All workloads of the warm-up
92 were performed with a 10.5° uphill incline on the treadmill (Woodway, Weil am Rein,
93 Germany). During each workload HR, VO_2 and RER were measured as means between the 3-
94 4.5 min of each workload. Subjective rating of perceived exertion (RPE) was evaluated
95 according to the Borg-scale (6 to 20) (39). Following the warm-up, a 5-min break was used
96 for blood sampling (pre-sample) for determination of lactate and glucose, recording HR and
97 providing final instructions to the subjects. The goal for each subject was to run for as long as
98 possible during each VO_{2max} performance test. Performance was measured as time to

exhaustion. Participants did not receive information regarding time, velocity or physiological measurements during the tests. The criteria for reaching VO_{2max} was as described above for the pre-test. Encouragement was given during all tests from the test leader, who was blinded to treatment to eliminate any bias. Maximal oxygen uptake was measured in $ml \cdot min^{-1}$. Elite athletes are normally weight stable, and therefore the same weight was used at the four tests for calculation of VO_{2max} in $ml \cdot kg^{-1} \cdot min^{-1}$. After finishing the VO_{2max} performance tests, the subjects were given a 5-min break before taking post-exercise measurements of lung function and filling out questionnaires.

Measurement and calculation of O_2-deficit: During the-standardized incremental tests, VO_2 was measured between 2.5-4.5 min of each 5 min work period, and the mean determined as a subject`s oxygen cost for the velocity. Oxygen uptake at the four velocities tested (7, 8, 9 and 10 $km \cdot h^{-1}$) during the sub-maximal exercise was used to construct a linear regression estimate of oxygen cost for the different running velocities used during the VO_{2max} performance tests as previously described by Medbø et al. (38). Collection of expired air started 15 seconds before starting the VO_{2max} performance tests and was continued until subjects reached task failure (when they stepped off the treadmill). The difference in estimated oxygen cost, and measured oxygen uptake was then calculated as subjects` O_2-deficit. In the present study, O_2-defecit was not adjusted for the contribution of the body's oxygen stores to the energy supply.

Measurement of VO_2 and ventilation: Oxygen consumption and RER were measured with an Oxycon Pro metabolic system (Jaeger Hochberg, Germany). Before each test the Oxygen Pro was calibrated with a gas mixture composed of O_2 and CO_2 (14.93% O_2 and 5.99 % CO_2) and normal air (20.90 % O_2 and 0.04 % CO_2). Volume was calibrated manually using a pump containing 3 liters of volume (Calibration Syringe, Series 5530, Hans Rudolph Inc., MO, USA). During all testing, expired air was collected using a mouth V2-

mask (Hans Rudolph Instr., USA) in combination with a nose clip and directed into a mixing chamber (Oxycon Pro) and analyzed with a turbine (Triple V volume transducer). Both the hose and V2-mask were tested for leakage prior to each individual test. Heart rate (HR) was measured using a HR monitor (Polar RS 800, Finland), were the error of measurement as stated by the company is ± 1 %.

Lung function: Spirometry was measured by maximum expiratory flow volume loops according to guidelines from the European Respiratory Society (ERS) (40), and recorded as forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC) and forced expiratory flow in 50 % of FVC (FEF_{50}). Lung function measurements were performed using a MasterScreen Pneumo Jaeger® (Würzburg, Germany) and reference values used are according to Quanjer et al. (41).

Fractional exhaled Nitric Oxide (FE_{NO}): FE_{NO} was measured by the single breath online technique according to American Thoracic Society (ATS)/ERS guidelines (40). The subject was in a seated position and instructed to breathe quietly. To avoid potential contamination from ambient NO, the subjects inhaled NO-free air close to total lung capacity, immediately followed by a full exhalation for at least 6 seconds at a constant flow of 50 $ml \cdot s^{-1}$. The constant flow rate was maintained with the aid of a visual feedback system. The expiratory pressure was kept between 5-20 mmHg to close the soft palate and eliminate nasal NO. FE_{NO} measurements were assessed prior to pulmonary function tests and were recorded as a mean value from three successive reproducible plateaus. A chemiluminescence analyzer, EcoMedics CLD 88 Exhalyzer® (Eco Medics AG, Duerten, Switzerland) (measurement range of 0.1-5000 parts per billion (ppb) was used and calibrated daily with a certified concentration of NO.

Pre-testing information: All subjects were informed to only perform light training for the 48 hours preceding each VO_{2max} performance test. To minimize variation in pre-

exercise glycogen stores, diet and exercise diaries were used to standardize food intake and training for each subject. Subjects were asked to prepare for the tests as they prepared for competitions. They were instructed to follow the same training and diet regime before each test, and to refrain from caffeine the last 24 h before each test day. Seven out of the 23 subjects in the study had a high intake of caffeine products on a daily basis (>150 mg). On each of the four main test days, subjects arrived at the laboratory at the same time (±15 min) of the day for each of their tests. The first two tests were performed with a washout period of three days between them. Before test three, a washout period of four days was imposed and subjects performed test three and four the following week on the same weekdays as tests one and two.

Blood analyses: Capillary blood samples for measurements of glucose and lactate were taken from the fingertip after skin puncture using a Saft-T-Pro Plus (Accu-Check, Mannheim, Germany). For measurement of blood lactate blood samples were drawn into a 50 µl capillary tube, and 20 µl pipetted into the YSI 1500 SPORT analyzer (Yellow Springs Instruments Life Sciences, Yellow Springs, OH). The analyzer was calibrated with a 5.0 mM lactate stock solution before each test. Values between 4.95 mM and 5.05 mM were accepted. Capillary blood glucose was measured with a HemoCue Glucose 201+ analyzer (HemoCue Glucose 201+, Ängelholm, Sweden) as previously described (42)

Caffeine and placebo intake: Caffeine (Coffeinum, Oslo Apotekerproduksjon, Oslo, Norway) was dissolved in a cordial concentrate (Fun Light) at 3 mg/ml concentration at the Norwegian School of Sports Sciences. Ingestion of caffeine (4.5 $mg \cdot kg^{-1}$) or placebo (Fun Light without additions; indistinguishable from caffeine) occurred 45 minutes before the standardized warm-up. Therefore, the VO_{2max} performance test started 75 min after caffeine ingestion.

Questionnaires: Questionnaires were used to evaluate motivation and "current fitness" using a scale from 1-100 (9). Sleep habits was evaluated by asking approximate sleep duration (hours) the 24 hours prior to each test. In addition, for each trial subjects were asked what product they believed they had received 30 min after ingestion and again before leaving the laboratory.

Statistical analysis: All data are presented as means ± SD. A two-way ANOVA for repeated measures was used to examine differences in HR, lactate, VO_2, glucose, and RPE during 2 submaximal workloads between the two treatments. If treatment differences were observed, a paired t-test was used to test differences at workloads. In exploratory analyses, multiple linear regression was used to disentangle if any caffeine effect on performance could be explained by changes in VO_{2max}, HR_{max}, VE_{max}, O_2-deficit or blood lactate by sequentially adjusting for each of these variables. Similarly, we examined to what extent the caffeine effect on VO_{2max} could be explained by HR_{max}, VE_{max}, O_2-deficit and blood lactate.

Results

Caffeine improved time-to-voluntary exhaustion (performance) in both testing weeks (Table 1). In the first week, caffeine increased time to exhaustion by 18 s (355 ± 41 vs. 373 ± 40 s, p <0.001) compared to placebo, and in the second week by 21 s (355 ± 44 vs. 376 ± 45 s, p <0.001). The average effect was 19.4 s (5.45%; P<0.001; Figure 2A; Table 1). Time to exhaustion was highly reproducible, with no statistical differences between the two placebo trials (p=0.78) or the two caffeine trials (p=0.74). The intra-class correlation coefficients (ICC) for time-to-exhaustion were 0.94 and 0.90 in placebo and caffeine trials, respectively.

Caffeine ingestion also increased mean maximal oxygen uptake from 75.8±5.6 to 76.7±6.0 $ml \cdot kg^{-1} \cdot min^{-1}$ (0.9 $ml \cdot kg^{-1} \cdot min^{-1}$; 1.2%; p<0.003) compared to placebo (Table 1; Figure 2B). Intraclass correlation coefficient (ICC) values for VO_{2max} were > 0.95 for both

198 conditions. The O_2-kinetics were similar between caffeine and placebo except when
199 comparing the last minute where higher VO_{2max} was reached with caffeine (Figure 3). The
200 higher VO_{2max} after caffeine ingestion contributed to the longer running time during the
201 performance test, since statistical adjustment for VO_{2max} reduced the caffeine-induced effect
202 on running time from 19.4 s to 15.4 s (21% attenuation).

203 Heart rate and ventilation developed similarly during the performance tests with and
204 without caffeine (Figure 3). However, higher maximal HR and VE values were reached
205 during the last minute of the test after caffeine ingestion compared to placebo. Specifically,
206 HR_{peak} increased from 191 ± 8 to 193 ± 9 beats·min^{-1} ($p< 0.001$), and maximal ventilation
207 (VE_{peak}) increased from 187.8 ± 17.8 to 192.2 ± 15.3 L·min^{-1} ($p<0.001$) after caffeine
208 ingestion compared to placebo (Table 1). The caffeine-induced increase in VO_{2max} was
209 attenuated by 0.7 ml·kg^{-1}·min^{-1} ($p<0.001$) after adjustment for the increase in HR_{peak}. When
210 VO_{2max} was adjusted for VE_{peak}, the effect of caffeine on VO_{2max} decreased by about 50% and
211 was no longer significant ($p=0.11$). Despite a higher VE_{peak} after caffeine ingestion, breathing
212 frequency (BF) was not significantly elevated when VO_{2max} was achieved (60 ± 7 vs 59 ± 9
213 breaths·min^{-1}; Table 1). When running duration was adjusted for VO_{2max}, VE_{peak} and HR_{peak}
214 there was still 11.7 s ($p<0.001$) an improvement in time to exhaustion after caffeine ingestion
215 (40% attenuation).

216 The accumulated oxygen deficit during the performance test increased from 63.1±18.2
217 ml·kg^{-1} in placebo to 69.5±17.5 ml·kg^{-1} with caffeine ingestion ($p<0.02$; Table 1; Figure 2C).
218 ICC for measurements of O_2-deficit was 0.61 and 0.64, respectively for placebo and caffeine
219 trials. Blood lactate values were higher with caffeine compared to placebo (8.54±1.02 vs 7.94
220 mM±1.06; $p<0.001$; Table 1). Calculations showed that the anaerobic processes (O_2-deficit)
221 covered 14.7±3.1 and 15.0±2.7% of total O_2-cost in placebo and caffeine trials. When time to
222 exhaustion was adjusted for both O_2-deficit and lactate concentration, the effect of caffeine

was reduced from 19.4 to 13.2 s ($p<0.001$). With additional adjustment for $VO_{2max,}$ the effect of caffeine on time to exhaustion was reduced to 8.0 s (59% attenuation), but still significant ($p<0.001$). With further adjustment for VE_{peak} and HR_{peak} the caffeine effect on performance was further reduced to 7.1 s (63% attenuation), but remained significant ($p = 0.003$). Plasma glucose after the performance tests were higher in caffeine compared to placebo trials (7.9±1.1 vs 7.3±0.9 mM; $p<0.001$). The highest RER during the performance test was independent of test conditions (Table 1).

During the submaximal incremental testing, repeated measures ANOVA showed that oxygen uptake, HR, VE, BF, RPE and blood lactate increased progressively from the first to the last of the four workloads (Table 2). HR and VO_2 at submaximal loads were similar after placebo and caffeine (treatment effect: $p=0.077$ for means of the two tests), whereas VE and lactate were higher after caffeine than placebo ingestion ($p<0.001$), but no significant interaction was observed. RPE was lower after caffeine ingestion compared to placebo (treatment effect: $p<0.029$; Table 2) with post hoc analyses showing lower RPE at the two highest workloads after caffeine.

The lung function measurements FEV_1, FVC, FEF_{50} and FE_{NO} performed at arrival, 30 minutes post placebo or caffeine ingestion, and post VO_{2max} performance tests were not different between treatments (Table 3).

Based on the questionnaire, there were no differences between caffeine and placebo trials regarding self-reported “current fitness” and motivation. Prior to the performance tests, subjects reported motivation of 77 ± 14, 79 ± 16 (placebo), and 76 ± 14, 76 ± 14 (caffeine) before tests (75 = high/very high), and 79 ± 17, 82 ± 12 (placebo), and 79 ± 14, 81 ± 14 (caffeine) after the performance tests. Ratings pre-test “current fitness” were 62 ± 11, 62 ± 13 (PLA), and 61 ± 13, 63 ± 12 (caffeine) (65 = high) before, and 62 ± 11, 65 ± 14 (placebo), and 67 ± 15, 79 ± 14 (caffeine) after the performance tests. Furthermore, the subjects were

unable to sense which product they received during the different trials, with 50% answering "uncertain" to the question. Of the subjects who answered that they thought they knew the treatment (caffeine or placebo), about 50% guessed wrong both pre and post testing independent of treatment ingestion. Hours of sleep, training, intake of food and liquid intake before tests also did not differ, confirming that subjects had followed instructions regarding training, food, liquid and caffeine consumption for the 48 h prior to each test.

Discussion

We confirmed the primary hypothesis that caffeine increases maximal oxygen uptake in elite endurance athletes. Caffeine also increased maximal heart rate and maximal ventilation, and the exploratory statistical analyses showed that both parameters contributed to the increase in VO_{2max}. The increase in VO_{2max} was small (1.2%), but explained about 4 s (~20%) of the improved performance (run time-to-exhaustion). Accumulated O_2-deficit and lactate during the performance test was also higher after intake of caffeine. Overall, these mechanisms accounted for 63% of the caffeine-mediated improvement in performance.

Recently, we observed that caffeine intake induced higher oxygen uptake during a 10-min time trial compared to maximal oxygen uptake during an incremental test without caffeine in professional cross-country skiers (9). The present study was designed to test the hypothesis that caffeine increases VO_{2max} in elite endurance athletes during running. The finding that caffeine increased VO_{2max} from 75.8±5.6 to 76.7±6.0 $ml \cdot kg^{-1} \cdot min^{-1}$ (1.2%) confirms our hypothesis. Caffeine is normally not believed to increase maximal oxygen uptake (3;17;43) and the small increase in VO_{2max} observed in this randomized placebo-controlled cross-over study may therefore be questioned despite it being highly significant (p-value of 0.003). However, several facts support that the increase is real. First, the 23 participants were tested twice with and twice without caffeine intake during two consecutive

weeks under standardized training and diet the days before all tests, and the effect was reproducible. Second, the participants were elite endurance athletes of national and international level (five participants are medalists in Olympic or U23 World Championship) accustomed to intense efforts, and the ICC for VO_{2max} were > 0.95 with and without caffeine. Third, more than 20% of the increase in running performance after caffeine intake was explained by the increase in VO_{2mas} according to our statistical analyses. Fourth, more than 50% of the increase in VO_{2max} could be explained by likely physiological mechanisms. Finally, the finding is also supported by our previous study showing higher maximal oxygen uptake during a 10 min double poling time-trial after intake of caffeine compared with VO_{2max} during an incremental test without caffeine (9), and recent studies reporting that caffeine increases VO_{2max} in moderately trained males (44) and mice (19).

The classical view is that VO_{2max} is determined by the delivery of oxygen to the active muscles, and therefore maximal cardiac output (HR · stroke volume) during running in healthy subjects (21;24). In agreement with other studies, HR_{peak} was 2 bpm higher during the caffeine trials (2), which would increase cardiac output, assuming stroke volume was maintained (24). Statistical analyses suggest the increased HR_{peak} explained 0.2 $ml \cdot kg^{-1} \cdot min^{-1}$ (22%) of the caffeine-induced increase in VO_{2max}. The heart expresses all four isoform of adenosine receptors (45) which are blocked by caffeine, and adenosine is used to treat supraventricular tachycardia (46). However, the role of adenosine receptors on heart rate is not completely clear (47). In the present study, caffeine did not influence heart rate at submaximal loads as expected (2;10). Intake of caffeine normally increases plasma concentrations of adrenaline and noradrenaline during maximal exercise (2;9), and stronger adrenergic stimulation may explain the higher heart rate after caffeine intake.

Elite endurance athletes often develop hypoxemia during maximal exercise (37;48;49). In the present study, several participants had VO_{2max} higher than 80 $ml \cdot kg^{-1} \cdot min^{-1}$, and

298 subjects with higher VO_{2max} have a greater oxygen desaturation upon reaching VO_{2max} than
299 less trained subjects (49). Several studies have found that reduced O_2-saturation can limit
300 maximal oxygen consumption for highly trained athletes due to arterial desaturation (33-
301 35;37;48). Limitation of O_2-saturation in elite endurance athletes is also supported by the fact
302 that mild hyperoxia (26 % O_2) increases VO_{2max} in highly endurance trained subjects but not
303 in moderately trained subjects (36). The higher maximal ventilation after caffeine, with
304 similar BF as the placebo trial, improves conditions for O_2-saturation. However, caffeine has
305 previously been reported to increase VE_{peak} during maximal exercise, without improving
306 VO_{2max} (34;48). The increased ventilation may also increase expiration of CO_2, and we have
307 previously found that plasma bicarbonate at exhaustion is lower after intake of caffeine
308 compared to placebo (18). However, the higher ventilation could also be driven by higher
309 central command.

310 Bronchioles express adenosine receptors, and adenosine contributes to physiological
311 and pathophysiological regulation of bronchoconstriction (50;51). In the present study,
312 caffeine increased VE_{peak}, as well as ventilation during submaximal intensities. The caffeine-
313 induced increase in ventilation at submaximal intensities is well-documented (2;10), and
314 could result from bronchodilation. In the present study, however, caffeine did not improve
315 forced expiratory volume during the first second (FEV_1), forced vital capacity (FVC) or
316 expiratory flow at 50 % of FVC (FEF_{50}), although it has been reported that caffeine causes a
317 small increase in FEV_1 (52). Interestingly, our statistical analyses supported the notion that
318 the increased ventilation after intake of caffeine contributed to the higher VO_{2max}. Adjustment
319 for the higher ventilation after caffeine intake, reduced the caffeine-induced increase in
320 VO_{2max} by 50% and the effect of caffeine on VO_{2max} was no longer significant ($p=0.11$),
321 suggesting ventilation *per se* is an important pathway by which VO_{2max} is increased by
322 caffeine in elite endurance athletes.

323 The incremental performance test was designed to optimally measure VO_{2max} and
324 lasted 355 s (5 min 55 s) during the placebo trial. Caffeine improved running duration by 19.4
325 s (5.5%) during the performance test in agreement with previous studies (2;7;9). When time-
326 to-exhaustion was adjusted for caffeine-mediated increase in VO_{2max}, VE_{peak} and $HR_{peak,}$
327 running duration was reduced from 19.4 to 11.7 s ($p<0.001$). These data suggest that
328 improved aerobic power explained nearly 40 % of the increased performance after intake of
329 caffeine.
330 The remaining improvements after caffeine compared to placebo, might be anaerobic
331 processes since exercise economy is not influenced by caffeine (Table 1). It is well-
332 documented that plasma lactate is higher at exhaustion after intake of caffeine (2;9). Although
333 plasma lactate is the byproduct from anaerobic glycolysis and an indirect measure of
334 anaerobic work, the higher lactate with caffeine intake supports larger anaerobic contribution.
335 In the present study, O_2-deficit was higher in caffeine than in placebo (69.5±17.5 versus
336 63.1±18.2 ml $\cdot kg^{-1}$). The magnitude of O_2-deficit agrees with previous studies (38), and
337 anaerobic processes accounted for ~15 % of the energy cost during the VO_{2max} time-to-
338 exhaustion performance test. Caffeine increases anaerobic work capacity during Wingate tests
339 and tests up to 6-7 minutes (11;53-55). Performance also has been reported higher after
340 caffeine intake during 4 km cycling time trials, in which anaerobic processes highly
341 contribute (53;55). Doherty reported that caffeine increased maximal accumulated oxygen
342 deficit by 11% in highly trained male athletes when running until exhaustion at ~125% of
343 VO_{2max} (13). These results are very comparable to results in the present study where 10 %
344 increase in O_2-deficit was observed. Although aerobic energy production contributed to most
345 of the energy requirement in the present study, there is no doubt that accumulated oxygen
346 deficit and lactate were key physiological components in delaying development of fatigue at
347 the end of the incremental test.

348 The mechanisms by which caffeine increases anaerobic capacity is not clear. It is well-
349 documented that caffeine reduces rate of perceived exertion at submaximal load (4;9;10), and
350 a common explanation is that caffeine increases performance simply by reducing pain and
351 discomfort. However, it has been reported that caffeine intake reduces interstitial potassium
352 during high intensity exercise (56) and improved potassium handling may improve
353 performance (57). This effect of caffeine could be indirectly on muscles or via elevated
354 adrenaline concentration. In the present study, statistical adjustments for O_2-deficit and lactate
355 reduced the caffeine-mediated improvement in performance from 19.4 to 13.2 s (~30%;
356 $p<0.001$). Therefore, these results show that higher O_2-deficit and lactate are contributing
357 physiological factors to the improved running duration during the VO_{2max} performance test.
358 Caffeine has well-defined effects at the molecular level and caffeine is an adenosine
359 receptor antagonist, inhibits phosphodiesterase, inhibits PI-3 kinase, inhibits glycogen
360 phosphorylase a, and stimulates Ca^{2+}-release from sarcoplasmic reticulum at high
361 concentrations (20;58;59). Data from our previous studies suggest that plasma caffeine
362 concentration was ~30 µM (9), and this concentration inhibits most adenosine receptors (20).
363 However, this knowledge may be of limited importance for understanding of the
364 physiological effects of caffeine on performance as adenosine receptors are expressed broadly
365 throughout the human body. A consistent finding is that caffeine reduces RPE, which will
366 allow higher work capacity. The mechanisms are unclear, but blocking adenosine receptors
367 reduces pain (60;61). The reduced pain sensation may increase effort and performance, which
368 again will drive higher heart rate. However, caffeine also improves ventilation (10;33), which
369 will reduce hypoxemia and therefore increase performance.
370 Caffeine influences a number of tissues and physiological processes, which
371 collectively improves performance. Our data show that caffeine appears to increase both
372 aerobic and anaerobic capacity during the ~6 min time-to-exhaustion test. Statistical analyses

with sequential adjustment suggest that the higher aerobic capacity contributed an additional 7 s whereas anaerobic processes contributed and additional 6 s of the 19.4 s improvement in performance. Interestingly, adjustment for the increases in VO_{2max}, ventilation, heart rate, O_2-deficit and lactate reduced the improvement in performance (running time) to 7 s. Therefore, we are able to explain ~63 % of the effect via plausible physiological mechanisms for the caffeine-mediated increase in performance during the ~6 min performance test.

The strength of the present study is that the performance protocol was designed to test maximal oxygen uptake and the tests with and without caffeine were performed twice. It is also a strength that the study was performed in highly endurance trained subjects accustomed to exhaustive exercise. Another strength is that the effects of caffeine on both aerobic and anaerobic capacities were examined. However, one limitation of the study is that we did not directly measure cardiac output and O_2-saturation in arterial blood, since we suggest that these two mechanisms contribute to the performance enhancing effect of caffeine. However, measurements of maximal cardiac output during maximal exercise is challenging. It would also have been interesting to measure blood levels of CO_2 and bicarbonate to investigate if the increased ventilation reduced CO_2. However, the increase in VO_{2max} was only 1.2%, which makes it difficult to determine the mechanisms by which caffeine increases maximal oxygen uptake.

In conclusion, the present study shows for the first time that caffeine increases VO_{2max} in elite athletes, which contributed significantly to improving time to exhaustion during a high intensity performance test. Our data suggest that increased ventilation and maximal heart rate contribute to the higher VO_{2max}. Caffeine also increased O_2-deficit and lactate at exhaustion, which contributed to improved performance. The present study shows that caffeine improves several physiological mechanisms, which collectively contributes to significant improvement in high intensity endurance performance.

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Legends

Figure 1. Experimental design. A: Top line shows pre-tests and main testing during the 3 weeks used to complete the VO_{2max} test for one subject. B: The bottom figure shows the test procedure for all VO_{2max} performance tests. Prior to the VO_{2max} test, subjects performed a standardized warm-up (incremental test) consisting of four intensities all lasting five minutes.

Figure 2. Effect of caffeine on time-to-exhaustion, maximal oxygen uptake and oxygen-deficit. A) Individual and mean time-to-exhaustion at the performance test. duration, VO_{2max} and O_2-deficit obtain during the VO_{2max} performance tests after placebo (open symbols) or caffeine (filled symbols). B) Percent change in running duration, VO_{2max} and O_2-deficit following caffeine consumption compared to placebo for each subject. Values are listed as means ± SD. * Significant different from placebo trials ($p<0.05$)

Figure 3: A) The 30 s measurements for VO_2, HR, VE, BF and RER during placebo (open symbols) and caffeine (filled symbols) VO_{2max} performance tests B) The last 120 seconds for each individual shown as mean for the group for VO_2, HR, VE, BF and RER. Values are listed as means ± SD. * Significant different from placebo trials ($p<0.05$).

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