

DISSERTATION FROM THE  
NORWEGIAN SCHOOL OF  
SPORT SCIENCES  
**2017**

Hans Kristian Stadheim

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## ABSTRACT

This thesis consists of five studies with additional unpublished results where the **aim** was to examine the effects of caffeine (CAF) ingestion on factors considered determining for endurance sport performance and fatigue. In total 65 male sub-elite and elite endurance athletes volunteered to participate, with twelve subjects participating in more than one study. All subjects included were trained endurance athletes with a  $\dot{V}O_{2\max}$  in the range of 67.2 to 90.5 (mL·kg<sup>-1</sup>·min<sup>-1</sup>). All studies included in the dissertation were performed in a randomized, double-blind, placebo-controlled, crossover design at the Norwegian School of Sports Sciences in the Department of Physical Performance, between 2012 and 2016.

**Results** from the five studies demonstrate that CAF ingestion of 3-6 mg·kg<sup>-1</sup> is an effective stimulant for improving endurance performance. Improvements were greater during time until voluntary exhaustion (~8-20%) trials compared to time trial (TT) testing (~1-5%). Independent of mode, approximately 75% of test subjects improved their performance following CAF ingestion. The studies in this thesis suggest that CAF has a wide range of potential effects on several physiological and psychological mechanisms important for endurance performance. Results displaying improvements following CAF ingestion are closely related to CAF's hypoalgesic effects in reducing rate of perceived exertion and pain, consequently allowing subjects to maintain higher exercise intensity with similar exertion. Novel findings in the thesis were that CAF ingestion increased aerobic power ( $\dot{V}O_{2\max}$ ), fractional utilization of  $\dot{V}O_{2\max}$ , anaerobic power (O<sub>2</sub>-deficit) and counter movement jumping heights, but led to no change in work efficiency, pacing strategy or substrate utilization.

**The conclusion** of the thesis is that CAF is an efficient ergogenic aid for improving endurance performance independent of mode (time until voluntary exhaustion vs TT), duration (10-120min), muscular usage (double poling vs running), or geographical condition (sea level vs altitude) in top athletes.

## SAMMENDRAG

Denne doktoravhandlingen består av fem studier hvor **hovedmålet** var å studere hvordan koffeininntak påvirker fysiologiske og psykologiske egenskaper en vet er av stor betydning for høy utholdenhetsprestasjon. Totalt deltok 65 mannlige, utholdenhetsrente forsøkspersoner, og av disse deltok 12 personer i mer enn ett studie. Alle forsøkspersonene var på sub- eller elitenivå i sin utholdenhetsidrett og hadde en  $\dot{V}O_{2max}$  mellom 67.2 til 90.5 ( $mL \cdot kg^{-1} \cdot min^{-1}$ ). Studiene i avhandlingen ble gjennomført på en randomized, double-blind, placebo-controlled, crossover design på Norges Idrettshøyskole ved seksjon for fysisk prestasjonsevne mellom 2012 og 2016. Samlet viser resultatene fra avhandlingen at et koffeininntak på 3-6  $mg \cdot kg^{-1}$  gir god effekt i å bedre utholdenhet. Forbedringen var større under tid til utmattelse (~8-20%) enn når bestemte distanse/testløp (~1-5%) ble gjennomførte (time trial). Uavhengig av koffeininntak og studie design forbedret omtrent 75 % av forsøkspersonene fysiskprestasjonsevne.

**Resultatene** fra avhandlingen viser at koffein påvirker flere fysiologiske og psykologiske egenskaper som er av stor betydning for høy utholdenhetsprestasjon. Spesielt virker effekten av koffein å ha betydning for å redusere opplevelsen av smerte og anstrengelse under aktiviteten. Forsøkspersonene klarte samtidig å vedlikeholde en høyere intensitet og varighet på prestasjonstestene. Videre er økningen i aerob kapasitet ( $\dot{V}O_{2max}$  og utnyttelse av denne), anaerob kapasitet (akkumulert oksygen underskudd), samt økningen av counter movement hopphøyde, nye resultat innen forskningsområdet. Forbedringen av disse fysiologiske egenskapene virker å være viktige for å kunne forklare koffeinens prestasjonsfremmende effekt. Resultatene viser derimot at koffeininntaket hverken påvirket arbeidsøkonomi, pacing strategi eller energisubstrat bruk under testene.

**Konklusjonen** på bakgrunn av resultatene i doktoravhandlingen er at koffein virker å være et effektivt middel for å heve utholdenhetsprestasjonen. Resultatene er uavhengig av type prestasjonstest (tid til utmattelse eller en time trial), varighet (10-120 min), muskulatur benyttet (armer vs. bein) eller om testen er gjennomført ved havnivå eller i høyden for sub- og elite idrettsutøvere.

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## LIST OF PAPERS PUBLISHED DURING THE DOCTORAL PERIOD

- I. *Caffeine increases performance in cross-country double-poling time trial exercise.*  
Stadheim HK, Kvamme B, Olsen R, Drevon CA, Ivy JL, Jensen J.  
Med Sci Sports Exerc. 2013 Nov;45(11):2175-83.
- II. *Caffeine and performance over consecutive days of simulated competition.*  
Stadheim HK, Spencer M, Olsen R, Jensen J. Med Sci Sports Exerc. 2014 Sep;46(9):1787-96.
- III. *Caffeine improves performance in double poling during acute exposure to 2,000 m altitude.*  
Stadheim HK, Nossum E.M, Olsen R, Spencer M, Jensen J. J Appl Physiol (1985). 2015 Oct 22:jap.00509.2015.

### *In Manus*

- IV. *Caffeine improves exercise performance, maximal oxygen consumption and accumulated oxygen deficit*  
Stadheim HK, Stensrud T., Brage. S., & Jensen J.  
Manuscript will be submitted to either MSSE, JAP or SJMSS
- V. *Caffeine improves prolonged running performance*  
Stadheim HK & Jensen J.  
Manuscript submitted to Scandinavian Journal of Medicine and Science in Sports



## ABBREVIATIONS AND ACRONYMS

What	Abbreviations
Anaerobic Threshold	AT
Arteriovenous oxygen difference	a-vO <sub>2</sub> diff
Bicarbonate	HCO <sub>3</sub> <sup>-</sup>
Beats per minute	BPM
Caffeine	CAF
Cardiac Output	$\dot{Q}_c$
Carbohydrates	CHO
Central Nervous System	CNS
Counter Movement Jump	CMJ
Cross-country Ski Poling Performance Test	C-PT
Double Poling	DP
Free Fatty Acids	FFA
Heart Rate	HR
Hemoglobin	Hb
Intraclass Correlation	ICC
Lactate	LA-
Maximal Oxygen Consumption	$\dot{V}O_{2max}$
Maximal Accumulated Oxygen Deficit	MAOD
Minute Volume	MV
O <sub>2</sub> -saturation	SpO <sub>2</sub>
Placebo	PLA
Rate of Perceived Exertion	RPE
Respiratory Exchange Ratio	RER
Standard Deviation	SD
Stroke Volume	SV
Time Trial	TT
Ventilation	V <sub>E</sub>
World Anti Doping Agency	WADA

# 1. INTRODUCTION AND RATIONALE FOR THE THESIS

## 1.1 History of Caffeine

Caffeine (CAF) is reported to have been consumed by humans since the Stone Age in certain areas of the world by chewing the seeds, bark, or leaves of plants containing CAF to ease fatigue, stimulate awareness, and elevate mood (58). According to Chinese legend the famous Chinese emperor and herbalist *Shennong* discovered tea by accident, making tea (*cha*), and CAF intake common in China from around 3000 BC (62). However, the earliest credible evidence of CAF consumption, and knowledge of the coffee tree, is from the middle of the fifteenth century, in the Sufi monasteries of Yemen in southern Arabia (12). From Mocha in Yemen, coffee spread to Egypt and North Africa, and by the 16th century, it had reached the rest of the Middle East, Persia, Turkey, Italy and then later the rest of Europe, where it became a popular drink in the southern regions (12). Since it became recognized that coffee contains stimulant compounds, firstly coffee itself and later CAF in its chemical form have been subject to some forms of regulation (31, 44, 172). The chemical substance CAF ( $C_8H_{10}N_4O_2$ ) was isolated from coffee in 1820 by the German chemist, Friedlieb Ferdinand Runge, and is a bitter, white, crystalline xanthine alkaloid (50).

**Table 1.1:** CAF levels in drinks and foods available in regular grocery stores in Norway, and typically found in grocery stores around the world.

Product	Serving (ml)	Caffeine (mg)	Numbers of servings for an 80 kg person to obtain		
			3 mg	6 mg	9 mg
Coffee	250 (1 cup)	60 (12-169)*	4 cups	8 cups	12 cups
Tea	250 (1 cup)	27 (9-51)*	9 cups	18 cups	27 cups
Hot chocolate	250 (1 cup)	5-10	24 cups	48 cups	72 cups
Coca Cola	500 (1 bottle)	53†	2250 ml	4500 ml	6750 ml
Red Bull energy drink	250 (1 can)	80†	3 cans	6 cans	9 cans
Ice coffee	250 (1 bottle)	50 (15-100)*	5 bottles	10 bottles	15 bottles
Dark chocolate bar	60 g (1 bar)	25 (10-50)*	600 g	1200 g	1800 g

\* Depending on brand and how the item is made, levels of CAF vary.

† Amount in Norway

In 2009 the global consumption of CAF was estimated at ~120,000 tons per year, with consumption of 150 million bags (each bag containing 60 kg) of coffee beans in 2015. This makes CAF one of the world's most widely consumed products, with about 90% of the adult

western population consuming CAF on a daily basis by ingestion of products such as coffee, tea, Red Bull, Coca Cola or other caffeinated 'energy' drinks (161). These products are primarily consumed for taste and enjoyment, but they are also consumed by athletes, students and workers solely to improve awareness, performance and/or working capacity (160, 161).

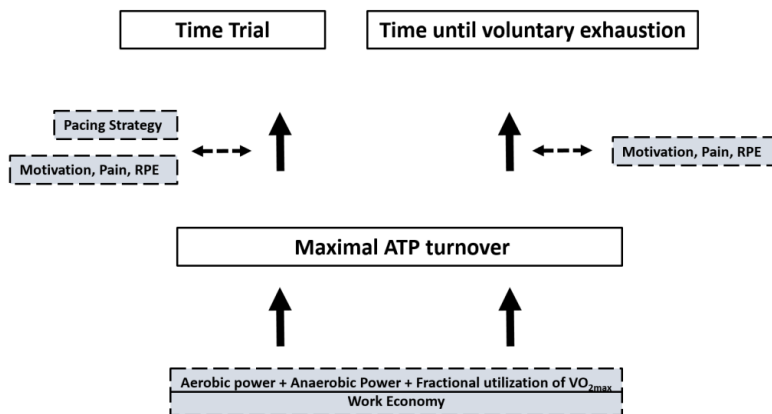
### ***1.2 Caffeine and scientific research***

The immense popularity of CAF has prompted decades of investigations into its ergogenic potential in exercise performance, dating back as far as the 1900s (148). One of the first scientific studies, by Rivers and Webber in 1907, showed that an oral ingestion of 300 mg CAF increased two test subjects' ability to perform biceps curls with a 4.5 kg weight until exhaustion (148). Later, in the 1970s and 1980s, the Costill laboratory focused on CAF as a way to increase performance in aerobic sports events. The classic study by Costill et al. (1978) found that nine cyclists improved their capacity to cycle until exhaustion at 80% of  $\dot{V}O_{2max}$  after ingesting 300mg of CAF (35). Since then, cycling studies by Graham (86-89) and Spriet (32, 88, 158) have given further support to these results and provided further insight into the mechanisms underlying CAF's ergogenic potential. Today the positive effects of ingesting appropriate quantities (approx. 3-6 mg · kg<sup>-1</sup> bodyweight) of CAF on exercise performance are well documented (4, 24, 75, 83, 161). Furthermore, results show that independent of the type of exercise activity (e.g. cycling, running or rowing), CAF can improve exercise performance in both short duration activities (where anaerobic power is of primary importance) (7, 42), and prolonged duration sports (where aerobic processes mainly determine performance) (24, 83). Likewise, CAF has been shown to be beneficial regardless of whether performance is measured as time to exhaustion (84, 99), or time to complete a set amount of work during time trial (TT) testing (47, 100). Therefore, CAF appears to be an effective stimulant for optimizing athletic performance and endurance capacity during both TTs and time to voluntary exhaustion as illustrated in Appendices I and II, with studies reporting improved performance following CAF ingestion (24, 42, 83).

### ***1.3 Caffeine, endurance, fatigue and athletic performance***

Determining factors for endurance capacity and athletic performance have been widely discussed and debated by exercise physiologists during the last decade (6, 48, 135, 177). The complexity involved in determining CAF's mechanisms of action has led to a plethora of research

investigations on human endurance potential, which have shown that several factors are important in maintaining high performance (1, 33, 107). Despite the development of exhaustion and fatigue being a complex phenomenon, exhaustion represents the inability to sustain further exercise at a required power or workload (72, 125). Endurance can therefore be defined as muscles' ability to resist fatigue, and maintain a given force for a prolonged period of time (6). Understandably, endurance athletes have always searched for ways to boost energy production and performance. A clear example of this in international sports competitions is the use of blood doping (101, 102). The effectiveness of blood doping is based on the fact that human endurance performance is characterized by one simple requirement; the necessity to sustain repeated muscle contraction. Endurance performance is therefore directly limited by the human body's capacity to produce and effectively use our primary energy unit, adenosine trisphosphate (ATP). An athlete's maximal ATP turnover, as illustrated in Figure 1.1, will therefore essentially determine both TT and time to voluntary exhaustion performance. To improve TT or time to voluntary exhaustion performances post CAF ingestion would require a higher production or more effective usage of ATP as illustrated in the model proposed by di Prampero (1, 6). Di Prampero's model has been shown to be a good model for explaining endurance performance since energy is required independent of activity and duration, and energy cannot be created or destroyed, only changed from one form to another (56). This means that increased work output or duration post CAF ingestion would require an increased energy provision by increasing  $\dot{V}O_{2max}$  (1.5.1), fractional utilization of  $\dot{V}O_{2max}$  (1.5.2), or accumulated O<sub>2</sub>-deficit (1.5.3), unless the energy produced is used more efficiently by improving work economy (1.5.4) (5, 6, 48). However, many studies have also found that an athlete's ability to pace (1.4.2), push and sustain high levels of pain (1.4.1) during endurance exercise can have a major effect on performance outcome, thus affecting the parameters of the di Prampero model (25, 65, 66, 132). These factors could therefore be important from an applied sports perspective if we are to better understand the mechanisms of CAF's ergogenic potential.



**Figure 1.1:** A simplified model illustrating the interaction of determining factors for endurance performance. The figure is based on the model by di Prampero (1, 6) with some modifications, and will be used as a framework in this thesis for explaining CAF's effect on endurance performance in athletes.

#### ***1.4 Caffeine and mechanisms of action***

The physical and psychological changes following CAF ingestion seem to be related to the unique molecular structure of CAF, allowing its passage through all biological membranes in the human body (70). Initially, a glycogen-sparing theory was proposed by Costill and colleagues in 1978, since improved performance following CAF ingestion was associated with increased concentrations of free fatty acids (FFA), adrenaline and lower RER values during prolonged submaximal cycling until exhaustion (35, 99). Since elite runners maintain intensities of ~80% of  $\dot{V}O_{2max}$  during half-marathon and marathon competitions, a sparing of glycogen following CAF ingestion during such activities would improve performance by avoiding glycogen depletion and maintaining a larger fractional utilization of  $\dot{V}O_{2max}$  (1, 33, 107). However, several studies have failed to observe an increase of glycogen content in muscles, or of RER-values post CAF ingestion during prolonged exercise, despite increases in FFA and adrenaline (83, 84, 84, 86). Furthermore, this hypothesis could not explain improved performance of short duration, where anaerobic power is important and glycogen content is not likely to be a limiting factor for maximal ATP turnover. A number of studies have observed that CAF ingestion can reduce the rate of perceived exertion (RPE) and muscular pain while subjects perform the same relative workload (54, 127). The reduction of RPE and pain is believed to be associated with CAF's ability to inhibit adenosine receptors throughout the body (70, 182). A reduction in RPE and pain

following CAF ingestion could explain the improved exercise intensity and duration during exercise tasks of both short and long duration.

Because of the observed variation in performance improvements post CAF ingestion, several studies have investigated CAF and dose response relationships. Based on the available literature, optimal levels of CAF ingestion are highly individual (47, 83, 157), but seem to occur with dosages of 3-6 mg · kg body wt<sup>-1</sup> (47, 83). Higher dosages (9-12 mg · kg body wt<sup>-1</sup>) do not seem to result in additional improvements, but rather lead to stronger side effects such as headaches or nausea (4, 139). Oral ingestion of a concentrated CAF dosage between 3-6 mg · kg body wt<sup>-1</sup> would typically result in a peak plasma CAF concentration of ~20-50 µmol/L, approximately 45 min post ingestion (83). These plasma CAF concentrations would significantly inhibit adenosine receptors in the human body, resulting in reduced neural signals related to perception of pain and effort (RPE) during exercise (67, 70, 162, 181).

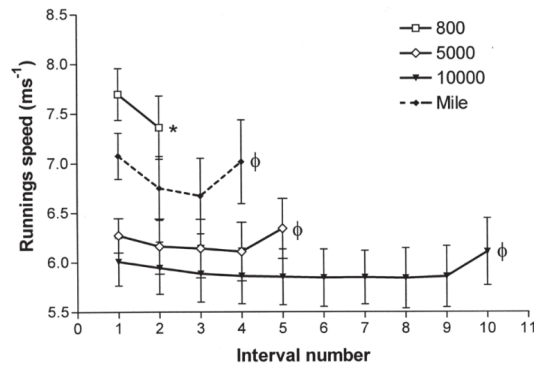
#### ***1.4.1 Caffeine, Pain and RPE***

During exercise, RPE and pain perception involve the collective integration of afferent feedback from cardiorespiratory, metabolic, and feed-forward mechanisms enabling individuals to evaluate how hard or easy an exercise task feels at any point in time (60). There is a strong correlation between RPE and pain and increased work intensity (61, 151). A reduction in pain and RPE following CAF ingestion would therefore in most situations be beneficial for improved exercise performance (60). The importance of being able to tolerate high levels of pain and RPE in endurance performance is clear from several studies in the area (25, 132), and is illustrated by the observation of pain relievers improving endurance performance by reducing self-felt pain and RPE during exercise (119, 127, 166). Because of similar responses during exercise after CAF ingestion, CAF is today found in combination with several popular pain relievers, such as paracetamol, ibuprofen and aspirin, making them more effective (25, 141). Furthermore, RPE and pain have been demonstrated to be remarkably good psychophysiological indicators, not only to predict exercise capacity, but also to explain changes in pace and pacing strategy during endurance performance tasks (60).

#### ***1.4.2 Caffeine and Pacing Strategy***

An optimal pacing strategy can be described as the most efficient use of physiological resources during athletic competition, and is crucial for optimal athletic performance (65, 66, 169). Optimal pacing appears to depend on many factors, including the length of the exercise task, the exercise performed, ambient temperature and altitude (169). The concept was first systematically studied in 1958, when researchers concluded that the best way to run a middle distance event was to delay the maximum effort until as late in the run as possible (149). Studies manipulating pacing by forcing athletes to start faster or slower than their best performances have found best performances occur with a faster start in short events (less than 80 sec) and sports where resistive drag forces are low (Fig. 1.2) (169). In contrast, during prolonged exercise (3 min or more) optimal performance is observed with an even or negative pacing strategy, characterized by increasing power output (velocity) at the end of the event as shown in Figure 1.2 (65, 169). Athletes generally learn optimal pacing strategies in training and competitions (132, 159). Top athletes are therefore generally extremely good at pacing themselves based on interpretations of feedback from pulmonary, cardiovascular and skeletal system responses during exercise (65, 159, 169). It is therefore a question of whether the reduction of RPE and pain following CAF ingestion for athletes is beneficial for performance outcomes in all situations. Critically low muscle pH,  $\text{HCO}_3^-$  and high blood LA- values at early or inappropriate times during a race have in many circumstances been associated with a reduction in velocity (64, 65). An increased starting velocity during the early stages of a TT following CAF consumption could increase blood LA-, reduce blood  $\text{HCO}_3^-$  and pH (106). Early and increased intracellular perturbations would also probably lead to unfavorable conditions in skeletal muscles for high performance, which could, among other things, reduce athletes' fraction utilization of  $\dot{V}\text{O}_{2\text{max}}$  during exercise (106).

Unfortunately, very few studies have discussed CAF's effect on pacing strategy, despite the enormous amount of research on endurance performance. In addition, an inhibition of  $\text{A}_1$  and  $\text{A}_{2\text{A}}$  adenosine receptors would have a much broader effect upon factors and mechanisms considered limiting and important for high endurance performance than would pacing or reduction of pain and RPE (8, 182).



**Figure 1.2:** Pacing strategy used to set running world records, from Tucker et al. (169). The interval numbers for the long distances (5000m and 10 000m) are divided per 1000m, while the shorter events ( 800m and mile) are divided per 400m of running.

### 1.5 Caffeine and energy production

Despite the immense research in the area, surprisingly few studies have actually measured or reported several important determining factors for energy and power production during endurance performance that could verify whether CAF ingestion can improve maximal ATP turnover. These factors include  $\dot{V}O_{2max}$ , accumulated  $O_2$ -deficit, fraction utilization of  $\dot{V}O_{2max}$ , and work economy ( $O_2$ -cost). Certainly, a reduction in RPE and pain alone could not explain higher velocity or duration post CAF consumption, since increased work output and duration would require an increased production or more efficient usage of energy (ATP) (1, 6).

Increased HR (23, 47, 100), ventilation (28-30, 40, 103, 144), and blood lactate accumulation (9, 10, 74, 85) are three of the most consistent observations associated with improved TT performances post CAF ingestion, and only rarely have increases in these values not been observed when measured (83). Moreover, the increase in HR is consequently typically associated with increased work intensity (Appendix I) (3, 21, 23, 47, 78, 80, 100, 140, 142).

Likewise, the few studies measuring  $\dot{V}O_2$  during TTs have found the increase in HR,  $V_E$  and velocity to be associated with subjects consuming larger amounts of oxygen, but unfortunately most studies do not measure or discuss these parameters (41, 57, 63, 80, 85, 158). The increases in HR,  $V_E$ ,  $\dot{V}O_2$  and blood lactate accumulation (LA-) suggest that CAF ingestion may increase aerobic and anaerobic processes for the production of energy (ATP).



### 1.5.1 Aerobic power ( $\dot{V}O_{2max}$ )

By the beginning of the 20<sup>th</sup> century, exercise physiologists believed delivery of oxygen was the single most crucial factor for predicting and understanding all endurance performance and aerobic muscle work (171). Since then, the highest rate at which oxygen can be taken up and used by the human body during severe exercise has been termed the maximal oxygen uptake ( $\dot{V}O_{2max}$ ) (6). The term is based on the scientific work of Hill, Lupton and colleagues from 1923-1924 (93, 94), and the principle known as the plateau was introduced and established as a result of experiments by Taylor and colleagues in 1955 (163). The plateau phenomenon is founded on the principle that  $\dot{V}O_2$  increases linearly with increased workload ( $O_2$ -cost), to a point where no further increase is observed, despite a larger  $O_2$ -demand due to increased workload (163, 164). Although limiting factors and test protocols for reaching  $\dot{V}O_{2max}$  in humans have been highly controversial and much discussed since the introduction of the concept in the 1920s (133, 134, 177), there is however today a broad agreement that  $\dot{V}O_{2max}$  is an important factor for predicting endurance sport performance (5, 6, 11, 37, 49), representing the integrated capacity of pulmonary, cardiovascular (*central*) and skeletal muscle (*peripheral*) systems to uptake, transport and use  $O_2$  (6, 43, 112, 143). Unfortunately, to date no studies have thoroughly investigated the potential of CAF to increase  $\dot{V}O_{2max}$  despite studies reporting increased  $\dot{V}O_2$  associated with both increased HR,  $V_E$ , adrenaline and velocity during TT performances (57, 63, 85, 158).

### 1.5.2 Fractional utilization of $\dot{V}O_{2max}$

Despite  $\dot{V}O_{2max}$  seeming to be the main factor for predicting endurance performance in several sports, the fraction utilization of  $\dot{V}O_{2max}$  a subject can sustain during competition has been shown during prolonged exercise to have a large impact on performance outcome (33, 36, 39, 104, 120). The anaerobic threshold (AT) is closely related to the fraction utilization of  $\dot{V}O_{2max}$  during prolonged endurance exercise ( $\geq 15$  min) (104, 106). The AT describes an estimation of the intensity at which the blood concentration of LA- begins to exponentially increase during exercise (82). The increase in LA- concentration in the blood during high exercise intensities is due to increased muscle glycolysis. However, if the intensity is reduced below AT, accumulated LA- will be converted back into glucose by the liver (82, 121). Numerous ways exist to determine AT, resulting in diverse "thresholds" for LA- vs. power output, but all seem to

correlate well with prolonged exercise performances (167). It has been highlighted that peripheral limitations for aerobic power, in addition to athletes' ability to withstand increasing pain and substrate utilization, are important physiological parameters for explaining differences in fraction utilization of  $\dot{V}O_{2max}$  between athletes (5, 6, 107, 122). However, the effect of CAF ingestion on athletes' fraction utilization of  $\dot{V}O_{2max}$  has rarely been a topic of discussion, despite its potential to improve both power output and performance during caffeine TTs (57, 63, 85, 158).

### ***1.5.3 Anaerobic power – Accumulated oxygen deficit***

The most accurate way of estimating maximal anaerobic power or capacity is the accumulated oxygen deficit (O<sub>2</sub>-deficit) (123, 136, 146). The O<sub>2</sub>-deficit is calculated based on the difference between the amount of O<sub>2</sub> consumed and the O<sub>2</sub>-cost to maintain a given workload as described in the classic study by Medbo et al (123). Anaerobic capacity is firstly of high importance during competitions with durations between  $\geq 120$  seconds. During exercise above or close to an athlete's  $\dot{V}O_{2max}$ , O<sub>2</sub>-deficit is also shown as an important determinant for high performance (123, 136, 146). However, several studies have found that metabolic acidosis is also an important contributing factor to fatigue during prolonged high-intensity exercise (105, 128). A high O<sub>2</sub>-deficit has, among other factors, been shown to be important for performance during a 5000 m event (179), because the build-up of a large O<sub>2</sub>-deficit in the early stages of exercise sets the biochemical environment within the muscle cells (121). Several pacing strategy studies have shown that early perturbations during endurance performance can directly dictate subsequent muscle metabolic responses, and therefore have a large impact on performance later in the event, (65, 169). Furthermore, in modern endurance sports, athletes may not finish competitions in the shortest time possible. This is for example the case in most mass start races, where the decisive factor for results is a sprint towards the end (22). This was clearly illustrated in the 2016 Olympics during both the 5000m and 10000m event where the winner's (Mo Farah) finishing laps were performed at 52.83 and 53.48 seconds, consequently being 33.8% and 34.8% faster than the average time taken per lap over the whole distance. Therefore, sustained energy provision via aerobic processes is required to maintain muscle contraction during competitions of longer durations. An improved O<sub>2</sub>-deficit following CAF ingestion, consequently improving maximal ATP turnover, could therefore be of great importance during prolonged mass starts and TTs, as well as during short-term, high intensity exercise.

#### ***1.5.4 Work Economy – O<sub>2</sub>-Cost***

$\dot{V}O_{2max}$ , fraction utilization of  $\dot{V}O_{2max}$  and O<sub>2</sub>-deficit together decide the highest power output or highest maximal ATP turnover during endurance exercise and testing (6). However, several studies have found that in endurance sports, work economy is an important and decisive factor in determining performance outcomes (111, 154). In exercise science, work economy defines the ratio between external mechanical work done and oxygen consumed during steady state exercise (6). In other words, a high work economy means an athlete has a small O<sub>2</sub>-cost for a given workload. During submaximal steady state exercise there is a linear relationship between velocity and oxygen uptake, and the ratio between these factors describes the work economy for a subject in that specific activity (6). However, since work economy is closely related to technique, subjects can display different work economies during different activities (106). Well-trained athletes with higher percentages of type I fibers are reported to maintain a higher power compared to subjects with a smaller amount of type I fibers, despite athletes displaying a similar  $\dot{V}O_{2max}$  and AT (97). It is therefore suggested that elite endurance athletes with a low  $\dot{V}O_{2max}$  compensate for this by exceptional work economy (38, 116). A few studies have found work economy to be affected following CAF ingestion (83). However, it has recently been suggested that augmented strength and motor-unit recruitment, rather than a sparing of glycogen or reduction in perceived pain and effort, may underlie CAF's ergogenic effect on endurance exercise (16, 83, 175). Several studies have found that CAF ingestion can promote and increase power production (7, 16, 175). Specifically, CAF ingestion has been found to improve maximal voluntary contraction (MVC) as well as counter movement jumping (CMJ) height (16, 18, 83, 114), and it is reported that increased power (e.g. by strength training) can improve work efficiency and also reduce RPE during exercise (90, 124, 137, 175). Improved strength following CAF ingestion could therefore very well reduce the subjective rating of RPE and improve work efficiency, postponing the development of fatigue, and consequently improving exercise performance.

#### ***1.6 Caffeine and athletic performance***

The use of CAF by athletes is mainly driven by its reported ability to improve exercise performance. From a sport performance perspective, CAF is also unregulated and legal to buy

and use in nearly all jurisdictions, in most countries of the world (2, 115). In fact, the substance has been widely used by endurance athletes in competitions since its removal from the World Anti-Doping Agency list in 2004 (44, 160). As illustrated in Figure 1.1, endurance performance is complex. Several factors are inter-correlated with each other, affecting the maximal ATP turnover. Training status and amount of active muscle mass engaged, in addition to where it is located (arm vs. legs) can have a major impact on the pulmonary, cardiovascular and skeletal muscle responses to exercise and on athletes' aerobic and anaerobic power (6, 26, 49, 145, 170). However, there are to date insufficient studies that have specifically investigated the effects of CAF ingestion on endurance capacity for top trained athletes with high  $\dot{V}O_{2\max}$  values (from 70 to 90 ml · kg<sup>-1</sup> · min<sup>-1</sup>) (24). The reasons for the lack of research on this specific group of athletes and humans are many, but probably related to the time athletes would have to contribute to conduct quality experimental studies. It could be argued that for this group of humans, CAF in most circumstances most likely would be an efficient ergogenic aid in reducing RPE and self-felt pain consequently improving exercise performance. Conversely, one could expect top athletes to be extremely good at pushing themselves and to cope with high RPE and pain during competitions, in relation to their optimal pacing strategies for overall performance (64-66, 168, 169). Furthermore, the overall lack of results measuring underlying mechanisms such as  $\dot{V}O_2$ -consumption, accumulated O<sub>2</sub>-deficit, work economy (O<sub>2</sub>-cost) and pacing strategy is needed to better understand how higher work output or duration is maintained after a reduction in RPE and pain following CAF ingestion for explaining endurance performance improvements.

## 2. AIMS OF THE THESIS

The overall aim of the present thesis was to investigate physiological mechanisms ( $\dot{V}O_{2max}$ , accumulated O<sub>2</sub>-deficit, fraction utilization of  $\dot{V}O_{2max}$ , work economy), and psychological factors (pain, RPE, pacing strategy) by which CAF could improve endurance performance in top endurance athletes. Five studies were conducted in order to address the aim of the thesis.

**Paper I** - The aim of the study was to investigate the effects of CAF ingestion on performance during a DP 8 km TT. Sub-aims were to evaluate the effects of CAF ingestion on HR, lactate, work economy, RPE/pain and substrate utilization at various exercise intensities.

**Paper II** - The aim of the study was to investigate the effects of CAF ingestion on DP performance, fractional utilization of  $\dot{V}O_{2max}$ , HR, lactate, pacing strategy, pain, RPE, work economy and muscular damage in athletes undertaking a DP TT performance on two consecutive days.

**Paper III** - The aim of the study was to investigate the effects of CAF ingestion on DP performance during acute exposure to hypoxia (altitude 2000m). It was important to investigate how CAF affected important factors for high endurance performance, including fractional utilization of  $\dot{V}O_{2max}$ , HR, lactate, pacing strategy, RPE, pain,  $V_E$ , blood HCO<sub>3</sub><sup>-</sup>, and pH when subjects performed both TT and time until task failure (time until exhaustion) tests.

**Paper IV** - The aim of the study was to investigate the effects of CAF ingestion on performance,  $\dot{V}O_{2max}$ , oxygen deficit and work economy while running.

**Paper V** - The aim of the study was to investigate the effects of CAF ingestion during a prolonged running exercise until voluntary exhaustion at 80% of  $\dot{V}O_{2max}$ . The study also aimed to see how CAF ingestion affected running work economy, HR, substrate utilization, RPE, pain, and muscular power (CMJ).

### 3. METHODS

Detailed information for the individual studies is provided in the published papers section. General information about the five studies is given in the following sections.

#### 3.1 Subjects

In total 65 male elite endurance athletes volunteered to participate in the five studies. Twelve of the subjects participated in more than one study. The characteristics of the subjects are summarized in Table 6.1. All studies were conducted according to the Declaration of Helsinki and sent for approval and reviewed by the Regional Ethics Committee (Norway) before the initial trials commenced. The first two studies were approved by the Regional Ethics Committee, while for study three, four and five the Regional Ethics Committee concluded "*an approval was not needed in order to performed the study as described*". Before participation all subjects gave their written consent to participate after being informed of the purposes of each study, and the risks involved. Inclusion criteria for all studies were that subjects should be: Male;  $\dot{V}O_{2\max -run}$  above  $65 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; and training seriously and competing in the Norwegian national cross-country skiing cup or similar endurance competitions for running, track and field or orienteering in the upcoming season. The training time per year varied from 500-900 hours between athletes. Their  $\dot{V}O_{2\max}$  while running ( $\dot{V}O_{2\max -run}$ ) varied from 67.2 to  $90.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , and their  $\dot{V}O_{2\max}$  while DP ( $\dot{V}O_{2\max -pol}$ ) from 56.8 to  $86.6 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ . Of the subjects included in the test period 2012-2015:

- Four subjects won medals in their specific Norwegian National Championships
- Three subjects competed regularly on the World Cup stage in Cross Country skiing
- Two subjects won the under 23 years World Championships in their specific sport
- 75% of all test subjects had a top 30 ranking in the national championship in their specific endurance sport during the test period (2012-2016).
- 25% of included subjects had a  $\dot{V}O_{2\max}$  higher than  $80 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$

\*Specific information for each study is summarized in Table 3.1.

Before all experimental trials (48h) subjects were instructed to perform light training (and no strength training), and to prepare as they would do before a competition. To minimize variation in pre-exercise glycogen stores, diet and exercise diaries were used to standardize food intake and

training for each subject. Test subjects were instructed to try to follow the same training and diet regimen before all tests, and a standardized washout period was observed before each performance test. Subjects also had to refrain from CAF consumption during the 24 h period before each test. Based on baseline plasma CAF concentrations, instructions were followed by all subjects in the studies. For each main test, subjects arrived at the laboratory at the same time ( $\pm$  15 min) and on the same day of the week.

**Table 3.1:** Subjects' characteristics for Studies I-V

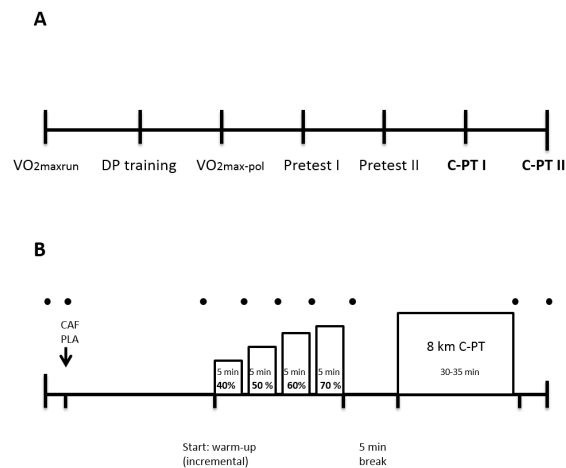
Study	n	Age (yrs.)	Height (cm)	Body mass (kg)	$\dot{V}O_{2\max\text{-run}}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	$\dot{V}O_{2\max\text{-pol}}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )
<b>I</b>	10	20.0 $\pm$ 1.0	181.0 $\pm$ 1.0	73.9 $\pm$ 2.3	69.3 $\pm$ 1.0	63.2 $\pm$ 1.5
<b>II</b>	8	20.0 $\pm$ 1.0	180.4 $\pm$ 1.7	70.6 $\pm$ 2.9	78.5 $\pm$ 1.6	70.5 $\pm$ 1.6
<b>III</b>	13	21.9 $\pm$ 2.7	180.0 $\pm$ 3.7	77.4 $\pm$ 5.6	72.6 $\pm$ 5.7	62.9 $\pm$ 6.8
<b>IV</b>	23	24.0 $\pm$ 1.0	182.1 $\pm$ 1.3	73.5 $\pm$ 1.6	75.9 $\pm$ 5.8	-
<b>V</b>	11	23.9 $\pm$ 2.8	181.9 $\pm$ 3.0	76.6 $\pm$ 7.1	71.0 $\pm$ 5.6	-

### 3.2 Study design

All studies in the dissertation were performed using a randomized, double-blind, placebo-controlled, crossover design. Specific designs for each study are provided below. All testing was carried out at the Norwegian School of Sports Sciences (120 m altitude), in the Department of Physical Performance. Testing was performed from 2012-2015. Tests were performed during athletes' preparation period (April-October). In total, there were two dropouts during the five studies, one each in studies III and IV, both due to sickness. Few side effects after CAF ingestion were registered during testing. One subject reported cramps during the last period of the DP performance test in study I, and one subject reported nausea post testing in study V. Questionnaires post testing for all five studies revealed most subjects were unable to recognise the difference between consuming CAF and placebo (PLA) prior to the different tests (Table 4.7). Approximately 25% of subjects participating in each study were able to detect or guessed when they had consumed CAF/PLA. However, approx. 25% of subjects also guessed/reported the "wrong answer", meaning stating they had consumed CAF/PLA when they had received the opposite treatment.

### Study I

The aim of the study was to investigate the effects of CAF ingestion on performance during peripheral limitations, such as double poling while using the arm muscles. Ten highly trained male cross-country skiers ( $\dot{V}O_{2\max}$  running,  $69.3 \pm 1.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) performed an 8 km time trial performance following PLA or CAF ingestion. Performance was assessed as time to complete the TT. Prior to the  $\dot{V}O_{2\max}$  performance testing a standardized incremental test (warm-up) was completed at set submaximal workloads to evaluate possible changes in DP work efficiency. During both incremental and performance testing HR,  $\dot{V}O_2$ , Time, LA-, glucose, RPE, VE, RER, plasma CAF, glycerol, FFA and adrenaline concentrations were evaluated. All testing was performed on a DP ergometer (Thoraxtrainer, Holbæk, Denmark). CAF ( $6 \text{ mg} \cdot \text{kg}^{-1}$ ) or PLA was ingested 75 min before the time trial performance test.



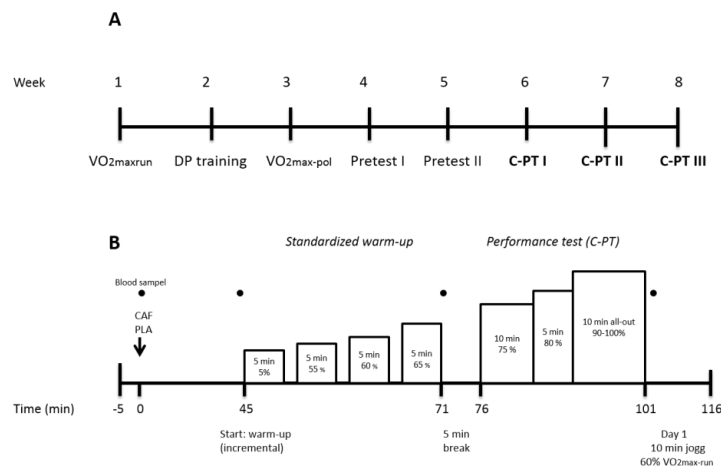
**Figure 3.1:** Experimental design of the 8 km cross country double poling test. (A) Shows tests and training performed during the 6-week period to prepare for the two performance tests in double poling (CP-T). (B) Shows the test procedure for the performance tests. Prior to the CP-T, subjects performed an incremental test consisting of four intensities for 5 minutes. Similar protocol to the CP-T was completed at Pretest-1 and Pretest-2, except that caffeine/placebo was not administered and no blood samples were taken. Abbreviations:  $\dot{V}O_{2\max}$ -run, Training in thorax trainer,  $\dot{V}O_{2\max}$ -pol, Pre-test I, Pre-test II, C-PT I and C-PT II.

### Study II

The aim of the study was to evaluate the effects of CAF ingestion ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) on performance, muscular damage and work economy when competing on consecutive days. Eight highly trained male cross-country skiers ( $\dot{V}O_{2\max}$  -run  $78.5 \pm 1.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) participated in the study.



Performance was assessed as distance covered during a 10-min all-out C-PT. Prior to the performance testing a standardized incremental test (warm-up) was completed at set submaximal workloads to evaluate possible changes in DP work efficiency. During both incremental and performance testing HR,  $\dot{V}O_2$ , time, LA-, glucose, RPE, muscular pain, VE, RER, plasma CAF, adrenaline and creatine kinase concentrations were evaluated. All testing was performed on a DP ergometer (Thoraxtrainer, Holbæk, Denmark), and oral CAF or PLA was ingested 75 min before the all-out C-PT.



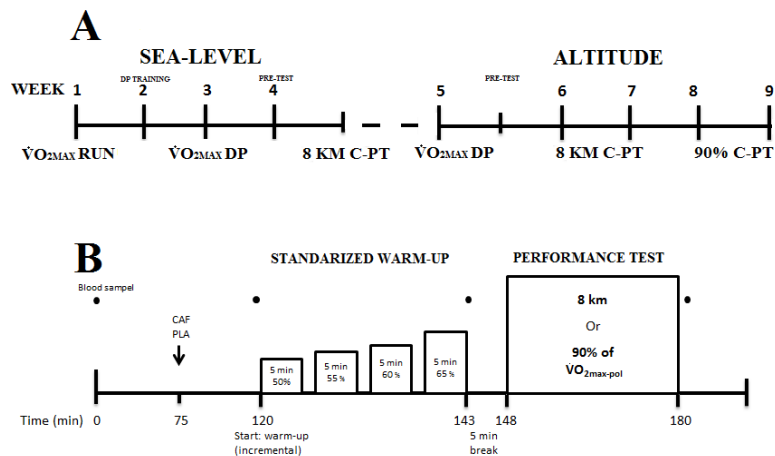
**Figure 3.2:** Experimental design. Top line shows tests and training performed during the 8 weeks of familiarization for the three, 2-day performance tests in double poling (C-P-T). The bottom figure shows the test procedure for all performance tests. Prior to the C-PT, subjects performed a standardized warm-up (incremental tests) consisting of four intensities all lasting 5 minutes. A similar protocol to the C-PT was performed during pretests 1 and 2, except that caffeine/placebo was not administered, and no blood samples were taken.

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

### Study III

The aim of the study was to investigate the effects of CAF ingestion ( $4.5 \text{ mg}\cdot\text{kg}^{-1}$ ) on DP performance, the pulmonary system and HR, when subjects were exposed to acute hypoxia (2000 m). Thirteen male sub-elite cross-country skiers ( $\dot{V}O_{2max} 72.6 \pm 5.68 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) were included. Testing was carried out in a hypobaric chamber, at 800 mBar, ( $P_{iO_2} \sim 125 \text{ mmHg}$ ) corresponding to  $\sim 2000 \text{ m}$  above sea level. The first four weeks of the study were performed at sea-level conditions (120 m altitude,  $\sim 960 \text{ mBar}$ ). The remaining five test weeks were carried out during acute (2 h) hypoxia in a hypobaric chamber (Norsk Undervannsteknikk A/S, Haugesund,

Norway). Performance was assessed using **1**) an 8 km cross-country DP time-trial (C-PT), and **2**) time until task failure at a set workload equal to  $\sim 90\%$  of DP  $\dot{V}O_{2max}$ . Prior to the performance testing a standardized incremental test (warm-up) was completed at set submaximal workloads to evaluate possible changes in DP work efficiency. During both incremental and performance testing HR,  $\dot{V}O_2$ , time, LA-, glucose, RPE, muscular pain, VE, RER,  $HCO_3^-$ , pH, plasma CAF, and adrenaline concentrations were evaluated. All testing was performed on a DP ergometer (Thoraxtrainer, Holbæk, Denmark), and oral CAF or PLA was ingested 75 min before the performance tests.

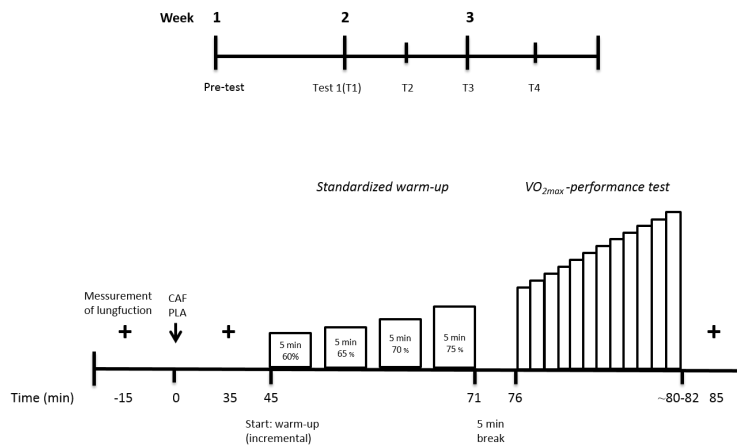


**Figure 3.3:** Experimental design for all performance tests (C-PTs). Prior to the C-PTs, subjects performed a standardized warm-up (incremental tests) consisting of four intensities of 5 min duration. A similar protocol to the C-PTs was completed in pretests, except that caffeine/placebo were not administrated, and no blood samples were drawn.

#### Study IV

The aim of the study was to test the effect of CAF ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) on maximal oxygen consumption during a standardized  $\dot{V}O_{2max}$  performance test. Twenty-three elite endurance trained male athletes ( $\dot{V}O_{2max-run} 76.3 \pm 1.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) participated in the study. Prior to the  $\dot{V}O_{2max}$  performance testing a standardized incremental test (warm-up) was completed at set submaximal workloads to evaluate possible changes in running work efficiency. Performance was assessed as time,  $O_2$ -deficit and  $\dot{V}O_{2max}$  during the standardized  $\dot{V}O_{2max}$  performance test. Oral CAF or PLA was ingested 45 min before a standardized warm-up protocol performed as an incremental test. During both incremental and performance testing HR,  $\dot{V}O_2$ , time, LA-, glucose,

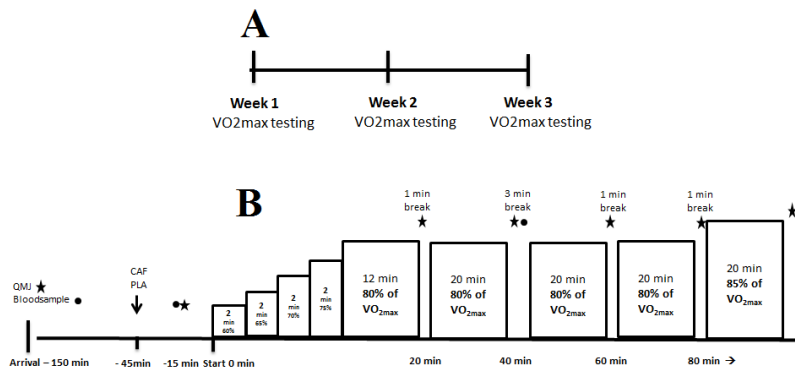
RPE,  $V_E$ , RER and BF were evaluated. All testing was performed on a treadmill (Woodway, Weil am Rein, Germany) with an uphill incline.



**Figure 3.4:** Experimental design. **A:** top line shows pre-tests and main testing during the 3 weeks used to complete the test for one subject. **B:** bottom figure shows the test procedure for all Stadheim  $\dot{V}O_{2max}$  performance tests. Prior to the tests, subjects performed a standardized warm-up (incremental tests) consisting of four intensities all lasting 5 minutes.

## Study V

The aim of the study was to evaluate the effects of CAF ingestion ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) on prolonged running performance, work efficiency, CMJ, substrate utilization and RPE during full-body exercise. Eleven male sub-elite endurance athletes ( $\dot{V}O_{2max} 71.0 \pm 5.6 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) performed tests involving prolonged running at a work load/velocity equal to  $\sim 80\%$  of  $\dot{V}O_{2max}$  until task failure. The test was built up as an interval with sets lasting 20 min. Between each set subjects performed counter movement jumps on a power platform. When starting the performance tests subjects completed a standardized warm-up protocol lasting 8 min. The standardized warm-up consisted of four, 2 -min workloads, equivalent to a velocity equal to  $\sim 60$ ,  $\sim 65$ ,  $\sim 70$  and  $\sim 75\%$  of subjects  $\dot{V}O_{2max}$ . During both incremental and performance testing HR,  $\dot{V}O_2$ , time, La-, glucose, RPE, muscular pain,  $V_E$ , RER, adrenaline and creatine kinase concentrations were evaluated. All testing was performed on a treadmill (Woodway, Weil am Rein, Germany) with an uphill incline of  $5.3^\circ$ , and oral CAF or PLA was ingested 75 min before the all-out C-PT.



**Figure 3.5:** Experimental design. Top panel (A) shows the tests and training performed during the 3 weeks of testing. The bottom panel (B) shows the test procedure for all performance tests.

### 3.3 Familiarization

Despite all subjects participating in the three DP studies being highly trained cross-country skiers who were very familiar with the DP technique, all subjects underwent a 3 to 4-week training protocol to familiarize themselves with the double poling ergometer, as illustrated in Figure 3.6. Furthermore, to ensure high reliability, and that there was no learning effect as a result of repeated TTs or performance tests, at least one initial pre-performance test without supplementation was performed before the main tests (Studies I-IV). Since the 8 km C-PT was used twice during the test period, a pilot study was completed with four well-trained athletes ( $\dot{V}O_{2\max\text{-pol}} 57.5 \pm 2.2$ , running  $62.1 \pm 2.5$ ) who were all previous cross-country skiers. As illustrated in Figure 3.6 a minimum of at least two pre-tests was needed to obtain satisfactory reliability and reproducibility as an effect of repetitive testing. There was a clear learning, technical and/or physiological adaptation between the first and second test that leveled off between tests three and four. However, after the first pre-test CV, variations in the 8 km C-PT were small (around 1~2%), and showed little variation from test to test after the familiarization procedure.

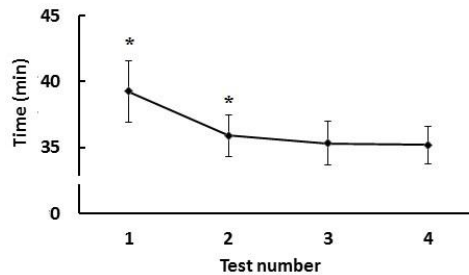


Figure 3.6: Double poling times during the 8 km C-PT for the pilot test group. Values are listed as means  $\pm$  SD \* Significant different from next test.

### 3.4 Test equipment used

#### *Thorax Trainer – CC-POL (Studies I-III)*

The cross-country double poling ergometer used in the studies was a Thoraxtrainer Elite (Thoraxtrainer, Holbæk, Denmark). Ski poles used during all testing were Swix CT1 (Swix, Lillehammer, Norway) and pole length was standardized to  $85 \pm 2\%$  of subject's height. The ski poles were attached to two sleds that moved independently and were connected to a flywheel that provided resistance. A computer displayed work output (W),  $\text{km} \cdot \text{h}^{-1}$ , and poling frequency in real time. Resistance in the Thoraxtrainer is generated by air pressure. The Thoraxtrainer Elite was set at level one (easiest) of ten available levels during all testing to optimize technique. For more information about the DP technique and the Thoraxtrainer, see the studies by Bojsen-Moller et al. (2010) and Van Hall et al. (2003).

#### *Treadmill (Studies I-V)*

The treadmill used in all studies was a Woodway (Woodway, Weil am Rein, Germany). An uphill incline of either  $5.3^\circ$  or  $10.5^\circ$  was used during all testing.

#### *Counter movement jumps (CMJs) (Study V)*

A force platform from Biomekanikk AS (Oslo, Norway), with a sandwich construction with one Vetek VZ563YH-200 kg load cell (Hantverksvägen 15. 76493 VÄDDÖ, Sweden) in each corner to calculate force, was used in study V. Calculation of CMJ heights was done using the impulse method (113). The software (Biojump, Oslo, Norway) samples force data with a frequency of 2000 Hz, using a Butterworth low pass filter with a cut-off frequency of 120 Hz.

#### *HR monitor (Studies I-V)*

HR was measured during all tests using an HR monitor from Polar RS 800, (Finland). The error of measurement was less than  $\pm 1\%$  as stated by the manufacturer.

#### *Oxygen consumption (Studies I-V)*

Oxygen consumption, ventilation, and RER were measured with the Oxycon Pro metabolic system (Jaeger, Hochberg, Germany) for all studies except study 3 where a  $V_{\max}29$  (Sensormedics, Yorba Linda, CA, USA) was used during testing in hypoxic conditions. The equipment for measurement of  $\dot{V}O_2$  was calibrated prior to tests using gas mixtures with known concentrations of  $O_2$  and  $CO_2$  (14.93%  $O_2$  and 5.99%  $CO_2$ ) and normal air (approximately 20.95%  $O_2$  and 0.039%  $CO_2$ ). Volume was calibrated manually using a 3-liter pump (Calibration Syringe, Series 5530, Hans Rudolph Inc., MO, USA). During testing, subjects used a mouth V2 mask (Hans Rudolph Inc., USA) in combination with a nose bracket. Expired air was sampled through a hose into the mixing chamber (Oxycon Pro) and analyzed with a turbine (Triple V volume transducer).

#### *Hypobaric chamber (Study III)*

In study III a hypobaric chamber (Norsk Undervannsteknikk A/S, Haugesund, Norway) was used for simulating hypoxic conditions. Here air pressure was reduced to 800 mBar, equivalent to  $\sim 11.5$  psi, or  $\sim 590$  mmHg, simulating an altitude of  $\sim 2,000$  m above sea level at  $17^\circ C$ . To ensure maintenance of atmospheric gas concentrations (20.95%  $O_2$  and 0.039%  $CO_2$ ) during all trials, concentrations were continuously measured for both fractional concentration of inspired  $CO_2$  ( $F_iCO_2$ ) with the Vaisala GMT222 Carbon Dioxide Transmitter (Vaisala, Stockholm, Sweden) and fractional concentration of inspired  $O_2$  ( $F_iO_2$ ) with the PMA30 M&C  $O_2$  analyzer (Marseille, France). During the first two hours (rest) of acute altitude exposure, approx.  $\sim 1$  l·min<sup>-1</sup> oxygen was added to maintain atmospheric gas concentrations of air. During physical activity oxygen consumption increased, thus additional oxygen was added to maintain a stable  $F_iO_2$ . On the basis of the pretests, approx.  $\sim 3$  l·min<sup>-1</sup> of extra oxygen was added to cover the enhanced usage of oxygen during physical activity, but was adjusted (increased or reduced) according to observed  $F_iO_2$  values for each individual hypoxic trial. Three gas scrubbers containing Sofnolime filters and circulating fans worked as  $CO_2$  traps to try to ensure a stable  $F_iCO_2$  concentration.

However, during the later stages of the C-PTs (~5–10min), CO<sub>2</sub> production from the subjects exceeded the CO<sub>2</sub> removal capacity of the three scrubbers. This resulted in an enhanced F<sub>I</sub>CO<sub>2</sub> concentration of the air inside the chamber with post values of CO<sub>2</sub> between 0.05–0.08%. Even though CO<sub>2</sub> concentration increased, it never exceeded 0.08%; these CO<sub>2</sub> values are not considered dangerous for subjects and are unlikely to influence test results. During rest and at sea level testing F<sub>I</sub>CO<sub>2</sub> concentrations were 0.04% as expected.

### ***3.5 Blood samples***

Blood samples were drawn from the subjects' median cubital veins using a BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). A 7 ml blood sample was drawn for all blood samples and placed in tubes containing EGTA/glutathione [20 µl 0.2 M glutathione and 0.2 M EGTA per ml blood] for analysis of adrenaline, noradrenaline, glycerol, FFA, creatine kinase and/or FFA. Blood samples were immediately placed in ice water and centrifuged at 2,500 rpm for 10 min at 4 °C (Heraeus Megafuge 16R centrifuge, Thermo Electron, Osterode am Harz, Germany). Thereafter, plasma was divided into three plasma aliquots (Microtube Superspin, VWR International, West Chester, PA, USA) and stored at -80 °C until analysis.

#### *Plasma measurements of caffeine*

Plasma CAF concentrations following ingestion of different CAF dosages (3, 4.5 and 6 mg · kg<sup>-1</sup>) were measured in studies 2, 3 and 5. Sample preparation was done using 200 µL plasma and the subsequent measurements of caffeine and theophylline (internal standard; IS) by liquid chromatography mass spectrometry with electrospray ionization (LC-ESI-MSMS) were performed according to a method previously described by Wang et al (173). In brief, 200 µL plasma was added to 100 µL 18 µg/mL IS in MeOH-water (50:50, v/v) and 100 µL MeOH-water (50:50, v/v). After vortex mixing for 30 s, an aliquot (3 mL) of diethyl ether-dichloromethane (3:2, v/v) was added and vortexed for 2 min, followed by centrifugation at 3,000 rpm for 10 min. The upper organic layer was transferred to a 4 mL sample vial and evaporated to dryness at 40 °C under a gentle stream of nitrogen. Residues were then dissolved in 600 µL of mobile phase followed by vortex-mixing for 30 s. A 1 µL aliquot of the reconstituted solution was injected into the LC-ESI-MSMS system.

#### *Glycerol and FFA*

Glycerol and FFA were only measured in study II. Glycerol was measured using a kit based on a colorimetric method (Randox Laboratories Ltd; Antrim, UK) and concentration of non-esterified fatty acids (FFA) was measured with a kit from DIALAB (Weiner Neudorf, Austria) according to the manufacturer's instructions.

#### *Catecholamines*

Plasma adrenaline and noradrenaline were measured with a Cat Combi Elisa kit (DRG Instruments GmbH, Marburg, Germany) according to the manufacturer's instructions.

#### *Creatine Kinase*

Plasma creatine kinase was measured according to the manufacturer's 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 (63).

#### *Lactate, Bicarbonate ( $\text{HCO}_3^-$ ) and Glucose*

Capillary samples from the finger tips were obtained using a Saft-T-Pro Plus (Accu-Check, Mannheim, Germany) for measurements of glucose, lactate and bicarbonate. For measurement of blood lactate, capillary blood samples were drawn into a 50  $\mu\text{l}$  capillary tube and a 20  $\mu\text{l}$  pipette was used to draw blood into the analyzer (YSI 1500 SPORT; Yellow Springs Instruments, Yellow Springs, OH, USA). The analyzer was calibrated with a 5.0  $\text{mmol}\cdot\text{l}^{-1}$  lactate stock solution before each test, and between the submaximal workloads and main tests. Calibration values between 4.95  $\text{mmol}\cdot\text{l}^{-1}$  and 5.05  $\text{mmol}\cdot\text{l}^{-1}$  were accepted. Under normal circumstances the errors of measurement are reported to be  $\pm 2\%$  for blood lactate values between 0 and 5  $\text{mmol}\cdot\text{l}^{-1}$  and  $\pm 3\%$  for values between 5 and 15  $\text{mmol}\cdot\text{l}^{-1}$ . Blood glucose measurements were obtained using a HemoCue glucose 201<sup>+</sup> (Ängelholm, Sweden). For measurements of bicarbonate, a 125  $\mu\text{l}$  capillary tube was filled with capillary blood, and then analyzed using an ABL 80 Flex (Radiometer, Brønshøj, Denmark).

#### *Subjective rating of Pain and RPE*



Subjective ratings of perceived exertion (RPE) were obtained using the Borg scale (6 to 20). Muscular pain in arms and legs was determined on a 1–10 point scale as described by Ritchie and Hopkins for each workload (20). Questionnaires used to evaluate motivation, "current fitness", and sleep quality used a scale from 1-100 (147).

### **3.6 Statistics**

Except for study I, which presented all data as SEM in the paper (but as SD here in the thesis), the remaining studies presented data as means  $\pm$  SD. Differences in performance after CAF ingestion compared to PLA were evaluated using a paired t-test, where the level of significance was set at  $p \leq 0.05$ . A two-way ANOVA for repeated measures was used to elicit differences in  $\dot{V}O_2$ , HR, LA,  $HCO_3^-$ , glucose,  $V_E$ , muscular pain, and RPE during submaximal workloads between the two treatments. If a significant f-ratio was found, a paired t-test was used to test differences between treatments for the different workloads. All data were tested for normal distribution using the Shapiro-Wilk test. Statistical analyses were performed using SPSS, and GraphPad Prism 6. For analysis of magnitude-based interference, typical error (CV) and interclass correlation (ICC), spreadsheets by Will Hopkins were used (95, 178). Here the level of significance was set at  $p \leq 0.05$ . Performance data were log-transformed to reduce the non-uniformity of error and then back-transformed to obtain the percentage difference in the means between the treatment conditions. Precision of estimation was indicated with a 90% confidence interval (96). For more detailed information about each study's statistics, see the individual papers.

## 4. RESULTS

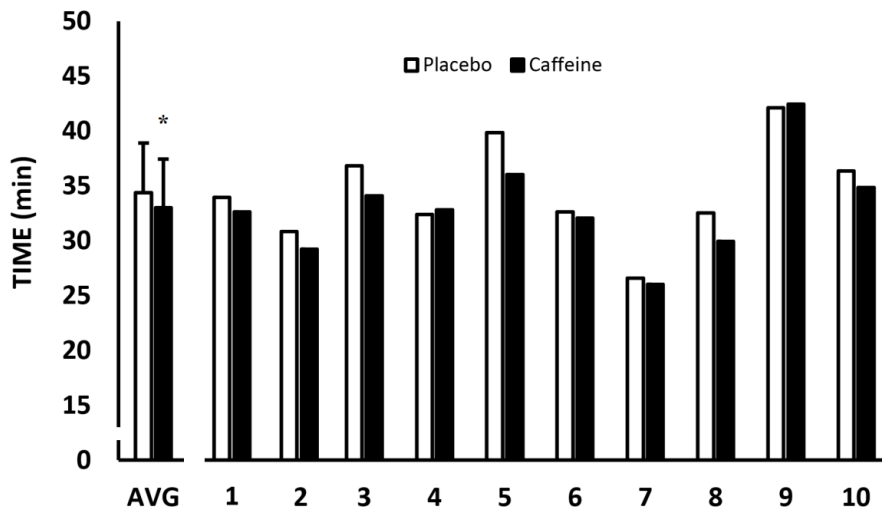
The results chapter summarizes the main findings from the five studies, according to the overall aim of the thesis. Detailed results from specific studies are found in the original papers at the end.

### 4.1 Caffeine and exercise performance (Studies I-V)

CAF (3, 4.5 or 6 mg·kg<sup>-1</sup>) ingestion approximately 60 min prior to performance testing improved athletic sport performance at sea level, acute altitude, during repeated testing, and when engaged in exercise activity mainly limited by arm (DP) or leg muscles (running) (Table 4.1).

Improvements were greater when measured as time until voluntary exhaustion (studies III-V) (5-20%) compared to TT testing (studies I-III) (~1-5%). However, no dose response (3, 4.5 or 6 mg·kg<sup>-1</sup>) was observed for improvements during performance testing (Table 4.1 and Fig 4.9).

Furthermore, independent of mode, approximately 75% of all athletes ( $\dot{V}O_{2max} \geq 70 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) showed improved performance following CAF ingestion (Figure 4.1, Table 4.1).



**Figure 4.1:** Mean and individual time for athletes receiving PLA (open) or CAF (filled) to complete the 8 km TT in study I. As illustrated typically ~75% of subjects improved their performance following CAF ingestion. Values are listed as means  $\pm$  SD. \* Significant difference from PLA trials ( $p < 0.05$ )

**Table 4.1:** Summary of key performance and physiological data from the five studies of the thesis.

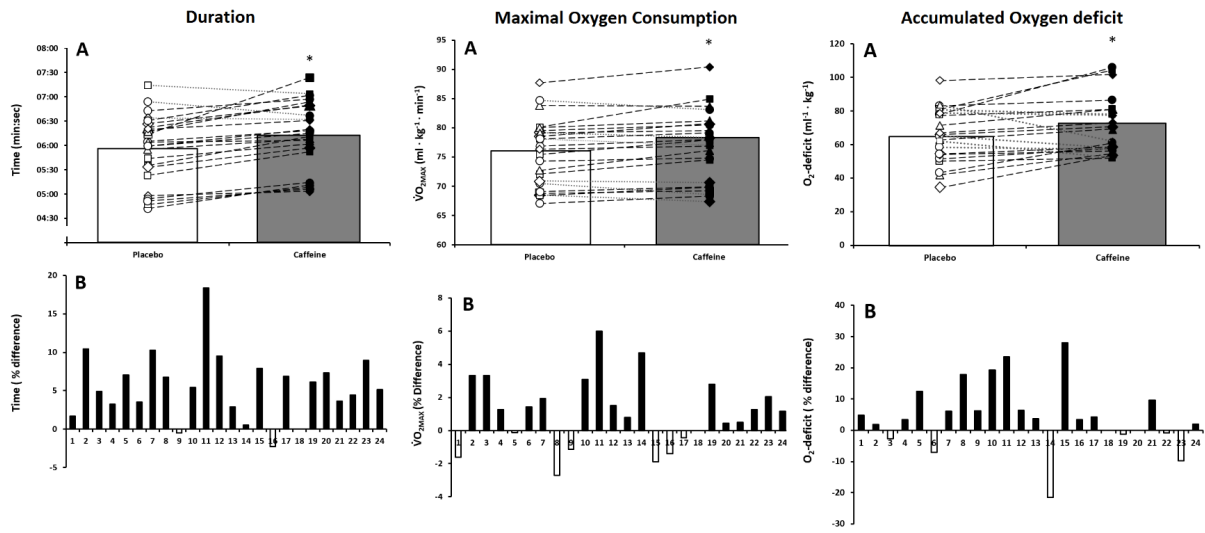
Study	What-duration-test type	Dosage (mg·kg <sup>-1</sup> ) <sup>1</sup>	Performance (velocity/time)	Quality Interference	Improved performance (N)	$\dot{V}O_2$ (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	HR (beat·min <sup>-1</sup> )	$V_E$ (L·min <sup>-1</sup> )	$O_2$ -deficit (ml · kg <sup>-1</sup> )	$O_2$ -cost (ml · kg <sup>-1</sup> · min <sup>-1</sup> )
<b>I</b> <i>Sea Level</i>	8 km DP - ~30 min (Time Trial)	6	↑ (4.0% faster)	Very Likely (p<0.01)	80.0% (n=10)	NM	↑	NM	NM	↔
<b>II</b> <i>Sea Level</i>	25 min DP (Time Trial two days in a row)	3 & 4.5	↑ (Day 1: 4.2%) ↑ (Day 2: 5.1%)	Very Likely (p≤0.04) Very Likely (p≤0.02)	87.5% (n=8) 87.5% (n=8)	↑	↑	↑	NM	↔
<b>III</b> <i>Altitude</i>	8 km DP - ~30 min (Time Trial)	4.5	↑/↔ (0.9% faster)	Likely/Possibly (p≤0.22)	69.0% (n=13)	↑	↑	↑	NM	↔
<b>IV</b> <i>Sea Level</i>	90% of $\dot{V}O_{2max}$ DP- ~10 min (Time until exhaustion) $\dot{V}O_{2max}$ performance test - ~6 min (Time until exhaustion)	4.5	↑ (20.5% longer) ↑ (5.5% longer)	Very Likely (p≤0.02) Very Likely (p<0.01)	69.0% (n=13) 69.6% (n=23)	↔	↑	↔	NM	↔
<b>V</b> <i>Sea Level</i>	80% of $\dot{V}O_{2max}$ - ~70-80 min (Time until exhaustion)	4.5	↑ (10.0% longer)	Very Likely (p≤0.02)	90.9% (n=11)	↔	↑	↔	NM	↔

↔ No difference between treatments, ↑ Significantly higher following CAF ingestion compared to PLA (p<0.05), ↓ significantly lower following CAF ingestion compared to PLA (p<0.05), NM = Not measured during performance testing.

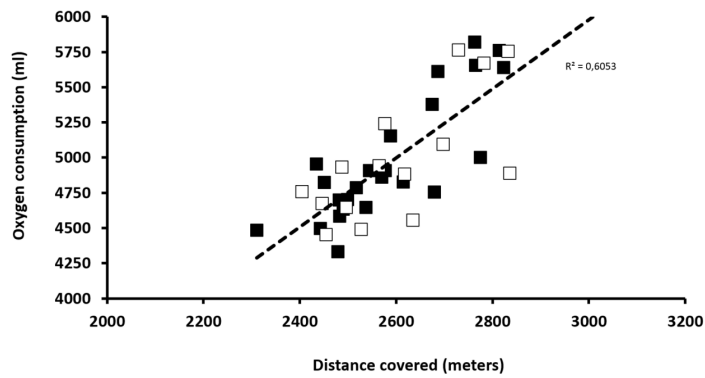
#### 4.2 Caffeine and $\dot{V}O_{2\max}$ (Studies II and IV)

The results from study II showed a higher  $\dot{V}O_2$ -uptake post CAF ingestion for DP. Specifically, the DP  $\dot{V}O_{2\text{peak}}$  increased by 2.5% from  $70.5 \pm 3.3$  to  $72.3 \pm 4.0$  ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) following CAF consumption during the 10 min all-out test (Fig 4.4). This corresponded to all test subjects (8 out of 8) reaching new DP  $\dot{V}O_{2\text{peak}}$  values during performance testing. Post measurements of DP  $\dot{V}O_{2\text{peak}}$  following the last performance test of the study revealed that no subjects reached similar  $\dot{V}O_{2\text{peak}}$  values to those observed during CAF TT testing during PLA conditions. CAF ingestion increased  $\dot{V}O_{2\max}$  while running (Fig 4.2). Specifically, results from study IV showed that subjects increased their  $\dot{V}O_{2\max}$  by 1.2% from  $75.9 \pm 5.8$  during PLA trials to  $76.7 \pm 6.0$  ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) after CAF consumption. Data showed that 16 out of 23 (69.6%) subjects improved their  $\dot{V}O_{2\max}$  following CAF ingestion. ICC values for  $\dot{V}O_{2\max}$  measurements were very high ( $\geq 0.95$ ) for both conditions, and  $O_2$ -kinetics were similar between CAF and PLA tests until the last minute of testing. Furthermore, statistical results from study IV showed the higher  $\dot{V}O_{2\max}$  after CAF ingestion contributed to improved running duration in the performance test, since adjustment for  $\dot{V}O_{2\max}$  reduced the CAF effect on increased running time from 19.4 s to 15.4 s.

In both studies II and IV the increase in  $\dot{V}O_{2\max}$  was associated with subjects reaching higher  $HR_{\max}$  and  $V_{E\max}$ . Specifically,  $HR_{\max}$  increased from  $185 \pm 6$  (PLA) to  $189 \pm 5$  (CAF3mg) and  $188 \pm 6$  (CAF4.5mg) beats per minute (bpm) in study II, and from  $192 \pm 6$  to  $194 \pm 8$  bpm in study IV. This corresponded to a 2.2% and 1.1% higher  $HR_{\max}$ , with 87.5% and 78.3% of test subjects reaching new  $HR_{\max}$  values. Maximal ventilation ( $V_{E\max}$ ) in study IV increased from  $187.8 \pm 17.8$  ( $\text{L} \cdot \text{min}^{-1}$ ) (PLA) to  $192.2 \pm 15.3$  during running CAF testing ( $p < 0.001$ ), and despite higher  $V_{E\max}$  breathing frequency (BF) was not significantly higher after CAF ingestion ( $60 \pm 7$  vs  $59 \pm 9$  breaths  $\cdot \text{min}^{-1}$ ,  $p < 0.07$ ). In study II HR increased from  $164 \pm 24$  (PLA) to  $170.8 \pm 21.6$  (CAF3mg) and  $172.1 \pm 22.8$  (CAF4.5mg) during DP CAF testing. Importantly, after adjustment for the increase in  $HR_{\max}$  the increase in  $\dot{V}O_{2\max}$  was attenuated to  $0.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $p < 0.001$ ), and furthermore with ~50%, after adjustment for  $V_{E\max}$ , was no longer significant ( $p = 0.11$ ). Furthermore, when running duration was adjusted for  $\dot{V}O_{2\max}$ ,  $V_{E\max}$  and  $HR_{\max}$  there was still a 11.7 s ( $p < 0.001$ ) improvement in running duration following CAF ingestion compared to PLA, to which anaerobic processes could most likely have contributed.



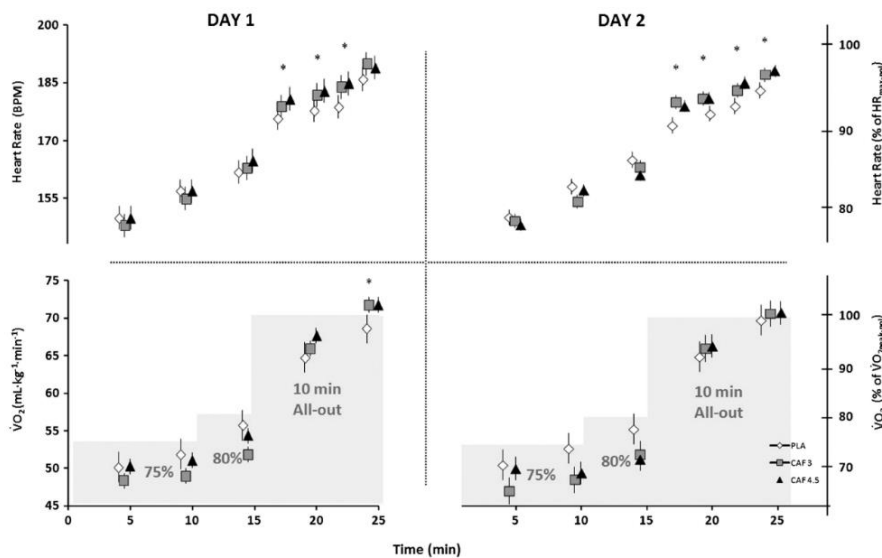
**Figure 4.2:** A) Individual and mean performance data for: Time,  $\dot{V}O_{2max}$  and  $O_2$ -deficit obtained during the  $\dot{V}O_{2max}$  performance tests after PLA (open symbols) or CAF (filled symbols). B) Percent change in running duration,  $\dot{V}O_{2max}$  and  $O_2$ -deficit following CAF consumption compared to PLA for each subject. Values are listed as means  $\pm$  SD. \* Significant different from PLA trials ( $p < 0.05$ )



**Figure 4.3:** Illustrates the correlation between distance covered during the 10 min all-out test in study II and oxygen consumption ( $ml \cdot min^{-1}$ ).

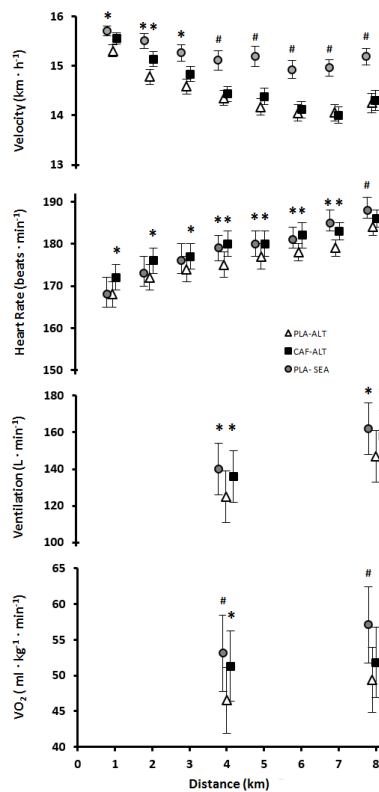
### 4.3 Caffeine and Fractional utilization of $\dot{V}O_{2max}$ (Studies II, III and V)

Fractional utilization of  $\dot{V}O_{2max}$  was increased following CAF ingestion during DP in study II. The increase in fractional utilization of  $\dot{V}O_{2max}$  was associated with higher velocity and improved TT performance (Fig 4.4, Appendix I). Furthermore, the results showed that with the same relative workloads and velocity (75 and 80% of  $\dot{V}O_{2max}$ ) no difference in oxygen consumption was observed between treatments (Fig. 4.4, Table 4.3). These results are comparable with the results from study V while running, where no difference in  $\dot{V}O_2$  was observed between PLA or CAF trials while maintaining 80%  $\dot{V}O_{2max}$  (Fig 4.7). During altitude testing (study III) CAF ingestion increased velocity and fractional utilization of  $\dot{V}O_{2max}$  in the first half of the 8 km TT, associated with improved performance (Fig 4.5). However, during the second half of the TT no difference in fractional utilization of  $\dot{V}O_{2max}$  was observed between treatments, associated with similar velocity and no difference in performance ( $p \leq 0.76$ ). Overall 8 km TT performance was not significantly improved ( $p \leq 0.22$ ), but the 0.9% reduction in time usage following CAF ingestion was associated with a "possible effect" based on magnitude-based statistics.



**Figure 4.4:** Higher fractional utilization of  $\dot{V}O_{2max}$  was observed post CAF (3 and 4.5 mg.kg<sup>-1</sup>) ingestion in study II, associated with higher velocity, HR and improved exercise performance. Data are given as mean  $\pm$  SD. \* Significant difference between treatments ( $p < 0.05$ ).

The increase in fractional utilization of  $\dot{V}O_{2max}$  was associated with higher HR during CAF trials compared to PLA in studies II and III. Specifically, HR increased from  $180 \pm 4$  to  $184 \pm 3$  bpm in study II and from  $184 \pm 7$  to  $187 \pm 7$  bpm in study III (Fig 4.4, 4.5). These results are similar to the findings in study I, where HR increased from  $174 \pm 2$  to  $179 \pm 3$  bpm during the 8 km TT following CAF ingestion compared to PLA. However,  $\dot{V}O_2$  during TT testing was not measured, to ensure optimal test conditions for test subjects. In contrast to the TT results, no difference in fractional utilization of  $\dot{V}O_{2max}$  or HR was detected between subjects while maintaining the same workload/velocity following CAF or PLA ingestion in studies III and V. These results are comparable to all standardized warm-up  $\dot{V}O_2$  results performed prior to all performance tests (Table 4.3, Appendix II).



**Figure 4.5:** Velocity, HR,  $V_E$  and  $\dot{V}O_2$  displayed as means during PLA and CAF 8 km DP TTs at sea level, and acute altitude (2000 m) from study III. Values are listed as means  $\pm$  SD. \* Significant difference between PLA and CAF altitude ( $p < 0.05$ ). # Significant difference between PLA sea level and CAF/PLA altitude ( $p < 0.05$ ).

#### ***4.4 Caffeine, O<sub>2</sub>-deficit and lactate accumulation (Studies I-V)***

In study IV, O<sub>2</sub>-deficit was on average increased by 10.1%, from 63.1±18.2 in PLA to 69.5±17.5 (ml·kg<sup>-1</sup>) during CAF trials (Fig 4.2). These results corresponded to 16 out of the 23 subjects (69.6%) having higher O<sub>2</sub>-deficits after CAF ingestion. Calculations show anaerobic processes (O<sub>2</sub>-deficit) covered approx. 15% of total energy costs during both CAF and PLA trials. Specifically, O<sub>2</sub>-deficit covered 14.7±3.1% and 15.0±2.7% of total O<sub>2</sub>-cost for PLA and CAF trials respectively. Importantly, when the running duration for the performance test was adjusted for both oxygen deficit and LA- concentration, the effect of CAF ingestion was reduced from 19.4 to 13.6 s (p<0.001). For measurements of oxygen deficit, the ICC was 0.61 and 0.64 respectively for PLA and CAF trials. The increase in O<sub>2</sub>-deficit was associated with a 7.0% increase in blood LA- accumulation post performance testing. Specifically, blood LA- values increased from 7.94 ±1.0 mM post the PLA trials to 8.54±1.0 mM post CAF testing (p<0.001).

Furthermore, in all five studies in the thesis, higher LA- accumulation was observed post CAF performance trials compared to PLA. The increases in LA- were observed independent of TT; time until voluntary exhaustion; duration (10-110 min); activity (DP vs. running); or test conditions (sea level vs. altitude) (Table 4.2).

Furthermore, in study III, CAF ingestion resulted in a significant decrease in HCO<sub>3</sub><sup>-</sup> values after finishing the performance tests. Specifically, HCO<sub>3</sub><sup>-</sup> values after finishing the performance trials were 16.5±2.3 and 14.5±1.9 (PLA) compared to 13.7±2.0 and 12.9±1.7 (CAF) respectively for the 8 km and 90% C-PT. These results were also reflected in a reduction in blood pH between treatments post the 8 km TT from 7.33±0.05 (PLA) to 7.29±0.05 (CAF) (p<0.01).

#### ***4.5 Caffeine and work economy (Studies I-V)***

Collectively the results from all five studies showed no change in work economy, measured as oxygen cost between PLA and CAF trials, during either low (60-65%) or high intensity exercise (80-90 % of  $\dot{V}O_{2max}$ ) (Table 4.3). Furthermore, these results were observed regardless of activity (DP vs running), test conditions (sea level vs. acute altitude) and duration of the test (incremental testing vs. time until task failure). Results also showed that the increase in  $\dot{V}O_2$ , HR,  $V_E$  or LA- was only observed with increased work output/velocity (Table 4.2, Figure 4.5).



However, in study V, the improved running duration following CAF consumption was associated with increased CMJ heights (Fig 4.6), but no difference in work economy ( $O_2$ -cost) during performance testing. Furthermore, in studies I and II, subjects used similar numbers of DP strokes to complete the tests while maintaining a higher work output, but higher  $\dot{V}O_2$  was observed during the CAF trials. Specifically, subjects used  $1932 \pm 132$  (PLA) and  $1942 \pm 126$  (CAF) strokes to complete the 8 km time trial in study I. In study II they used  $618 \pm 42$ ,  $625 \pm 32$  and  $619 \pm 26$  strokes during the 10 min all-out test on day one, and  $635 \pm 32$ ,  $621 \pm 22$  and  $623 \pm 29$  on day two respectively for PLA, CAF 3 and  $4.5 \text{ mg} \cdot \text{kg}^{-1}$ .

**Table 4.2:** Blood LA- post performance testing for studies I-V.

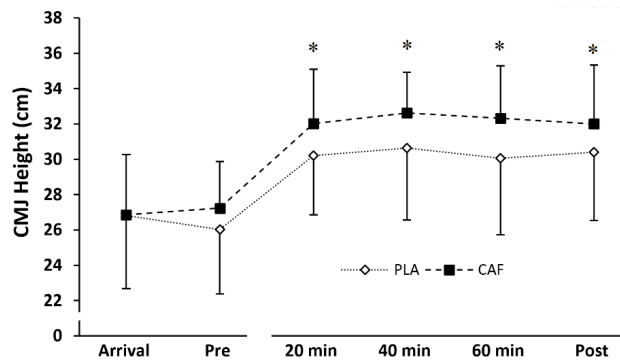
Study	Activity	Treatment	Post
<b>I</b> (DP- Sea level)	DP- Time trial	PLA	6.7±0.3
		CAF (6 mg · kg <sup>-1</sup> )	8.2±0.3*
<b>II</b> (DP- Sea level)	DP- Time trial	PLA	6.2±0.2
		CAF (3 mg · kg <sup>-1</sup> )	7.7±0.6*
		CAF (4.5 mg · kg <sup>-1</sup> )	7.5±2.7*
<b>III</b> (DP-Altitude)	DP- Time trial	PLA	6.9±1.5
		CAF (4.5 mg · kg <sup>-1</sup> )	8.2±1.6*
	DP- Time until voluntary exhaustion	PLA	8.3±2.1
		CAF (4.5 mg · kg <sup>-1</sup> )	9.8±2.1*
<b>IV</b> (Running- Sea level)	Running - Time until voluntary exhaustion	PLA	8.00±1.13
		CAF (4.5 mg · kg <sup>-1</sup> )	8.65±0.94*
<b>V</b> (Running- Sea level)	Running - Time until voluntary exhaustion	PLA	3.5±1.4
		CAF (4.5 mg · kg <sup>-1</sup> )	4.22±1.7*

Data are given as mean ± SD. \* Significant difference between treatments ( $p < 0.05$ ). Results from study II are taken from day I.

**Table 4.3:**  $\dot{V}O_2$  ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) during low (60-65%) and high intensive exercise (80-90% of  $\dot{V}O_{2\text{max}}$ ) when DP (I-III) and running (IV-V) in sea level and altitude conditions following PLA or CAF ingestion.

Study	Treatment	Low intensive exercise % of $\dot{V}O_{2\text{max}}$		High intensive exercise of $\dot{V}O_{2\text{max}}$
		60% ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )	65% ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )	80-90% ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )
<b>I</b> (DP- Sea level)	PLA	36.7±4.0	44.3±5.8	<b>No Data</b>
	CAF (6 mg)	36.3±2.5	43.0±3.3	
<b>II</b> (DP- Sea level)	PLA	41.8±3.5	45.2±4.2	55.8±1.0
	CAF (3 mg)	40.9±2.5	44.2±3.6	51.9±4.8
	CAF (4.5mg)	42.0±3.1	44.6±3.5	54.4±5.9
<b>III</b> (DP- Altitude)	PLA	33.2±4.0	35.1±3.6	50.3±4.7
	CAF (4.5mg)	32.7±3.6	35.5±3.6	50.3±6.1
<b>IV</b> (Running- Sea level)	PLA	44.8±3.7	49.3±4.2	65.9±3.3
	CAF (4.5mg)	45.1±3.8	49.6±4.1	66.0±2.9
<b>V</b> (Running- Sea level)	PLA	<b>No data</b>		57.7±3.8
	CAF(4.5mg)			58.5±4.3

Data are given as mean ± SD. \* Significantly different from PLA ( $p < 0.05$ )



**Figure 4.6:** CMJ heights displayed as means from study V while running at 80% of  $\dot{V}O_{2\text{max}}$  until voluntary exhaustion. Values are listed as means ± SD. \* Significant difference between PLA and CAF ( $p < 0.05$ ).

**Table 4.4** General summary of physiological ( $\dot{V}O_2$ , HR,  $V_E$ , RER LA-) and psychological (RPE and pain) responses following CAF ingestion during submaximal steady state exercise loads from studies I-V. For specific data from the different test, see original paper at the end of the thesis

Study	Activity	$\dot{V}O_2$ (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	HR (beat·min <sup>-1</sup> )	$V_E$ (l · min <sup>-1</sup> )	RER	LA- (mmol)	RPE (6-20)	Pain (1-10)
<b>I</b>								
(DP- Sea level)	DP	↔	↔	↔	↔	↔	↓	Not measured
<b>II</b>								
(DP- Sea level)	DP	↔	↔	↔	↔	↔	↓	↓
<b>III</b>								
(DP-Altitude)	DP	↔	↔	↔	↔	↑	↓	↓
<b>IV</b>								
(Running- Sea level)	Running	↔	↔	↑	↔	↑	↔	Not measured
<b>V</b>								
(Running- Sea level)	Running	↔	↔	↔	↔	↔	↓	↓

↔ No difference between treatments, ↑ Higher following CAF ingestion compared to PLA, ↓ Lower following CAF ingestion compared to PLA ( $p < 0.05$ ). If physiological or psychological parameters were increased ↑ or decreased ↓ at least three of the four standardized submaximal steady state exercise loads where changed significantly ( $p < 0.05$ ) following CAF ingestion.

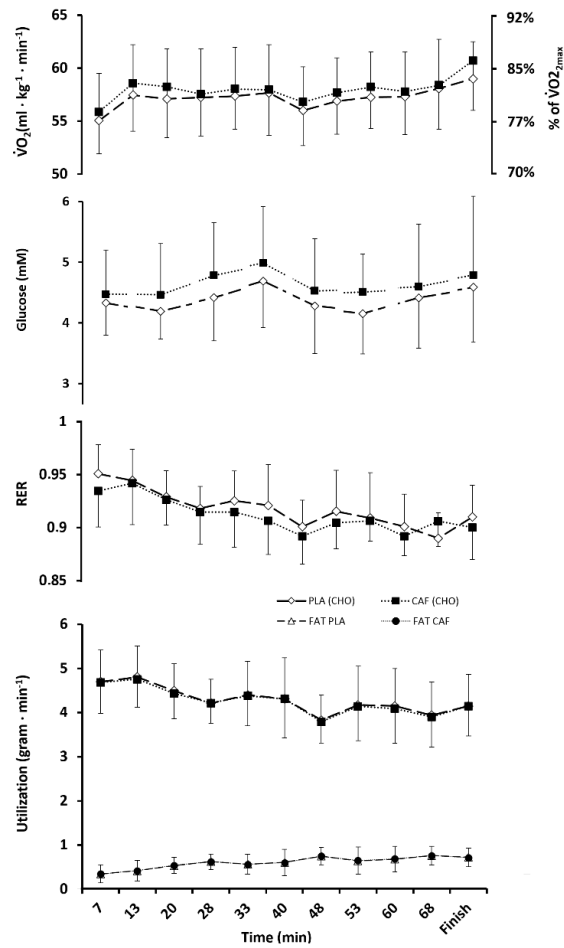
#### 4.6 Caffeine and substrate utilization (Studies I-V)

When comparing substrate utilization measured as RER values, no difference was observed between treatments (Table 4.4). This was also independent of muscular usage (DP vs running), duration (5 vs 90 min), and level of exercise intensity (low, 60-65% or high, 80-90% of  $\dot{V}O_{2max}$ ) (Table 4.4).

In study I, the observation of similar RER was associated with no difference in plasma concentration of FFA or glycerol between treatments (4.5). In study V, calculations of carbohydrate and fat oxidation during the performance trial showed no change following CAF ingestion. The stable and high glucose values observed in all studies during both PLA and CAF performance trials indicate that no subjects became hypoglycemic.

However, when comparing DP and running RER results for the same relative workload, higher RER values were observed for DP compared to running. Specifically, RER values for DP in study I at 60% and 70% of  $\dot{V}O_{2max}$  were  $0.97 \pm 0.01$  and  $0.98 \pm 0.01$  compared to  $0.91 \pm 0.01$  and  $0.89 \pm 0.01$  while running. Furthermore, LA- concentrations were also higher for DP compared with running at the same submaximal intensities (e.g. 70% DP,  $3.2 \pm 0.3$  mM, vs running,  $1.3 \pm 0.2$

mM), but similar concentrations between exercises were reached after finishing the  $\dot{V}O_{2max}$  tests while DP ( $8.1 \pm 0.4$  mM) and running ( $8.1 \pm 0.3$  mM).



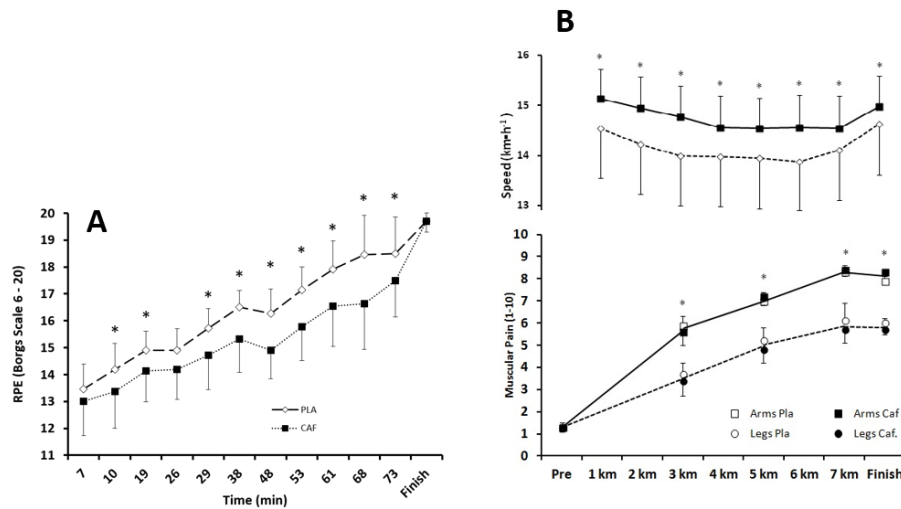
**Figure 4.7:**  $\dot{V}O_{2max}$ , glucose, RER and substrate utilization of carbohydrates and fat while running at 80% of  $\dot{V}O_{2max}$  until voluntary exhaustion. Values are listed as means  $\pm$  SD. \* Significant difference between PLA and CAF ( $p < 0.05$ ).

#### 4.7 Caffeine RPE and Pain (Studies I-V)

With increasing workload/duration (e.g. % of  $\dot{V}O_{2max}$ ) RPE increased in all five studies independent of product consumption, as illustrated in Figure 4.8A. However, while performing the

same relative workload, lower RPE and pain were observed during CAF trials compared to PLA (Table 4.4, Fig 4.8). As illustrated in Figure 4.8A, a clear reduction in RPE was displayed when subjects performed the same relative workload during running until voluntary exhaustion in study V. However, similar RPEs were observed between treatments at voluntary exhaustion, except a 10% longer duration was sustained during the CAF trial compared to PLA.

During TT studies at sea level (I-II) subjects maintained higher velocity during CAF tests compared to PLA while reporting similar RPE and pain (Fig 4.8B). In study III during the acute altitude test, similar responses for RPE and pain were observed as at sea-level conditions, but here the increased velocity in the first 4 km of the TT was not sustained in the second half.

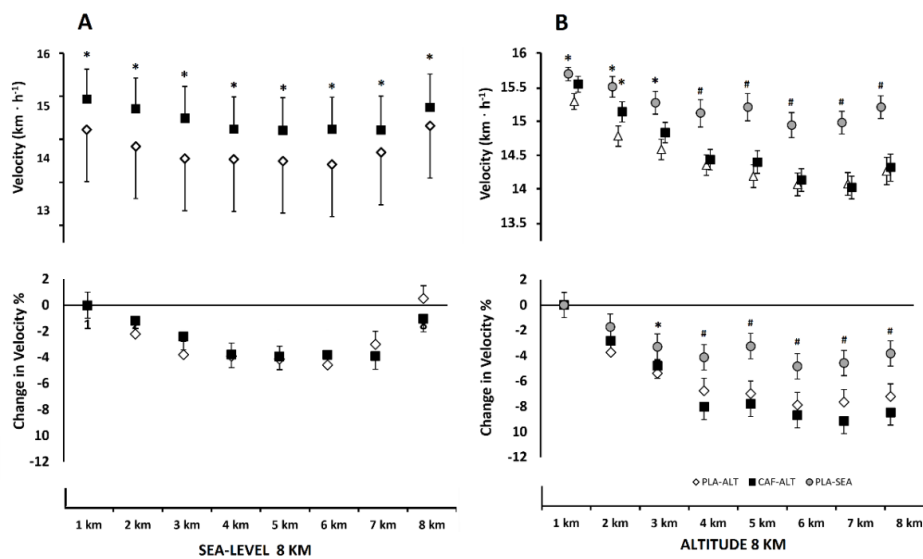


**Figure 4.8:** A: Reduction in RPE following CAF ingestion while running at equal velocity (approx. 80% of  $\dot{V}O_{2max}$ ), but similar RPE observed at exhaustion with improved running duration. B: Similar muscular pain during the 8 km DP TT but with higher velocity in the CAF trial compared to PLA. Figures taken from Paper I and V. Data are given as mean  $\pm$  SD. \* Significant difference between treatments ( $p < 0.05$ ).

#### 4.8 Caffeine and pacing strategy (Studies I-III)

No change in pacing strategy was observed between CAF and PLA TTs in studies I and II during sea level testing (Figure 4.4 and 4.8A). In the two studies, subjects increased velocity during CAF trials, but pacing throughout the TTs was similar, independent of treatment (Fig 4.8). In study III there was a tendency ( $p \leq 0.17$ ) toward a change in pacing strategy between CAF and PLA TTs. Results displayed a higher starting velocity in the first half of the TT, but relative to CAF's starting pace the reduction in velocity was larger in the second half compared to PLA. The larger

percentage reduction in velocity during the CAF trial was not significant ( $p \leq 0.17$ ) compared to PLA, but magnitude statistics showed a "possible effect" (Fig 4.8B). Furthermore, the pacing strategies both for PLA and CAF trials at altitude were different compared to sea-level testing (Fig 4.8). During sea level conditions a more even pacing was observed during the 8 km TT (Fig 4.9B), compared to a steady drop in intensity during acute altitude testing.



**Figure 4.9:** A: Higher velocity but similar pacing during CAF and PLA trials at sea level 8 km TT testing. B: Higher velocity and a small change in pacing during the first 4 km of the 8 km TT during altitude testing. Pacing was different between sea-level and altitude testing independent of product consumption. Figures based on results from Paper I and III. Data are given as mean  $\pm$  SD. \* Significant difference between treatments ( $p < 0.05$ ).

#### 4.9 Caffeine and blood data (Studies I-V)

Plasma CAF concentrations increased following CAF ingestion, and the highest plasma CAF concentrations were reached between 60-90 minutes post ingestion (Studies I, II and III). Plasma CAF concentration was dependent on CAF dosage ingested ( $3-6 \text{ mg} \cdot \text{kg}^{-1}$ ) (Fig 4.9). Post measurements of LA<sup>-</sup>, glucose (studies I-V), adrenaline (studies I-III), FFA, glycerol (study I), and CK (24h) (studies II and V) were higher, whereas HCO<sub>3</sub><sup>-</sup> and blood pH (study III) were lower (Table 4.5).

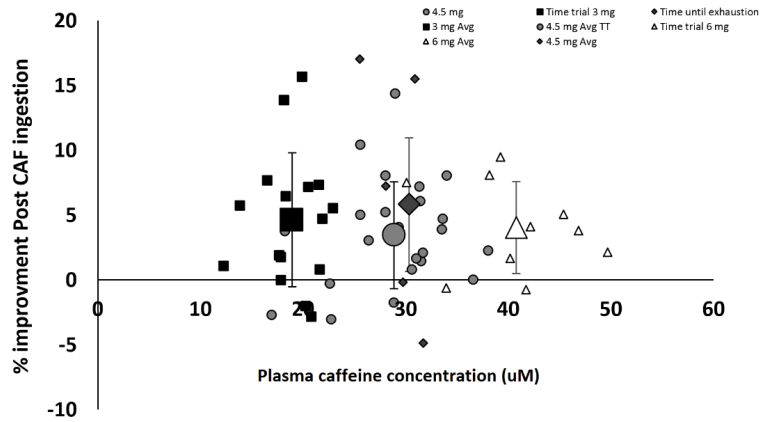
During submaximal exercise no difference was observed for the same parameters during the standardized warm-ups (incremental testing). In study IV, however, higher LA<sup>-</sup> values were also

observed during submaximal testing following CAF ingestion. (Table 4.4), and in study III a reduction in  $\text{HCO}_3^-$  was already evident post the standardized warm-up.

**Table 4.5** Summary of blood data post performance testing from the five studies.

Study	What	Dosage ( $\text{mg}\cdot\text{kg}^{-1}$ )	Caffeine ( $\mu\text{M}$ )	LA- ( $\text{mM}$ )	Glucose ( $\text{mM}$ )	Adrenaline ( $\text{nM}$ )	FFA ( $\text{mM}$ )	CK ( $\text{U/L}$ )
I	8 km DP (Time Trial)	6	38.7 $\pm$ 1.2	↑	↑	↑	↑	NM
II	10 min DP (Time Trial, two days in a row)	3	19.7 $\pm$ 0.8	↑	↑	↑	NM	↑
		4.5	28.6 $\pm$ 1.3					
III	8 km DP (Time Trial altitude) 90% of $\dot{V}O_{2\text{max}}$ DP (Time until voluntary exhaustion)	4.5	28.1 $\pm$ 7.6	↑	↑	↑	NM	NM
		4.5	30.3 $\pm$ 2.5	↑	↑	↑	NM	NM
IV	$\dot{V}O_{2\text{max}}$ performance test (Time until voluntary exhaustion)	4.5	NM	↑	↑	NM	NM	NM
V	80% of $\dot{V}O_{2\text{max}}$ (Time until voluntary exhaustion)	4.5	NM	↑	↑	NM	NM	↑

Data are given as mean  $\pm$  SD. NM = Not measured during performance testing.



**Figure 4.9:** Improvements following ingestion of different CAF dosages compared to plasma CAF concentrations in blood. Data based on results from studies I, II and III. Data are given as mean  $\pm$  SD.

#### 4.10 Caffeine and other performance data (Studies I-V)

No differences were observed between PLA and CAF regarding responses to questionnaires on “current fitness”, motivation, and amount of sleep (hours) before or after finishing performance testing in any of the five studies (Table 4.6) Motivation was on average rated as "very high", current fitness as "high" and no difference in sleep was observed the day prior to performance testing.

**Table 4.6** Summary of questionnaires where subjects rated motivation, current fitness and registered approx. hours of sleep prior to the performance testing that day.

Study	Pre PLA trial			Pre CAF trial			Post PLA trial		Post CAF trial	
	Motivation (1-100)	Current Fitness (1-100)	Sleep (Hour)	Motivation (1-100)	Current Fitness (1-100)	Sleep (Hour)	Motivation (1-100)	Current Fitness (1-100)	Motivation (1-100)	Current Fitness (1-100)
<b>I</b>	84±12	69±14	8.2±1.2	85±11	68±15	8.4±1.0	84±13	68±15	86±16	79±14*
<b>II - Day I</b>	78±15	69±16	8.5±0.9	83±15	74±11	8.1±0.8	79±16	67±13	85±10	79±9
<b>Day II</b>	82±14	71±15	8.7±0.7	81±13	70±12	8.7±0.7	81±16	73±17	86±10	75±12
<b>III - 8 km</b>	81±11	74±8	7.9±1.5	81±9	71±1.5	8.2±1.0	83±13	71±14	83±8	67±12
<b>90%</b>	80±12	69±10	7.7±0.8	82±12	73±11	7.6±1.7	77±10	61±10	80±15	69±11
<b>IV - Test I</b>	77±14	62±11	7.7±1.0	79±16	62±13	7.9±1.4	79±17	62±12	82±13	67±15
<b>Test II</b>	76±14	62±13	8.0±0.9	76±14	63±12	7.9±1.4	78±15	65±14	80±14	70±14
<b>V</b>	79±11	67±9	7.2±1.3	70±16	63±15	7.7±1.0	83±10	74±11	77±15	76±17
<b>Overall</b>	<b>80±3</b>	<b>68±4</b>	<b>8.0±0.5</b>	<b>80±5</b>	<b>68±5</b>	<b>8.1±0.4</b>	<b>81±3</b>	<b>68±5</b>	<b>82±3</b>	<b>72±8</b>
<b>Word rating</b>	<b>"Very high"</b>	<b>"High"</b>		<b>"Very high"</b>	<b>"High"</b>		<b>"Very high"</b>	<b>"High"</b>	<b>"Very high"</b>	<b>"High"</b>

Data are given as mean ± SD. \* Significantly different from PLA ( $p < 0.05$ )

Furthermore, questionnaires revealed that most subjects were unable to sense which product they received during the different trials, and had followed instructions given regarding training, food, liquid and CAF consumption in the 48 h prior to each C-PT.

**Table 4.7** Summary of questionnaires where subjects stated which product they believed they had consumed pre (approx. 90 min after CAF or PLA consumption) and post-performance testing.



Study	PLA						CAF					
	Pre			Post			Pre			Post		
	PLA	UNSURE	CAF	PLA	UNSURE	CAF	PLA	UNSURE	CAF	PLA	UNSURE	CAF
<b>I</b>	30%	50%	20%	30%	40%	20%	30%	50%	20%	30%	30%	40%
<b>II</b>	18.5%	62.5%	18.5%	44%	44%	12%	18.5%	62.5%	18.5%	12%	44%	44%
<b>III</b>	27%	57.5%	15.5%	31%	57.5%	11.5%	23%	43%	31%	15%	42.5%	42.5%
<b>IV</b>	20%	61%	20%	25%	55%	20%	20%	55%	25%	33%	33%	34%
<b>V</b>	27%	46%	27%	27%	46%	27%	56%	9%	35%	36%	37%	27%
<b>Overall I-V</b>	<b>24%</b>	<b>57%</b>	<b>19%</b>	<b>32%</b>	<b>50%</b>	<b>18%</b>	<b>26%</b>	<b>50%</b>	<b>26%</b>	<b>24%</b>	<b>38%</b>	<b>38%</b>

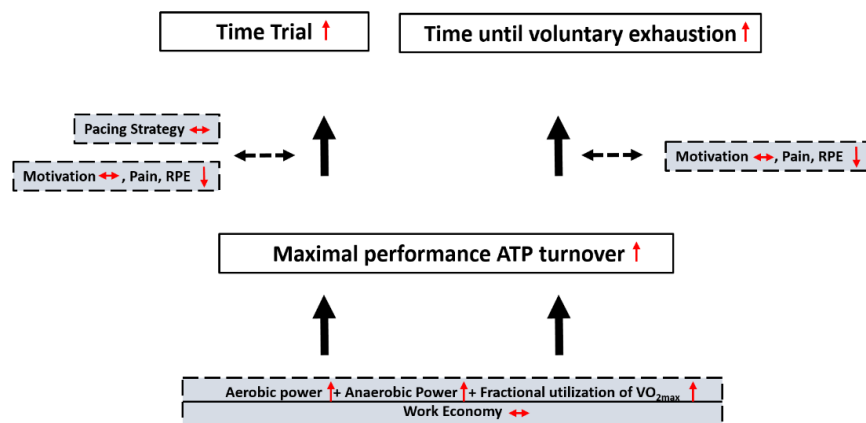
*Green color* indicates "right answer", *White color* "Unsure" and *Red color* "wrong answer". Data are mean values (%)

## 5. DISCUSSION

### 5.1 Caffeine and endurance performance (Studies I-V)

The main findings of the thesis were that CAF ingestion improved exercise performance during both TT (studies I-III) (~4%) and time until voluntary exhaustion (studies III-V) (~10%) (Table 4.1). Approximately 75% of all test subjects improved their endurance performance regardless of whether exercise was performed at sea level, at altitude, on consecutive days, or when undertaking exercise activities mainly limited by arm (DP) or leg muscles (running) (Fig 4.1 and 4.2).

A novel approach in the thesis was the use of top trained endurance athletes ( $\dot{V}O_{2\max} \geq 70 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) for whom the existing literature lacks solid results. Based on the results no dose response was observed after ingesting 3, 4.5 or 6  $\text{mg} \cdot \text{kg body wt}^{-1}$  CAF prior to exercise (Fig 4.9). Improvements following CAF ingestion were associated with a reduction of perception of pain and effort (RPE) during standardized workloads in all studies (Fig 4.6 and Table 4.4). Other mechanisms explaining CAF's ergogenic potential and novel findings were the increase in  $\dot{V}O_{2\max}$  (Fig 4.2),  $O_2$ -deficit (Table 4.2), fractional utilization of  $\dot{V}O_{2\max}$  (Fig 4.4), and the absence of change in work economy (Table 4.3), pacing strategy (Fig 4.9) or substrate utilization (Fig 4.7) following CAF ingestion.



**Figure 5.1:** In this thesis the different physiological and psychological factors from Figure 1.1 were evaluated after subjects consumed CAF/PLA prior to time trial and time until voluntary exhaustion testing. The red arrows indicate whether CAF ingestion improved ↑, reduced ↓, or led to no change ↔ after CAF consumption.

### 5.2 Caffeine and $\dot{V}O_{2max}$ (Studies II and IV)

There is today a broad agreement that  $\dot{V}O_{2max}$  is an important factor in explaining endurance sport performance (5, 6, 11, 21, 37, 49). In the present thesis  $\dot{V}O_{2max}$  was increased both in DP and running in studies II and IV. Results in both studies were associated with improved performance and increased HR and  $V_E$  following CAF ingestion compared to PLA. To the researcher's knowledge, no studies have so far specifically investigated or found that CAF ingestion can increase maximal oxygen consumption. Certainly, CAF ingestion has been observed to improve exercise performance despite the use of different muscle groups or test protocols in several studies (Appendix I) (24, 47, 83, 99, 100, 142). Furthermore, in the few studies that have measured  $\dot{V}O_2$  during TT performances, the increased velocity has been associated with a higher oxygen consumption (41, 57, 63, 80, 85, 158).

During maximal aerobic exercise, the "classical view" of maximal oxygen uptake ( $\dot{V}O_{2max}$ ) is that maximal rates of oxygen utilization and production of ATP in skeletal muscles are limited in most circumstances by the heart's ability to pump and deliver adequate amounts of oxygen to the working muscles (6, 112). It is estimated that 70-85% of limitations in  $\dot{V}O_{2max}$  during sea level testing are linked to cardiac output ( $\dot{Q}_c$ ), meaning that in most situations  $\dot{V}O_{2max}$  is primarily limited by  $\dot{Q}_c$  (6, 129). Supporting this assumption is the observation that elite endurance athletes primarily have higher  $\dot{V}O_{2max}$  due to higher  $\dot{Q}_c$  (17, 153). Furthermore, if  $\dot{Q}_c$  mainly limits  $\dot{V}O_{2max}$  in humans,  $\dot{V}O_{2max}$  is a physiological characteristic bound by the parametric limits of the Fick equation (17, 112, 152). The principles of the Fick equation state that if the arteriovenous oxygen difference (a-vO<sub>2</sub> diff) is unchanged, a higher HR and similar stroke volume (SV) should increase both  $\dot{Q}_c$  and  $\dot{V}O_2$  (17, 152). The increase in  $\dot{V}O_2$  associated with increased HR in studies II, and IV would fit very well with this theory, and might explain the improved oxygen consumption following CAF ingestion.

Increased HR associated with increased velocity during TT performance following CAF ingestion is one of the most consistent observations (Appendix I) (3, 21, 23, 47, 78, 80, 100, 140, 142). Although the inhibition of adenosine receptors has been closely linked to reduction in pain and RPE during steady state exercise (54, 68, 75) (see more in section 4.7), inhibition of adenosine receptors could also affect cardiovascular and pulmonary functions, since they are expressed in the human heart and lungs (68, 70). Certainly, the CAF dosages ingested in studies

II and IV would lead to peak plasma caffeine concentrations of 25-35  $\mu\text{M}$  (Fig 4.9), reducing adenosine receptor activation (70). In the human heart,  $A_1$  receptors, among others, inhibit adenylyl cyclase (69, 174), and a blockage of  $A_1$  receptors in the heart may increase the response to sympathetic activity, potentially removing a “safety break” in the heart resulting in improved contractility and/or pumping capacity (68, 133). Mice lacking  $A_1$  and  $A_2$  receptors is observed to have no effect of CAF on HR or activity compared to normal wild mice (182). The increase in HR during studies II and IV could therefore very well be associated with an inhibition of adenosine receptors (68, 182). Furthermore, although not measured in study IV, several studies have found CAF ingestion increases the release of epinephrine, as observed in study II (and studies I and III in this thesis). The observed increase in adrenaline could also have contributed to an increase in HR during CAF trials, and/or heart contractility qualities (108). Indeed, increased adrenaline in the bloodstream is observed to stimulate adrenergic receptors, resulting in higher HR and increased force of each individual heart contraction (51, 108).

An inhibition of  $A_1$  receptors in the lungs following CAF could also affect pulmonary functions during exercise (68, 70). Specifically, inhibition of the  $A_1$ -receptor in the lungs has been observed to lead to a bronchial dilation in the lungs, by which pulmonary functions for gas exchange during exercise might be improved (6, 68). Studies have found CAF to be a strong ventilatory stimulant, increasing both the sensitivity of peripheral chemoreceptors in untrained subjects and  $V_E$  during exercise in highly trained endurance athletes (28-30, 40, 103, 144). In study IV, no change in lung function (FENO, FEV1, FVC or FEF) was observed pre or post  $\dot{V}O_{2\text{max}}$  performance test between treatments. However, during sub-maximal and maximal exercise, higher  $V_E$  was observed following CAF ingestion. The observed increase in  $V_E$  in studies II and IV (also in study III) would have improved conditions for  $O_2$  saturation during maximal and high intensity exercise by e.g. increasing the mean transit time for the red blood cells passing the alveolar ducts (28, 165). Since no difference in breathing frequency (BF) was observed in either of the two studies, CAF at least improved the  $V_E$  vs BF ratio during maximal exercise. Several studies have found that maximal  $V_E$  indeed can limit maximal oxygen consumption for highly trained athletes during sea level exercise (28, 29, 165). This is perhaps even more evident during hypoxic testing where pulmonary function and  $O_2$  saturation directly represent limiting factors for  $\dot{V}O_{2\text{max}}$  and performance as observed in study III (73, 126, 176). It is also reported that an increase in  $V_E$  can counteract increased vagal nervous drive to the heart

due to input from pulmonary stretch receptors. Therefore increased  $V_E$  following CAF ingestion might also have contributed to the increased HR observed in studies I, II, III and IV (77, 155).

In the present thesis, neural activity, SV, a-vO<sub>2</sub> diff and  $\dot{Q}_c$  were not measured in any studies, so the effects of the increased HR<sub>max</sub> and V<sub>E</sub>max on O<sub>2</sub> delivery following CAF ingestion during DP and running should be interpreted with caution. Still, during both pre and the two main PLA tests in study IV, subjects were unable to reach the values for HR<sub>max</sub>, V<sub>E</sub>max and  $\dot{V}O_{2max}$  that were achieved during CAF testing. This was reflected in very high ICC values for  $\dot{V}O_{2max}$  of 0.95 during both PLA and CAF trials. Furthermore, the observation that the increase in  $\dot{V}O_{2max}$  after CAF consumption was attenuated to 0.7 ml · kg<sup>-1</sup> · min<sup>-1</sup> (p<0.001) after adjustment for the increase in HR<sub>max</sub>, and further more with ~50%, after adjustment for V<sub>E</sub>max. The changes in HR and V<sub>E</sub> therefore seem important for explaining increased  $\dot{V}O_{2max}$  following CAF ingestion. The observation of similar  $\dot{V}O_2$ , HR and V<sub>E</sub> in studies II and IV (and in all other studies in the thesis) while performing the same relative submaximal workloads (% of  $\dot{V}O_{2max}$ ) (Table 4.4), indicate that increases in these three physiological parameters were only observed with increased work intensity. These results are also comparable to observations from several other studies in the area (Appendix I and II) (34, 40, 53, 80, 140, 142). Furthermore, when running duration was adjusted for  $\dot{V}O_{2max}$ , V<sub>E</sub>max and HR<sub>max</sub> in study IV, duration was reduced from with 39.6 %, from 19.4 to 11.7s. It is therefore tempting to suggest that the ability to improve running duration during performance testing in study IV was closely related to the observed increase in  $\dot{V}O_{2max}$ , HR<sub>max</sub> and V<sub>E</sub>max, thus improving conditions for O<sub>2</sub> delivery and production of ATP in working muscles.

Results from studies II and IV therefore indicates that CAF ingestion can increase aerobic power, consequently increasing athletes' maximal performance ATP turnover, which would explain exercise improvements post ingestion.

### ***5.3 Caffeine and Fractional utilization of $\dot{V}O_{2max}$ (Studies I, II and III)***

The increase in  $\dot{V}O_{2max}$  following CAF ingestion indicates that the maximum rate at which oxygen is taken up and used for the production of ATP is increased (6, 49). However, the period of time that this specific intensity could be sustained would not be more than a few minutes (6, 48). During prolonged exercise tasks ( $\geq 10$  min) the fraction utilization of  $\dot{V}O_{2max}$  would therefore represent an important physiological parameter for explaining improved exercise performances

(33, 36, 39, 104, 120). Certainly, an increased velocity following CAF ingestion as observed in all TT studies would require a higher production of ATP if work efficiency was not improved, based on Di Prampero's model (48). No difference in work economy was evident as an effect of CAF ingestion in the studies in this thesis (further detail is provided in section 5.5). Therefore, one would expect increased oxygen consumption if the SV and a-vO<sub>2</sub> were unchanged since velocity and HR were higher during caffeine TT testing (section 5.2) (17, 152). Despite no measurements of  $\dot{V}O_2$  being performed in Study I, the results show an HR increase of 5 beats per min (BPM), and a velocity increase of  $0.6 \pm 0.04 \text{ km} \cdot \text{h}^{-1}$  during the CAF 8 km TT compared to PLA testing. Results from submaximal exercise intensities from the same study showed an increase of  $0.6 \text{ km} \cdot \text{h}^{-1}$  leading to an increase in O<sub>2</sub>-cost of  $3.2 \pm 0.5 \text{ ml}^{-1} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  which would require an increase of 6 bpm (assuming similar a-vO<sub>2</sub> diff and SV). Since HR was  $5 \pm 1$  bpm higher during the CAF TT compared to PLA, it suggests that the increased HR improved oxygen uptake and thereby fractional utilization of  $\dot{V}O_{2\text{max}}$ . This assumption was confirmed by the results from studies II and III with the observations of increased  $\dot{V}O_2$ , velocity and HR. Higher  $\dot{V}O_2$  values have also been observed with increased HR in previous studies following CAF ingestion (57, 63, 85, 158).

However, to date few studies have discussed the potential mechanisms that may explain higher fractional utilization of  $\dot{V}O_{2\text{max}}$  following CAF ingestion. Based on the results from this thesis it is possible that the mechanisms could be related to CAF's effect on HR and ventilatory functions by blocking adenosine receptors, or causing increased adrenaline release as explained in section 5.2. However, increased fractional utilization of  $\dot{V}O_{2\text{max}}$  could, during prolonged exercise, also be associated with a sparing of glycogen by avoiding glycogen depletion due to a shift in substrate utilization as suggested in the classic studies from the 1970s (35, 99). However, due to the duration and intensity maintained during the three TTs in the present thesis (10-30 min) this seems somewhat unlikely (for further detail see chapter 5.6). It has also been suggested that peripheral limitations such as capillaries per mm<sup>2</sup>, oxidative enzymes, fiber composition and mitochondrial size and number are important factors for a high fraction utilization of  $\dot{V}O_{2\text{max}}$  (17, 48). In contrast, the contrary observations of no change in work economy or substrate utilization and similar physiological responses (HR,  $\dot{V}O_2$ , LA-, RER, V<sub>E</sub>, glucose) during submaximal exercise (Table 4.3 and 4.4) indicate that peripheral mechanisms were not improved or affected

following CAF ingestion to an extent that would explain higher fractional utilization of  $\dot{V}O_{2max}$  (6).

It is however reported that athletes' ability to withstand increasing pain can have a large effect on fraction utilization of  $\dot{V}O_{2max}$  during exercise (5, 6, 107, 122). Therefore, a more likely explanation, based on the results from this thesis, is that the increased fractional utilization of  $\dot{V}O_{2max}$  was a direct result of the reduction in RPE following CAF ingestion (Table 4.4, chapter 5.6). If so, subjects increase their velocity during CAF TT to reach similar RPE, which would require an increased oxygen consumption (Chapter 5.7). It therefore seems that multiple mechanism could explaining the increased fractional utilization of  $\dot{V}O_{2max}$  following CAF ingestion. However, independent of what directly explains the improvements a higher fractional utilization of  $\dot{V}O_{2max}$  would increasing athletes' maximal performance ATP turnover, and performance.

#### ***5.4 Caffeine, O<sub>2</sub>-deficit and lactate accumulation (Studies I-V)***

Today the most accurate way of estimating anaerobic contribution during exercise is the accumulated O<sub>2</sub>-deficit (136, 146). Numerous studies have demonstrated that metabolic acidosis is an important contributing factor to fatigue during prolonged high-intensity exercise, since the build-up of a large O<sub>2</sub>-deficit in the early seconds of exercise establishes the biochemical environment within the muscle cells (65, 128, 169). Results from study IV also showed that anaerobic processes (O<sub>2</sub>-deficit) accounted for 14.7±3.1 and 15.0±2.7 % of total O<sub>2</sub>-cost in PLA and CAF trials, but CAF ingestion increased O<sub>2</sub>-deficit by 10.1% from 63.1±18.2 to 69.5.5±17.5 ml ·kg<sup>-1</sup> (p≤0.02) (Fig 4.2). Several studies have suggested the total amount of anaerobic energy produced during continuous exercise is fixed (156). However, as observed in study IV, CAF ingestion can promote anaerobic power, in agreement with other studies that observed CAF ingestion can increase anaerobic glycolysis during high-intensity exercise (52, 156). Specifically, Doherty (1998) observed that CAF ingestion (5 mg · kg body wt<sup>-1</sup>) improved running duration during a short-term exercise (~2-3 min) for 9 highly trained male athletes ( $\dot{V}O_{2max} \geq 60$ ). Performance was measured as running until exhaustion at a velocity of ~125% of  $\dot{V}O_{2max}$  on a treadmill with an uphill incline of 10.5%. The improvements were mainly explained by the 11.1% improvement in O<sub>2</sub>-deficit (52). These results are very comparable to the results from

study IV in terms of methodological design, CAF dosages and the observed improvement in O<sub>2</sub>-deficit (10.1%). However, in contrast to the study by Doherty et al., study IV is the first to show CAF can also increase O<sub>2</sub>-deficit during longer exercise tasks (6-6.5 min). Furthermore, statistical calculations show that when running durations were adjusted for both O<sub>2</sub>-deficit and LA-, the effect of CAF ingestion was reduced by 31 % from 19.4 to 13.2 s (p<0.001). Based on results from study IV the increases in both O<sub>2</sub>-deficit and LA- seem important for explaining improved running duration during the  $\dot{V}O_{2\max}$  performance test.

Additionally, all five studies of the thesis observed higher blood LA- following CAF performance trials compared to PLA (Table 4.2). Despite measurements of O<sub>2</sub>-deficit only being calculated in study IV, higher LA- post CAF trials compared to PLA indicate increased anaerobic glycolysis, and increased LA- following CAF ingestion has been observed in a large number of TT studies (Appendix I) (52, 78, 80, 98, 103, 142). Greater reductions in HCO<sub>3</sub><sup>-</sup> and pH during CAF performance tests were observed in study III, which also indicates that more H<sup>+</sup> efflux from muscles was buffered by blood HCO<sub>3</sub><sup>-</sup>. The reduction in HCO<sub>3</sub><sup>-</sup> and pH would also indicate higher anaerobic glycolysis during performance testing following CAF ingestion (15, 118, 128). Based on the overall results from all five studies in this thesis, it therefore seems that CAF is an effective stimulant of anaerobic processes for the production of ATP, thus explaining improvements in both running and DP performances at sea level and under hypoxic conditions.

### ***5.5 Caffeine and work economy (Studies I-V)***

During prolonged exercise, the aetiology of fatigue and performance is somewhat more complex than during short-term exercise (111, 154), but if simplified, the improved performance following CAF ingestion would require either a higher ATP turnover, or improved work economy (Figure 1.1) (48, 49). Since several studies have found work economy is an important and decisive factor between athletes for high performance in endurance sports (111, 154), it is not unlikely that this could also explain improvements following CAF ingestion in the present thesis.

To the author's knowledge, study V is the first study to investigate and demonstrate that CAF can improve prolonged running performance and reduce RPE by possibly affecting neuromuscular function, as judged by improved CMJ heights (Fig 4.6). Indeed, neuromuscular fatigue is often defined and quantified by measurements of changes in maximal voluntary contraction (MVC) which is observed to have a close relationship to CMJ heights (55, 71, 72, 124,



150, 183). In study V, no difference in CMJ heights was observed at arrival between treatments, but 30 min post CAF ingestion a tendency was already evident for increased CMJ compared to PLA ( $p \leq 0.11$ ). During performance testing, CAF ingestion resulted in consistently higher CMJs (Fig 4.6). In support of these findings, Bloms et al. reported that CAF ( $5 \text{ mg} \cdot \text{kg}^{-1}$ ) increased CMJ height, associated with increased peak force in 25 collegiate athletes (18). Despite study V being the first to perform CMJ measurements during prolonged running exercise following CAF ingestion, it is not the first to show that CAF can improve MVC or maximal power (16, 83, 114). The mechanisms explaining improved CMJ and muscular power following CAF ingestion are speculated to relate either to a direct effect on the muscle (e.g., maintaining electrolyte homeostasis or enhancing sarcoplasmic reticulum  $\text{Ca}^{2+}$  release) or the CNS (e.g. increasing motor unit recruitment) (16, 80, 83, 109, 110). CAF is reported to increase cortical and spinal neuron excitability by increasing motor unit recruitment, which also may explain increased MVC, CMJ and the reduction in RPE (16, 110). Specifically, Black et al. found CAF ingestion ( $5 \text{ mg} \cdot \text{kg}^{-1}$ ) resulted in an increase in MVC and motor unit recruitment, associated with improved performance due to a reduction in RPE (16). The improved CMJ height following CAF ingestion in study V could therefore very well have contributed to a reduction in RPE and improved work economy, since studies have reported strength training to improve running work efficiency by increased power production (90, 124, 137, 138).

However, when comparing submaximal low (60-65%) and high intensity exercise (80-100% of  $\dot{V}\text{O}_{2\text{max}}$ ) results in DP and running studies in this thesis, there seemed to be no difference in  $\text{O}_2$ -cost, that would indicate an improved work efficiency following CAF ingestion (Table 4.3-5). Furthermore, results from study II demonstrated that despite subjects using similar numbers strokes to complete the test, they increased their oxygen consumption associated with increased HR. Additionally, despite CAF increasing CMJ heights in study V, HR,  $V_E$  and  $\dot{V}\text{O}_2$  increased in a similar linear fashion independent of treatment, demonstrating similar work efficiency (Fig 4.7). Collectively the results of the thesis therefore seem to negate the suggestion that improved work economy can explain the performance improvements observed following CAF ingestion, or lead to a more effective usage of maximal performance ATP turnover post ingestion in top athletes.

### ***5.6 Caffeine and glycogen sparing (Studies I-V)***

Glycogen sparing, as proposed by Costill and colleagues in 1978, could have explained the improved performance in study V (35, 99). Since the runners maintained an intensity of ~80% of  $\dot{V}O_{2max}$ , (comparable to that maintained during half-marathon and marathon competitions (33, 107), a sparing of glycogen, thus avoiding glycogen depletion, could be important for maintaining the work intensity over a prolonged period of time (1, 33, 107). However, since no difference in utilization of fat or carbohydrate oxidation was evident based on RER values, these results would not support a sparing of glycogen following CAF ingestion (Fig 4.7). Furthermore, in the remaining four studies of the thesis both the duration and intensity during performance testing negate glycogen sparing as a significant factor in explaining exercise improvements. The results from the thesis are comparable to several other studies that failed to observe increased muscle glycogen content (muscle biopsies), despite the observation of increased FFA, glycerol or reduced RER values post CAF ingestion (Appendix II) (83, 84, 86). Furthermore, when comparing substrate utilization measured as RER values from all five studies during submaximal exercise no systematic difference is observed between treatments (Table 4.4). This is independent of muscular usage (DP vs running), duration (5 vs 90 min), and level of exercise intensity (low, 60-65% or high, 80-90% of  $\dot{V}O_{2max}$ ). The observation of stable high glucose values in study V and post all performance tests indicates that no subjects became hypoglycaemic. Therefore even if there had been a sparing of glycogen in the different studies it would probably have had little to no effect on performance outcome. It is therefore somewhat unlikely that glycogen sparing, as suggested in the classic study on cyclists by Costill et al. (1978), would give explanation to the improved performances seen in this thesis (59, 99).

Interestingly, results from DP studies have shown that the relative contribution of carbohydrate oxidation, measured as RER, is much higher in DP compared to running. Specifically, results from study I showed RER values at both 60% ( $0.97 \pm 0.01$  vs  $0.89 \pm 0.01$ ) and 70% ( $0.98 \pm 0.01$  vs  $0.91 \pm 0.01$ ) of  $\dot{V}O_{2max}$  were higher during DP compared to running, and these results were comparable to the findings in studies II and III. It is reported that substrate utilization, mitochondria (size and amount), muscles (type and adaptation) and capacity to extract oxygen from the blood are different in the arm muscles compared to those of the legs (26, 91, 92, 145). It is possible that the difference in RER values for DP vs. running are therefore a

consequence of these muscular differences, since while DP the arm muscles contribute as much as 40%–50% of the total O<sub>2</sub>-cost, and provide the main speed-generating force (5, 19, 170). One would therefore maybe expect that if CAF led to a sparing of glycogen it would perhaps be even more evident during an activity such as DP due to the high RER values compared to those obtained while running.

### ***5.7 Caffeine RPE and Pain (Studies I-V)***

During athletic competitions, the ability to tolerate high levels of pain and RPE is important for endurance performance (25). RPE and pain involve the collective integration of afferent feedback from cardiorespiratory, metabolic, and feed-forward mechanisms, enabling individuals to evaluate how hard or easy an exercise task feels at any point in time (60). There is therefore a strong relationship: RPE and pain increase with increased work intensity or duration during performance exercise (61, 151). This is clearly illustrated during submaximal incremental testing, TT and time to voluntary exhaustion performance testing in the thesis (Fig 4.8) (151). A reduction in pain and RPE following CAF ingestion would therefore in most situations be beneficial for improving exercise performance, as long as it does not negatively influence pacing strategies for athletes (60). In the five studies of this thesis, RPE and pain were reduced while performing similar workloads during both incremental and time until voluntary exhaustion testing following CAF ingestion compared to PLA (Table 4.4). In contrast, during TT testing (studies I-III) no difference in RPE or pain was observed between CAF and PLA trials, despite subjects maintaining a higher work output (Fig 4.8). Furthermore, in all tests, maximal effort (RPE) and pain were observed independent of treatment at completion of the different performance tests.

Several studies investigating the effects of CAF ingestion and endurance performance have concluded that improvements following CAF ingestion are linked to the inhibition of adenosine receptors, reducing RPE and pain due to their involvement and effects on nociception (25, 54, 68, 127). In this thesis no measurements of inhibition of adenosine receptors were performed, but the CAF dosage used (3-6 mg · kg<sup>-1</sup>) led to peak plasma CAF concentrations of ~ 25-50 μM, which are reported to reduce adenosine receptor activation and lead to the condition termed hypoalgesia (Figure 4.9) (68). Collectively, the results from the thesis demonstrate that CAF ingestion allowed subjects to maintain higher intensity with similar exertion during TT testing (Fig 4.8). It is therefore reasonable to speculate that in order to reach similar levels of pain and RPE, subjects

increased exercise intensity during TT, and duration during time until voluntary exhaustion testing. However, as discussed in section 5.5, one cannot rule out the possibility that the increase in CMJ height (study V) might have contributed to subjects perceiving workloads to be easier, thus reducing RPE.

The results from sea-level testing in the thesis observing reduced RPE following CAF ingestion seem comparable to previous studies in this area. However, during acute altitude testing (study III), the results suggest that a reduction of RPE and/or pain is not necessarily positive for performance outcome in all conditions. Indeed as shown in several pacing strategy studies (chapter 5.8), the ability to "sense" the right intensity is important for optimal pacing and performance outcome (67, 70, 162, 181)..

Moreover, the observation that CAF increased  $\dot{V}O_{2max}$ ,  $O_2$ -deficit and fractional utilization of  $\dot{V}O_{2max}$  raises the question of whether the methods used for evaluating RPE and pain following CAF ingestion are optimal. Certainly if CAF increases the production of ATP by changing physiological limitations such as affecting pulmonary or cardiovascular functions, the question is whether exercise at the same velocity or percentage of  $\dot{V}O_{2max}$  truly are the same. CAF increased  $\dot{V}O_{2max}$  in studies II and IV, and  $O_2$ -deficit in study IV. This means that at least during these studies the actual relative workload was lower in percentage terms during the CAF trial compared to PLA, since the four work loads of the incremental testing were specific percentages of  $\dot{V}O_{2max}$  set as a specific velocity based on inclusion testing. Based on the results from all studies in the thesis, during incremental testing a lower workload (% of  $\dot{V}O_{2max}$ ) would result in lower RPE. To the author's knowledge this thesis is the first to address this issue, because the observations of increased  $\dot{V}O_{2max}$  in studies II and IV are the first to date. However, it is clear that CAF has hypoalgesic effects from studies showing that CAF improves the effectiveness of over-the-counter pain-relieving drugs (13, 45, 46), and at the same velocity during submaximal exercise CAF did reduce subjects' RPE and pain. It is therefore hard to specifically conclude whether the workload is easier following CAF ingestion due to improved energy availability, inhibition of adenosine receptors, neuromuscular function (as judged by improved CMJ height) or a combination of all three.

### ***5.8 Caffeine and pacing strategy (Studies I-III)***

Athletes generally learn optimal pacing strategies through training and competitions (132, 159). Top athletes are therefore generally extremely good at pacing themselves based on interpretation of feedback from pulmonary, cardiovascular and skeletal muscle responses during exercise and competition (65, 159, 169). It is therefore surprising that so far the effects of CAF ingestion on pacing strategy have been given little attention.

The results from studies I and II showed no change in pacing despite a higher velocity throughout the CAF TTs under sea-level conditions (Fig 4.4 and 4.8A). Studies manipulating pacing by forcing athletes to start faster or slower than their best performances have found the best performances occur with an even or negative pacing strategy, typically characterized by increased power output (velocity) at the end as illustrated in Figure 1.2 (65, 169). The results from studies I, II and III (PLA) during sea-level conditions are comparable to the pacing observed in Figure 1.2. Although subjects increased their starting velocity following CAF ingestion, they maintained a relatively steady pace and increased it at the end, comparable to pacing in the PLA trials. The reason why increase exercise intensity is maintained following CAF ingestion seems related to CAFs effect to improve aerobic as well as anaerobic processes for production of energy during exercise (Chapters 5.2-4)

In contrast, during acute hypoxic testing a tendency toward a different pacing strategy was evident between the CAF and PLA trials (Fig 4.8B). During the CAF 8 km TT at altitude, athletes chose a higher starting velocity, which was quite similar to that observed during sea-level testing. However, the altered velocity was only maintained for the first half of the test following CAF consumption compared to PLA during acute exposure to altitude. The reason for these results could be that when exposed to hypoxia (altitude 2000m) the reduction in performance is highly related to pulmonary limitations in saturating hemoglobin while blood cells pass the alveolar ducts, due to reduction of partial pressure in the atmosphere (176). During sea-level testing, increased HR and  $V_E$  seem important in explaining the higher  $\dot{V}O_{2max}$  and fractional utilization of  $\dot{V}O_{2max}$  observed following CAF consumption (Chapter 5.2-5.3). It could therefore be that due to a reduction of partial pressure in the atmosphere during hypoxic conditions, an increase in HR and  $V_E$  (as observed through the whole 8 km TT following CAF ingestion) did not improve the conditions for  $O_2$ -saturation (Fig 4.5).

It is also possible that the increased velocity in the first 4 km of the CAF TT during hypoxic conditions increased blood LA- accumulation and reduced  $\text{HCO}_3^-$  and pH, leading to intracellular perturbations. Critically low muscle pH,  $\text{HCO}_3^-$  and high blood LA- values at early or inappropriate times during a race have in many circumstances been associated with a reduction in velocity and performance (64, 65). Indeed several studies have found that early and increased intracellular perturbations lead to unfavorable conditions in skeletal muscles, consequently reducing fraction utilization of  $\dot{V}\text{O}_{2\text{max}}$  (106). The fact that CAF improved the 90%  $\dot{V}\text{O}_{2\text{max}}$  performance test in study III, but not significantly the 8-km C-PT may indicate in this study that pacing strategy can become inefficient when CAF is ingested at altitude, (maybe even under sea-level conditions) due to CAF's hypoalgesic effects. Results from study III show that CAF's ability to lower sensations of pain and RPE was likely beneficial for higher performance during the time until task failure test since it requires no pacing strategy (intensity is predetermined). However, too high starting velocity due to a reduction of RPE and pain (exemplified in study III during the first 4 km of the 8 km TT) could lead to early intracellular perturbations and have a negative effect on overall performance. The results from study III therefore illustrates that a reduction in RPE or pain may therefore not always be beneficial in top trained athletes during conditions, since the ability to sense the right signals from the body is important for setting an optimal pacing for overall performance.

It is, however, important to note that when comparing the 8 km TT pacing results from study I at sea level with hypoxic conditions (Study III), the pacing changed during both PLA and CAF trials. It is therefore hard to say if the chosen pacing during CAF trials in hypoxia was optimal or not, since it reduced time usage by 0.9 % (though this was not statistically significant).

### ***5.9 Caffeine and other performance data***

In order to elicit differences between PLA and CAF ingestion it was important to ensure subjects were blinded, motivated, rested and at the same level of fitness before trials. Based on answers from questionnaires pre and post-performance testing, the test subjects in all five studies seemed to be successfully blinded (table 4.7). However, when ingesting CAF some subjects did report physiological reactions indicating this was not the case, as illustrated in this answer from a questionnaire in study I: *"I am quite sure I have consumed CAF today. I feel more awake, aroused and really can't wait to start pushing myself during the time trial!"* However, there were

also many subjects who were "sure" of consuming CAF or PLA when they in fact received the opposite treatment. These subjects also reported physiological effects after consuming "CAF", illustrated from this questionnaire in study III: *"Today I know that I have consumed CAF. There is no doubt. I feel like I cannot sit still. When I came I felt sleepy; now I feel energized and ready"*. Based on these illustrations it is therefore hard to specifically conclude whether subjects did experience physiological responses following CAF ingestion that led to them not being blinded. It may be that some subjects were guessing the right/wrong answers due to the "placebo effect".

Strict instructions were given to ensure that "current fitness" levels before each performance trial were equal. The amount of sleep showed no differences prior to performance testing that could have affected current fitness. Specifically, overall ratings of current fitness were similar between treatments for all trials, and based on the questionnaires, subjects appeared to be equally motivated before each trial (Table 4.6).

### ***5.10 Caffeine, doping and ethical considerations (Studies I-V)***

Athletes in endurance sports train hard and systematically over the whole year to develop the required capacity to win competitions that can be won or lost by a few seconds (14). An improvement of a few seconds could therefore have a big impact on results in real competitions. Collectively the results from the thesis demonstrate that CAF ingestion is an effective way to improve endurance performance. It is therefore natural to consider whether CAF ingestion should be banned from use in association with sporting competitions. One of the first who raised this question was Boje in 1939, who recommended CAF should be banned from all athletic competitions (83). Over the last 60 years CAF has either been forbidden or controlled at various times by sports organizations (44). From 1980-2003 CAF was included on the list of substances banned by the International Olympic Committees (IOC), where urine caffeine levels above  $12 \mu\text{g} \times \text{mL}^{-1}$  ( $\geq 62 \mu\text{mol}$ ) were deemed to be a doping offence (131). However, in 2004, caffeine was removed from the prohibited substances and methods list of the World Anti-Doping Agency (WADA) and IOC, meaning it could be used in athletic competitions (44). The drug is today on the monitoring program of WADA; *"substances which WADA want to monitor in order to detect patterns of misuse in sport"* (172). It is interesting to note from a doping perspective that in studies measuring urine concentrations of CAF, with a CAF intake of up to  $9 \text{mg} \cdot \text{kg}^{-1}$ ,

approximately one hour prior to exercise, the post-exercise urine levels of caffeine are below  $12 \mu\text{g} \times \text{ml}^{-1}$  (79, 117). This means that the dosage given in all five studies in this thesis would be legal to ingest even if CAF was on WADA and IOC's doping list. The question of whether it is ethical and/or fair to ingest CAF prior to competitions is therefore perhaps more important, since it seems ingestion would give some athletes an advantage compared to other contestants. However, one could argue that different athletes have beneficial advantages in other areas such as differences in equipment, training facilities, and in terms of how much money different countries use on preparation, such as types of training camps (e.g. altitude training) (180). It is therefore perhaps more important to discuss whether ingestion of CAF is unhealthy, or could lead to health risks. Most likely, top athletes who compete internationally are very good at pushing themselves. If you lower these subjects' perception of pain, they might push themselves to serious health risks, as shown with other stronger stimulating drugs in sports such as cycling (76). Even so, if high-profile athletes use CAF to improve performance, the risk is perhaps even larger that health problems might occur if CAF is ingested by children or by people in at-risk groups. However, based on studies investigating the effects of CAF ingestion (through coffee consumption) on a daily basis on health, the conclusion is that CAF ingestion does not seem lead to health risks, but rather reduces the risk of diabetes, Parkinson's and liver diseases (27, 81, 130). More research is however needed on top athletes who usually ingest CAF in its chemical form prior to competitions, rather than by drinking coffee.



## **6. CONCLUSION**

### **Study I**

In the first study, we found that CAF ingestion improved 8 km DP performance. The reduced time usage during the 8 km TT following CAF ingestion was associated with higher HR, adrenaline and lactate, but with no difference in pacing strategy, RPE or pain. During submaximal exercise, RPE was reduced while no difference was observed for work efficiency or substrate utilization following CAF ingestion. Improvements in performance therefore seemed related to reduced RPE allowing subjects to exercise at higher velocity and HR following CAF ingestion.

### **Study II**

In the second study, we found that CAF ingestion improved DP performance on consecutive days. Improvements were associated with increased fractional utilization of  $\dot{V}O_{2max}$ , HR, lactate, adrenaline and CK values. No difference was observed for pacing strategy, pain, RPE or work economy during TT testing following CAF ingestion compared to PLA. During submaximal exercise, a reduction in RPE and pain was evident, while no differences were observed for  $\dot{V}O_2$ , HR, lactate, adrenaline, CK or RER between treatments.

### **Study III**

In the third study, we found that CAF may improved performance during acute exposure to hypoxia (altitude 2000m). Results show that CAF ingestion improved DP time during time until voluntary exhaustion by 20.5%. CAF ingestion also reduced times during the first 4 km of a 8-km time trial, which was associated with increased fractional utilization of  $\dot{V}O_{2max}$ , HR, and  $V_E$ . There were, however, a non-significant effect for time usage for the whole 8-km TT despite a 0.9% reduction in time usage. The mechanisms underpinning improvements in both studies seem related to increased HR,  $V_E$ , epinephrine, LA-, reduced pain, RPE, pH and  $HCO_3^-$ . There was also a tendency for a change in pacing strategy during the TT performance following CAF ingestion, however not significant ( $p < 0.17$ ).

#### **Study IV**

In the fourth study, we found that CAF ingestion resulted in 5.5 % longer running duration during the performance test, which was associated with higher  $\dot{V}O_{2max}$  and  $O_2$ -deficit, while no difference was observed for work economy. The results indicated that the improved  $\dot{V}O_{2max}$  resulted from higher  $HR_{max}$  and  $V_{Emax}$ , while the increased  $O_2$ -deficit was associated with higher LA- during CAF exposure compared to PLA.

#### **Study V**

In the fifth study, we found that CAF ingestion improved running until voluntary exhaustion at 80% of  $\dot{V}O_{2max}$ . Improvements were associated with increased CMJ heights and reduced RPE. No difference was observed for work economy, HR,  $\dot{V}O_2$ , LA-, glucose, RER, fat or carbohydrate oxidation between the two treatments. However, higher LA- and HR were observed at CAF exhaustion compared to PLA.

#### **Overall conclusion (Studies I-V)**

Collectively the results from the five studies of the thesis demonstrate that CAF ingestion of 3-6 mg·kg<sup>-1</sup> is an effective aid for improving endurance performance in top athletes. Improvements are observed independent of mode (time until voluntary exhaustion vs TT), duration (10-120min), muscular usage (double poling vs running), or condition (sea level vs altitude) where approximately 75% of test subjects improved their performance following CAF ingestion. The reduction in both pain and RPE following CAF ingestion seem important for explaining exercise improvements. Novel results from the thesis are the observations that CAF ingestion increased aerobic power ( $\dot{V}O_{2max}$ ), fractional utilization of  $\dot{V}O_{2max}$  and anaerobic power ( $O_2$ -deficit), thus increasing energy availability (ATP turnover). No difference in work economy, pacing strategy or substrate utilization was however evident following CAF ingestion.

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# PAPER I

*Caffeine increases performance in cross-country double-poling time trial exercise.*

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# Caffeine Increases Performance in Cross-Country Double-Poling Time Trial Exercise

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<sup>1</sup>Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, NORWAY; <sup>2</sup>Department for the Chemical and Biological Work Environment, National Institute of Occupational Health, Oslo, NORWAY; <sup>3</sup>Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, NORWAY; and <sup>4</sup>Department of Kinesiology and Health Education, University of Texas at Austin, Austin, TX

## ABSTRACT

STADHEIM, H. K., B. KVAMME, R. OLSEN, C. A. DREVON, J. L. IVY, and J. JENSEN. Caffeine Increases Performance in Cross-Country Double-Poling Time Trial Exercise. *Med. Sci. Sports Exerc.*, Vol. 45, No. 11, pp. 2175–2183, 2013. **Purpose:** Caffeine (CAF) improves performance in both short- and long-duration running and cycling where performance relies on power output and endurance capacity of leg muscles. No studies have so far tested the effects of CAF while using the double-poling (DP) technique in cross-country skiing. When using the DP technique, arm muscles provide the speed-generating force and therefore play an important role in performance outcome. The metabolism of arm muscles differs from that of leg muscles. Thus, results from studies on leg muscles and CAF may not be directly applicable to exercises while using the DP technique in cross-country skiing. The purpose of our study was therefore to investigate the effects of CAF on exercise performance in DP. **Method:** Ten highly trained male cross-country skiers ( $\dot{V}O_{2\max}$  running,  $69.3 \pm 1.0 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) performed a placebo (PLA) and CAF trial using a randomized, double-blind, crossover design. Performance was assessed by measuring the time to complete an 8-km cross-country DP performance test (C-PT). CAF ( $6 \text{ mg}\cdot\text{kg}^{-1}$ ) or PLA was ingested 75 min before the C-PT. **Results:** CAF ingestion reduced the time to complete the 8-km C-PT from  $34:26 \pm 1:25$  to  $33:01 \pm 1:24 \text{ min}$  ( $P < 0.05$ ). The subjects maintained higher speed and HR throughout the C-PT, and lactate was higher immediately after the C-PT with CAF exposure compared with PLA. Subjects reported lower RPE at submaximal intensities during CAF compared with PLA, although HR was similar. **Conclusion:** CAF intake enhances endurance performance in an 8-km C-PT, where arm muscles limit performance. CAF ingestion allowed the participants to exercise with a higher HR and work intensity possibly by reducing perception of effort or facilitating motor unit recruitment. **Key Words:** EXERCISE, PAIN, RPE, ADENOSINE RECEPTORS, GLUCOSE, ADRENALINE

Many studies have demonstrated during the last 40 yr that caffeine (CAF) intake ( $3\text{--}9 \text{ mg}\cdot\text{kg}^{-1}$ ) improves endurance performance in activities such as cycling (25,26), running (7,21), and rowing (35). Furthermore, CAF intake can improve exercise performance in short- (28) or long-duration (12,27) activities, and improvement was shown when exercise performance was measured as time to exhaustion (25) or time to complete a set amount of work (7).

Multiple mechanisms have been proposed to explain improved endurance performance after CAF ingestion (20,36). Initially, CAF was observed and thought to have a carbohydrate-sparing effect (15,25). However, this hypothesis cannot explain improved performance in short-duration activity where glycogen content is not likely to be a limiting factor (28,35). Inhibition of adenosine receptors and increased motor unit recruitment by CAF are additional mechanisms reported to explain its ergogenic effects (1,9,14,19,20). Adenosine receptors are expressed in most tissues of the human body, and blockade of these receptors may affect both RPE and HR (2,3,18). Indeed, lower RPE has been reported at submaximal workloads after CAF ingestion (2,3,13), and higher HR has been reported in time trial studies after CAF ingestion compared with placebo (PLA) (3,7,26). CAF has also been shown to improve maximal voluntary contraction (MVC) (20). The mechanism explaining improved MVC is that CAF ingestion may directly affect the muscle (e.g., maintaining electrolyte homeostasis or enhancing sarcoplasmic reticulum  $\text{Ca}^{2+}$  release) or the CNS (e.g., increasing motor unit recruitment) (20,31).

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During endurance performance tasks lasting 10–90 min, the ergogenic effects of CAF have typically been shown for exercises dependent on endurance capacity of the leg muscles (e.g., cycling, running, and rowing) (7,26). During double poling (DP), the arm muscles provide the speed-generating force (37), and endurance capacity of the arm muscles therefore plays a crucial role in performance outcome. The metabolism of arm muscles differs from that of leg muscles, with studies observing that arm muscles have a higher percentage type 2 fiber compositions, extract less oxygen, and rely more on carbohydrate utilization during exercise (10,22,37). Because of observed differences between arm and leg muscles, CAF may act differently when an exercise performance is relying on endurance capacity of the arm muscles.

In cross-country skiing (XCS), DP has become an important technique because of changes in equipment, tracks, and types of competition (4). In races such as Marcialonga and Vasaloppet, DP capacity directly affects performance outcome. Furthermore, maximal oxygen uptake in a well-trained XCS athlete is lower during DP than that during running or diagonal skiing (5,23). The lower maximal oxygen uptake during DP may be due to a lower active muscle mass, indicating that cardiac output *per se* does not limit performance, or the fact that arm muscles have a lower mitochondria density and are unable to extract the same amount of oxygen as leg muscles (10,22,30).

The effect of CAF on exercise endurance performance while using the DP technique in XCS, where endurance capacity of the arm muscles plays a key role in performance outcome, has not been investigated. Thus, the purpose of our study was to determine the effect of CAF on exercise performance limited to the arms while using the DP technique. We hypothesized that ingestion of CAF will improve performance in DP where arm muscles produce the speed-generating force.

## MATERIALS AND METHODS

**Subjects and approvals.** Ten healthy highly trained male cross-country skiers participated. Physical characteristics of the subjects were as follows (mean  $\pm$  SE): age, 20.0  $\pm$  1.0 yr; height, 181.0  $\pm$  1.0 cm; weight, 73.9  $\pm$  2.3 kg; maximal oxygen uptake during running ( $\dot{V}O_{2\max\text{-run}}$ ), 69.3  $\pm$  1.0 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ; and maximal oxygen uptake during DP ( $\dot{V}O_{2\max\text{-pol}}$ ), 63.2  $\pm$  1.5 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ . All subjects trained systematically to compete in the Norwegian National Cross-country Skiing Cup. Additional inclusion criteria were that  $\dot{V}O_{2\max\text{-run}}$  was above 65 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ . The study was approved by both the Regional Ethics Committee and the Norwegian Medicine Agency. Subjects gave their written consent after being informed about the purpose of the study and risks involved. All testing was performed in the pre-season for the XCS (September–November).

**Experimental procedures.** The study was a randomized, double-blind, PLA-controlled, crossover design. Treatments included CAF (6 mg $\cdot$ kg $^{-1}$  body weight) and PLA

(vehicle only). CAF (Coffeinum; Oslo Apotekproduksjon, Oslo, Norway) was dissolved in a cordial concentrate Fun Light (3 mg $\cdot$ mL $^{-1}$ ) and was prepared by Ullevål Apotek Produksjon (Oslo, Norway). Before the 8-km cross-country DP performance tests (C-PT), the participants underwent a 4-wk training protocol to familiarize the participants with the DP ergometer and the C-PT. On day 1, participants performed a  $\dot{V}O_{2\max\text{-run}}$  test on a treadmill (Woodway, Weil am Rhein, Germany), and the highest HR was defined as HR $_{\max\text{-run}}$ . Oxygen consumption and RER were measured with an Oxycon Pro (Jaeger, Hoechberg, Germany), and air was collected during a mouth V2-mask (Hans Rudolph Instr., Shawnee, KS) in combination with a nose bracket. The  $\dot{V}O_{2\max\text{-run}}$  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° incline. A 1-min break was given between each workload where lactate was measured. After the last workload of the warm-up, subjects walked for 5 min at 5 km $\cdot$ h $^{-1}$  before stating the  $\dot{V}O_{2\max\text{-run}}$  test. Starting speed was 10 km $\cdot$ h $^{-1}$  with an incline on the treadmill of 10.5°. For each half minute, speed was increased with 0.5 km $\cdot$ h $^{-1}$  until subjects were unable to maintain the speed and jumped off the treadmill. All 10 subjects had to meet the first criterion and at least two of the three other criteria to reach  $\dot{V}O_{2\max\text{-run}}$ : 1) oxygen consumption reached a plateau, meaning  $\dot{V}O_2$  increased less than 1 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ , although speed was increased two times 0.5 km $\cdot$ h $^{-1}$ ; 2) RER values were above 1.10; 3) postblood lactate measurements were above 7.0 mmol $\cdot$ L $^{-1}$ ; and 4) RPE was  $\geq$ 19 on the Borg scale 6–20 (6).  $\dot{V}O_{2\max\text{-run}}$  was based on the average of the two highest measurements. Measurements of oxygen consumption were done every 30 s. Subjects with  $\dot{V}O_{2\max\text{-run}}$  higher than 65 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$  were included. Day 2 subjects performed 40 min of familiarization by DP training on the poling ergometer (Thoraxtrainer Elite; Thoraxtrainer, Holbæk, Denmark), with workloads ranging from 55% to 85% of their HR $_{\max\text{-run}}$ . Day 3 subjects performed a  $\dot{V}O_{2\max\text{-pol}}$  test on the poling ergometer; the highest HR was defined as HF $_{\max\text{-pol}}$ . Criteria for  $\dot{V}O_{2\max\text{-pol}}$  that were reached were the same as those for  $\dot{V}O_{2\max\text{-run}}$ . On days 4 and 5, the participants completed time trials identical with the final C-PT but without supplement or blood sampling. Pilot results indicated that performance stabilizes at the third 8-km time trial (data not shown).

Before each 8-km C-PT test, subjects performed a standardized warm-up protocol lasting 26 min. The warm-up was performed as an incremental test with four 5-min workloads, equivalent to 40%, 50%, 60%, and 70% of the subjects'  $\dot{V}O_{2\max\text{-pol}}$  with a 2-min break between each workload. HR,  $\dot{V}O_2$ , and RER were measured as means between the 3 and 4.5 min of each workload. Subjective RPE according to the Borg scale (from 6 to 20) was determined for each workload (6). After the warm-up incremental test, a 5-min break was used for blood sampling and preparation for the 8-km C-PT (Fig. 1). During the 8-km C-PT, subjects self-selected their speed with the goal of using as

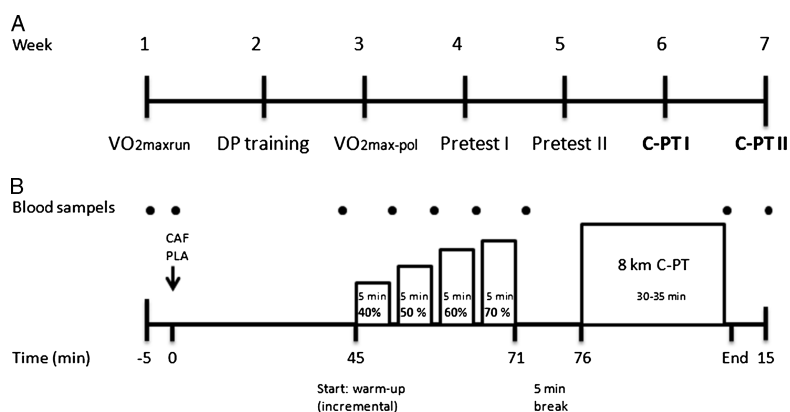


FIGURE 1—Experimental design of the 8-km cross-country DP test. (A) Shows tests and training performed during the 6-wk period to accustom for the two performance tests in DP (C-PT). (B) Shows the test procedure on the performance tests. Before the C-PT, subjects performed an incremental tests consisting of four intensities for 5 min. Similar protocol to the C-PT was completed at pretest 1 and pretest 2, except that CAF/PLA was not administered and no blood samples were taken.

little time as possible (time trial). Encouragement was given during the whole C-PT. Each C-PT was separated by 6 d (washout period), and subjects were instructed to abstain from hard-intensity training (80%–100% of HR<sub>max</sub>) or any strength training 48 h before testing. The tests were performed from September through November, which is a period before the start of the XCS season.

**The performance tests (C-PT).** Before the study started, a pilot study was performed to determine the variations in performance during four repeated 8-km C-PT. Four highly trained subjects ( $\dot{V}O_{2\max-pol}$ ,  $57.6 \pm 2.3$  mL·kg<sup>-1</sup>·min<sup>-1</sup>) all familiar with the cross-country DP technique participated. Results showed that each subject needed two 8-km C-PT trials before they had no further improvement in poling performance. The improvements in performance during the 8-km C-PT trials were as follows: test 1 versus 2, 8%; test 2 versus 3, 1.6%; and test 3 versus 4, 0.8%. The typical coefficient of variation in percentage change (CV%) between test 1 versus 2 was 3.2% [90% CL, 2.0–9.7; effect size (ES) = 0.33 small]; between test 2 versus 3, 1.1% (90 CL, 0.7–3.2; ES = 0.12 trivial); and between test 3 versus 4, 2.1% (90 CL, 1.3–6.2; ES = 0.24 trivial/small).

The subjects prepared for each 8-km C-PT as they would normally do before a competition. To minimize variation in preexercise glycogen stores, diet and exercise diaries were used to standardize food intake and training for each subject. Subjects were asked to refrain from CAF consumption the last 48 h before each experiment. Four subjects were regular CAF drinkers (100–250 mg·d<sup>-1</sup>). One subject (subject 7) who normally had a high daily intake of CAF (>300 mg·d<sup>-1</sup>) was allowed to consume one-fourth of his normal CAF amount 48 to 24 h before testing but refrained from CAF the last 24 h. Their last meal was consumed approximately 1.5 h before arriving at the laboratory on both testing days. Subjects were allowed to choose what this meal consisted of but were told to eat the same meal as they would normally do before

important competitions. This meal should be rich in carbohydrates and proteins. The training performed the last 48 h and food intake the day before were registered before the first C-PT. Subjects were instructed to follow the same training and diet procedure before the second test. After arriving at the laboratory, the subjects only consumed water before and during the testing. During the 8-km C-PT, no intake of water was allowed to mimic real-life competitions.

Each subject arrived at the laboratory at the same time of day for each of their C-PT. Upon arrival, subjects rested in a supine position for 15 min, and the lowest HR was recorded as resting HR for the day. Next, a catheter was placed (BD Venflon™ Pro, BD, Helsingborg, Sweden) in the cephalic vein and a 7-mL resting blood sample was drawn in tubes containing EGTA/glutathione (20 μL of 0.2 M glutathione and 0.2 M EGTA per milliliter of blood). Blood samples were centrifuged (10 min, 2500 rpm, 4°C), and three plasma aliquots were stored (Microtube Superspin; VWR International, West Chester, PA) at –80°C until analyses. Capillary blood was taken from a fingertip for measurement of glucose (HemoCue glucose 201<sup>+</sup>; HemoCue, Ängelholm, Sweden) and lactate (YSI 1500 SPORT; Yellow Springs Instruments, Yellow Springs, OH). The subjects then consumed either CAF or PLA. Thirty minutes after supplementation, resting HR was determined as mentioned, followed by a venous and capillary blood sample. Forty-five minutes after supplementation, the warm-up protocol commenced. Between each submaximal workload, there was a 2-min break where blood samples (5 mL) were drawn and prepared as described previously. After the venous blood samples, catheters were flushed with saline (B. Braun Melsungen AG, Melsungen, Germany), and capillary samples for measurement of glucose and lactate were taken. After each workload, subjects reported RPE according to the Borg scale (6).

During the 8-km C-PT, HR and speed were recorded each kilometer. At 3, 5, and 7 km, subjects were asked to report

pain in legs and arms on a scale from 1 to 10 (1 as no pain and 10 as unbearable/maximal pain). During the C-PT, subjects could see the remaining distance (m), and subjects were encouraged by a blinded test leader. Venous blood samples (7 mL) were taken 1 and 15 min after completion of the 8-km C-PT described previously.

**Thorax trainer—CC-POL.** The cross-country DP ergometer used in the study was a Thoraxtrainer Elite (Thoraxtrainer). Temperature in the test laboratory was between 22°C and 23°C on all test days. Ski poles used during all testing were Swix CT1 (Swix, Lillehammer, Norway), and length was standardized to 85% ± 2% of the 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 the work output (W), speed (km·h<sup>-1</sup>), and poling frequency in real time. Resistance in the Thoraxtrainer is generated by air pressure, and the mean barometric air pressure on days of PLA and CAF trials averaged 966 ± 4 and 970 ± 2.2 mm Hg, respectively. 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, see the studies by Bojsen-Moller et al. (5) and Van Hall et al. (37).

**Plasma CAF.** Sample preparation of 200-μL plasma and the subsequent measurements of CAF and theophylline (internal standard) by liquid chromatography–electrospray ionization–tandem mass spectrometry were performed according to a method previously described by Wang et al. (38). In brief, 200 μL of plasma was added 100 μL of 18 μg·mL<sup>-1</sup> internal standard in MeOH–water (50/50, v/v) and 100 μL of MeOH–water (50/50, v/v). After vortex mixing for 30 s, an aliquot (3 mL) of diethyl ether–dichloromethane (3/2, v/v) was added and vortexed for 2 min, followed by centrifugation at 3000 rpm for 10 min. The upper organic layer was transferred to a 4-mL sample vial and evaporated to dryness at 40°C under a gentle stream of nitrogen. Residues were then dissolved in 600 μL of mobile phase followed by vortex mixing for 30 s. A 1-μL aliquot of the reconstituted solution was injected onto the liquid chromatography–electrospray ionization–tandem mass spectrometry system.

**Plasma glycerol and nonesterified fatty acid.** Glycerol was measured with a kit based on colorimetric method (Randox Laboratories Ltd, Antrim, UK), and concentration of nonesterified fatty acids (FFA) was measured with a kit from DIALAB (Wiener Neudorf, Austria) according to description.

**Plasma catecholamines.** Plasma adrenaline and noradrenaline were measured with a Cat Combi ELISA kit (DRG Instruments GmbH, Marburg, Germany) according to description.

**Questionnaires.** Pain in arms and legs was evaluated by a 1- to 10-point scale described by Ritchie and Hopkins (34). Other questionnaires were used to evaluate motivation, day form, and sleep quality using a scale from 1 to 100.

**Statistical analysis.** All data in the study are presented as mean ± SEM, and differences in performance during the

8-km C-PT were evaluated by a paired *t*-test. A two-way ANOVA for repeated measures was used to elicit differences in HR, lactate,  $\dot{V}O_2$ , 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 Shapiro–Wilk test. For analyzing the typical error (CV) of the 8-km C-PT, a spreadsheet by Hopkins (24) was used. Statistical analyses were performed using GraphPad Prism 6, and the level of significance was set at *P* < 0.05.

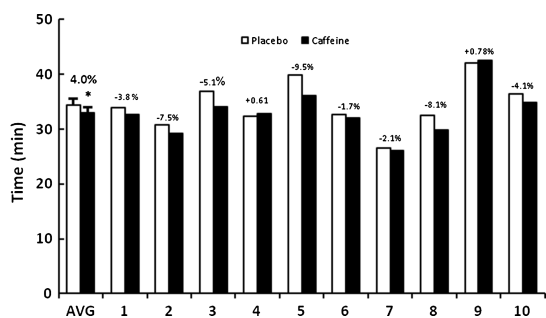
## RESULTS

### Performance Test (8-km C-PT)

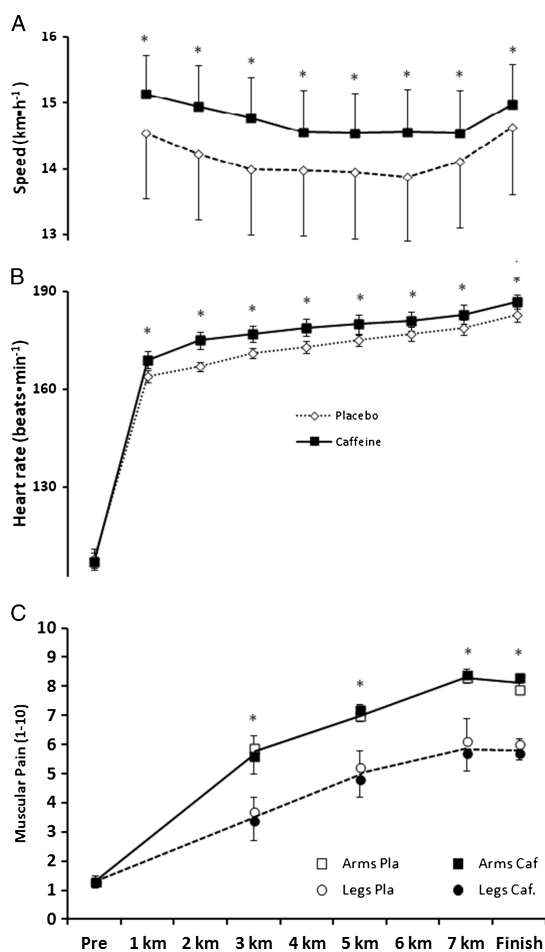
Subjects completed the 8-km C-PT 4% faster (*P* < 0.003) after ingestion of CAF (33:01 ± 1:24 min) as compared with PLA (34:26 ± 1:25 min) (Fig. 2). Total numbers of poling strokes to complete the C-PT did not differ between treatments (CAF, 1942 ± 126 strokes, and PLA, 1932 ± 132 strokes; *P* < 0.46). Moreover, 80% of the subjects performed the 8-km C-PT faster after CAF ingestion than PLA (Fig. 2). Mean speed was higher from start to finish after CAF ingestion compared with PLA (Fig. 3A). Mean speed for the 8-km C-PT was 14.5 ± 0.6 versus 13.9 ± 0.6 km·h<sup>-1</sup> (*P* < 0.003) after CAF and PLA ingestion, respectively. Mean HR during the C-PT was 179 ± 2 for CAF and 174 ± 2 for PLA (*P* < 0.00001) (Fig. 3B).

Plasma CAF concentrations were low in all subjects on arrival before both tests. Ingestion of 6 mg·kg<sup>-1</sup> of CAF increased plasma concentrations to 40.8 ± 1.9 μM before the 8-km C-PT, and it remained at a similar level throughout the exercise trial (Table 1), whereas ingestion of PLA resulted in no increase in plasma CAF concentration (Table 1).

Plasma adrenaline immediately after the C-PT was increased during both CAF and PLA but was higher after CAF than PLA, and it remained so at least 15 min after the 8-km



**FIGURE 2**—Effect of CAF on time trial performance in a DP ski ergometer. Average time and individual time for the 8-km C-PT without (open bar) and with (filled bar) CAF ingestion. \**P* < 0.05 compared with PLA. Subjects 3, 7, 8, and 10 were CAF users (300–600 mg·d<sup>-1</sup>). The rest were nonusers (<40 mg·d<sup>-1</sup>). All subjects except subjects 4 and 9 used less time after CAF ingestion.



**FIGURE 3**—Effect of CAF on mean speed, HR, and pain in arm and leg muscles during the 8-km C-PT. (A) Mean speed during performance tests in a DP without (open symbols) and with (filled symbols) CAF ingestion. (B) Mean HR during performance tests in a DP without (open symbols) and with (filled symbols) CAF ingestion. (C) Subjects reported muscular pain in arms (circles) and legs (squares) without (open symbols) and with (filled symbols) CAF at 3, 5, and 7 km. A scale from 0 to 10 (no pain to unbearable) was used. Values are presented as means  $\pm$  SEM. \*Significant difference between treatments ( $P < 0.05$ ). Data on muscle pain in legs and arms before exercise were collected retrospectively 3–4 wk after the test subjects had performed the two different 8-km C-PT.

C-PT (Table 1). Plasma concentration of noradrenaline increased in CAF and PLA at similar levels immediately after C-PT but was slightly higher in CAF 15 min after the C-PT. Plasma glycerol and FFA were similar (Table 2) for the two treatments. Blood concentrations of lactate and glucose increased during the 8-km C-PT and were higher after CAF compared with PLA (Table 2). The concentration of blood lactate and glucose remained elevated 15 min after the C-PT and remained higher after CAF compared with PLA. The same tendency was observed for glycerol (Table 2). There was no difference in the quantity of sleep before PLA and CAF tests ( $7.6 \pm 1.3$  and  $7.8 \pm 1.4$  h).

**Metabolism at resting and during incremental tests.** Resting HR and concentration of adrenaline, noradrenaline, glucose, FFA, or glycerol were not increased 35 min after CAF ingestion (Tables 1 and 2). Blood glucose concentration decreased during poling in PLA at 50% of  $\dot{V}O_{2max-pol}$  and higher intensities. In CAF, blood glucose was higher than PLA at 50% and 70% of  $\dot{V}O_{2max-pol}$  and only lower than resting values at 70% of  $\dot{V}O_{2max-pol}$  (Table 2). The HR increased similarly in CAF and PLA (Table 3). RER increased with increasing intensity in both PLA and CAF but was significantly higher during PLA compared with CAF at 70% of  $\dot{V}O_{2max-pol}$  (Table 3). Plasma concentration of lactate, FFA, and glycerol showed no differences at any exercise intensity (Table 2).

**Questionnaires.** During the incremental test, subjects reported lower RPE after CAF ingestion at 50%, 60%, and 70% of  $\dot{V}O_{2max-pol}$  (Table 3). Subjects reported “no muscular pain” in either arms or legs on arrival (Fig. 3C) but higher pain in arms than that in legs during the C-PT. Reported muscular pain in both arms and legs followed a similar pattern in PLA and CAF. Pain increased gradually during the C-PT in arms as well as legs for both treatments. In arms, pain was  $8.3 \pm 0.3$  and  $8.4 \pm 0.3$  (almost unbearable) for PLA and CAF at 7 km (Fig. 3C), whereas leg pain was only  $6.0 \pm 0.8$  and  $5.7 \pm 0.6$  (some/quite a lot) at 7 km. The motivation was high before both CAF and PLA trials [ $84.0 \pm 3.9$  and  $84.5 \pm 3.4$ , respectively, not significant (NS)], and subjects experienced similar “day form” ( $68.0 \pm 2.7$  and  $69.0 \pm 4.3$ , respectively; NS). Interestingly, subjects reported better “day form” after the 8-km C-PT after ingestion of CAF compared with PLA ( $78.5 \pm 4.2$  vs  $68.2 \pm 4.8$ ,  $P < 0.005$ ). The questionnaires revealed that the participants

**TABLE 1.** Plasma concentration of caffeine, adrenaline, and noradrenaline before, during, and after DP tests with and without CAF ingestion.

	Ingested	Resting Measurements		Submaximal 70%	8-km C-PT 1 min Post	8-km C-PT 14 min Post
		ArRes	35Res			
Caffeine ( $\mu$ M)	PLA	$0.16 \pm 0.07$	$0.11 \pm 0.05$	$0.10 \pm 0.05$	$0.09 \pm 0.05$	$0.09 \pm 0.05$
	CAF	$0.29 \pm 0.11$	$34.5 \pm 2.8^*$	$40.8 \pm 1.9^*$	$38.7 \pm 1.2^*$	$38.7 \pm 1.2^*$
Adrenaline (nM)	PLA	$0.28 \pm 0.02$	$0.29 \pm 0.05$	$1.24 \pm 0.14$	$3.47 \pm 0.66$	$0.52 \pm 0.07$
	CAF	$0.25 \pm 0.02$	$0.41 \pm 0.04$	$3.48 \pm 0.66$	$5.28 \pm 1.31^*$	$0.86 \pm 0.04^*$
Noradrenaline (nM)	PLA	$1.69 \pm 0.21$	$2.05 \pm 0.23$	$10.07 \pm 1.63$	$29.57 \pm 5.00$	$3.43 \pm 0.47$
	CAF	$1.98 \pm 0.18$	$2.65 \pm 0.45$	$10.01 \pm 1.59$	$31.95 \pm 6.54$	$4.86 \pm 0.71^*$

Blood samples were taken at arrival (ArRes), 35 min after CAF/PLA ingestion (35Res), after the last workload on incremental tests (70%), and after the C-PT.

Values are presented as mean  $\pm$  SEM.

\*Significantly different from PLA ( $P < 0.05$ ).



TABLE 2. Effect of CAF on blood metabolites before, during, and after DP tests.

	Resting Measurements		Submaximal DP (% of $\dot{V}O_{2max-pol}$ )				8-km C-PT		
	Ingested	ArRes	35Res	40	50	60	70	1 min Post	14 min Post
Glucose (mM)	PLA	5.0 ± 0.2 <sup>a</sup>	5.1 ± 0.3 <sup>a</sup>	4.7 ± 0.1	4.5 ± 0.1 <sup>b</sup>	4.5 ± 0.1 <sup>b</sup>	4.5 ± 0.1 <sup>b</sup>	7.1 ± 0.8 <sup>b</sup>	5.7 ± 0.3
	CAF	5.2 ± 0.2 <sup>c</sup>	5.1 ± 0.2 <sup>c</sup>	5.2 ± 0.2	5.0 ± 0.1*	4.8 ± 0.1 <sup>b</sup>	4.8 ± 0.1 <sup>b</sup>	8.8 ± 0.5*	6.7 ± 0.4*
Lactate (mM)	PLA		0.79 ± 0.07 <sup>d</sup>	1.40 ± 0.10	1.74 ± 0.15	2.31 ± 0.23	3.15 ± 0.29	6.7 ± 0.3	4.0 ± 0.3
	CAF		0.75 ± 0.04 <sup>*,c</sup>	1.70 ± 0.15*	1.92 ± 0.15	2.33 ± 0.22	3.5 ± 0.25	8.2 ± 0.3*	5.0 ± 0.3*
FFA (mM)	PLA	0.23 ± 0.02	0.19 ± 0.03	0.21 ± 0.03	0.25 ± 0.04	0.23 ± 0.04	0.24 ± 0.03	0.37 ± 0.06	0.38 ± 0.06
	CAF	0.24 ± 0.02	0.29 ± 0.06	0.33 ± 0.08	0.31 ± 0.08	0.31 ± 0.08	0.33 ± 0.07	0.44 ± 0.06	0.47 ± 0.09
Glycerol (μM)	PLA	24.4 ± 4.4	19.8 ± 4.0	28.5 ± 5.6	29.6 ± 5.6	34.1 ± 7.8	40.5 ± 8.0	104.8 ± 14.6	80.6 ± 17.7
	CAF	21.1 ± 2.4	28.1 ± 3.9	43.7 ± 9.0	50.8 ± 13.4	63.6 ± 16.1	68.4 ± 16.3	145.0 ± 19.2	136.2 ± 17.5

Blood sample were taken at arrival (ArRes), 35 min after CAF/PLA ingestion (35Res), after each workload on incremental tests, and after the C-PT.

Values are presented as mean ± SEM.

\*Significantly different from PLA ( $P < 0.05$ ).

<sup>a</sup>Missing data from two subjects.

<sup>b</sup>Significantly different from ArRes.

<sup>c</sup>Missing data from one subject.

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 the last 48 h before the C-PT showed that the participants had followed instructions.

#### Oxygen uptake and metabolism in DP and running.

Maximal oxygen uptake was higher during running than while DP ( $69.3 \pm 1.0$  and  $63.2 \pm 1.5$ , respectively;  $P < 0.05$ ). The highest HR achieved during the  $\dot{V}O_{2max}$  tests was achieved during running compared with when poling ( $194 \pm 2$  and  $189 \pm 1$ , respectively;  $P < 0.05$ ). HR at the same relative submaximal intensities (60% and 70% of  $\dot{V}O_{2max}$ ) was similar during running and poling ( $153 \pm 3$  and  $156 \pm 3$ , NS). Still, relative contribution of carbohydrate oxidation measured as RER was much higher during poling compared with running at both 60% ( $0.97 \pm 0.01$  vs  $0.89 \pm 0.01$ ,  $P < 0.05$ ) and 70% ( $0.98 \pm 0.01$  vs  $0.91 \pm 0.01$ , respectively,  $P < 0.05$ ) of  $\dot{V}O_{2max}$ . Lactate concentration was higher during poling compared with running at the same submaximal intensities (70%: poling,  $3.2 \pm 0.3$  mM, vs running,  $1.3 \pm 0.2$  mM;  $P < 0.05$ ), but similar concentrations between exercises were reached after finishing the  $\dot{V}O_{2max}$  tests (poling,  $8.1 \text{ mM} \pm 0.4$ , vs running,  $8.1 \pm 0.3$  mM).

## DISCUSSION

DP is an important technique for the best skiers in long-distance competitions such as Vasaloppet and Marcialonga.

The winner of the 90-km-long Vasaloppet race in 2013 (Jørgen Aukland) completed the race without any grip wax, using only the DP technique. Also, in normal XCS, DP capacity can determine the final outcome of competitions. Research on the effects of CAF on sports performance has traditionally focused on cycling and running where endurance capacity of the leg muscles determines power output and performance. In our study, maximal oxygen uptake was approximately 10% lower while using the DP technique compared with that of running. This is in accordance with previous studies (5,23), showing that even when highly trained in XCS DP, they are unable to obtain the same maximal oxygen uptake and  $HR_{max}$  as when running. This is most likely related to differences between arm and leg muscles in metabolism and oxygen extraction as a result of lower oxidative capacity and capillarization in the arm muscles (10,22,29), or the fact that a smaller muscles mass is being used. Thus, results from studies on leg muscles and CAF may not be directly applicable to exercises while using DP technique during XCS.

Indeed, leg muscles use a substantial amount of oxygen during DP (5,23,37). However, during DP, arm muscles contribute as much as 40%–50% of total  $O_2$  cost (5,37) and, more importantly, provide the main speed-generating force (37). Therefore, endurance capacity of the arm muscles plays a crucial role in performance outcome. Results of pain perception during the 8-km C-PT confirmed that arms limit

TABLE 3. Oxygen uptake, RER, HR, and perceived exertion during the incremental DP test.

What	Ingested	Submaximal DP (% of $\dot{V}O_{2max-pol}$ )			
		40	50	60	70
Speed average ( $\text{km} \cdot \text{h}^{-1}$ )	PLA	10.1 ± 0.3	11.4 ± 0.3	12.9 ± 0.3	14.1 ± 0.4
	CAF	10.0 ± 0.3	11.5 ± 0.3	12.9 ± 0.3	14.1 ± 0.4
$\dot{V}O_2$ ( $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )	PLA	24.0 ± 2.6	31.6 ± 1.4	37.3 ± 1.3	44.4 ± 1.9
	CAF	26.5 ± 0.5	31.3 ± 0.7	36.7 ± 0.9	43.0 ± 1.0
RER	PLA	0.92 ± 0.01	0.94 ± 0.01	0.97 ± 0.01	0.98 ± 0.01
	CAF	0.91 ± 0.02	0.93 ± 0.01	0.94 ± 0.02	0.96 ± 0.01*
HR (bpm)	PLA	112 ± 3	128 ± 3	142 ± 3	156 ± 3
	CAF	112 ± 3	126 ± 4	141 ± 4	156 ± 4
RPE (Borg scale 6–20)	PLA	8.0 ± 0.3	10.9 ± 0.4	12.7 ± 0.3	14.8 ± 0.3
	CAF	7.9 ± 0.4	10.2 ± 0.4*	12.2 ± 0.3*	14.1 ± 0.3*

Values presented as mean ± SEM.

\*Significantly different from PLA ( $P < 0.05$ ).

performance in DP because muscular pain was much greater in arms (almost unbearable) than that in legs (some pain) (Fig. 3C). Importantly, we show for the first time that CAF increases endurance performance in DP in which the main muscles used to produce power and speed are in the upper body.

The results from the present study are of significant interest for performance sports. CV results for reliability of the 8-km C-PT are small (around 1%–2%) and show little variation from test to test after the familiarization procedure (Fig. 1). The improvement of 4% after ingestion of  $6 \text{ mg}\cdot\text{kg}^{-1}$  CAF observed in the study would most likely effect results in real-life XCS competitions. Actually, a reduction of 1:25 min to complete the 8-km C-PT is the time difference between the winner and 12th place in the 2011 World Championships in XCS during the 15-km classic relay in Oslo.

It has been reported that CAF improves performance by 1%–5% in cycling (26), running (7), and rowing (8) studies. Our present study is in agreement with these studies. CAF was initially suggested to improve performance because fat oxidation was increased and glycogen was spared (11,25). The higher lactate concentration after the C-PT with CAF compared with PLA does not support that glycogen sparing was the reason for the improved performance. Several mechanisms contribute to the ergogenic effects of CAF (20,36). Many of these effects seem to be related to CAF affecting the CNS. CAF is an antagonist of adenosine receptors with particularly high affinity for  $A_1$  and  $A_{2A}$  receptors (16), and our plasma CAF concentrations of approximately  $40 \mu\text{M}$  (Table 1) would significantly reduce  $A_1$  and  $A_2$  receptor activation (16). Adenosine receptors are expressed in most tissues including brain, heart, skeletal muscles, and the vascular system (17). Blockade of adenosine receptors reduces somatic pain (14), improves MVC (39), as well as reduces perceived exertion at submaximal intensities (33,36). In our present study, CAF reduced RPE in DP at 50%, 60%, and 70% of maximal oxygen uptake (Table 3), which is in agreement with findings in running and cycling (2,3,13).

CAF-mediated reduction in pain perception or increased MVC may have contributed to the improved performance during the 8-km C-PT. The higher lactate concentration after CAF compared with PLA supports higher effort but does not explain alone the improved performance. The poling speed was higher in the 8-km C-PT with CAF, which would require a higher aerobic capacity and/or power production. Indeed, higher average power per stroke during DP must have been produced after CAF ingestion because the number of DP strokes used to complete the 8-km C-PT was similar in PLA and CAF.

CAF has been observed to improve MVC and muscular endurance (39). The effect of CAF on MVC seems to be related to improved motor unit recruitment (31) and not as a direct effect on the muscles (39). An improved MVC after CAF ingestion resulting from increased motor unit recruitment (31) would potentially increase DP power production.

Facilitation of motor unit recruitment might also reduce RPE as observed at submaximal workloads in the study. Although well-documented effect of CAF on MVC has only been reported in knee extensors (39), it is possible that the improved performance after CAF ingestion in the study came as a result of improved MVC. However, an improved MVC in arm muscles after CAF ingestion is not consistently documented (39).

In the present study, pain in the arms becomes intense in the arms (almost unbearable, Fig. 3C), and the ability to withstand pain and to maintain an effective DP technique is important for high performance. Our participants exercised at a higher intensity throughout the whole 8-km C-PT, which lead to higher HR and blood lactate accumulation in the CAF trial compared with PLA. Thus, we speculate that reduced pain sensation allows subjects to push themselves harder and exercise at a higher HR. The higher concentrations of lactate and adrenaline immediately after the 8-km C-PT with CAF ingestion support the idea that reduced pain sensation allowed higher discomfort, higher HR, and better effort.

CAF might have increased HR by direct action on adenosine receptors in the heart coupled to  $G_{ai}$  or by increasing sympathetic activity (16). However, we did not observe any effect of CAF on HR,  $\dot{V}O_2$ , or adrenaline concentration at rest or during submaximal loads (Tables 1 and 3). Increased HR after CAF consumption was only observed when speed was higher during the 8-km C-PT. No measurements of  $\dot{V}O_2$  were done during the 8-km C-PT, but the average HR increases with 5 bpm during the 8-km C-PT, and the speed was improved by  $0.6 \pm 0.04 \text{ km}\cdot\text{h}^{-1}$ . Results at the submaximal exercise intensities show that an increase of  $0.6 \text{ km}\cdot\text{h}^{-1}$  requires an increase in  $\dot{V}O_2$  of  $3.2 \pm 0.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  and HR of 6 bpm. Because HR was  $5 \pm 1$  bpm higher during the CAF trial compared with the PLA trial during the 8-km C-PT (Fig. 3B), it is tempting to suggest that the ability to maintain a higher HR during the CAF time trial increased oxygen uptake. A higher delivery of  $O_2$  to the active muscles in the arms might therefore have allowed for an increased production of power during DP.

In our present study, carbohydrate oxidation was much higher during arm exercise compared with running at similar relative intensities. It has been shown that blood glucose extraction is higher in arm muscle than that in leg muscle exercise (37), and the higher carbohydrate metabolism in arm muscles is explained by lower density of capillaries and mitochondria and less effective carbohydrate oxidation in arm muscles compared with leg muscles (10,22,37). Interestingly, blood glucose decreased during poling at all submaximal intensities in PLA, and blood glucose was higher in CAF compared with PLA at 50% and 70% of  $\dot{V}O_{2\text{max-pol}}$ . Recently, it has been shown that intake of CAF reduces contraction-stimulated glucose uptake in rat skeletal muscles (32). Thus, it is possible that CAF reduces glucose uptake in arm muscles. Interestingly, at 70% of  $\dot{V}O_{2\text{max-pol}}$ , RER was lower in CAF compared with PLA, although adrenaline, glycerol, and FFA were similar and glucose concentration was higher in

CAF. These data suggest that glucose uptake may have been impaired directly by CAF. Indeed, the higher glucose concentration in CAF may also be due to higher glucose production, and future studies will clarify the effect of CAF on glucose kinetic during arm exercise.

## CONCLUSION

We are the first to report that ingestion of 6 mg of CAF per kilogram of body weight enhances endurance performance in an 8-km DP time trial, an activity in which arm muscles limit performance. CAF ingestion allowed the participants to exercise with a higher HR during the time trial and experience reduced RPE at submaximal intensities. These

results are quite similar to those observed in studies where subjects are mainly limited by endurance capacity of leg muscles, e.g., cycling and running. We speculate that CAF-mediated improvement in performance comes as a result of reduced pain sensation, allowing subjects to exercise at higher speeds and HR.

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# PAPER II

*Caffeine and performance over consecutive days of simulated competition.*

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# 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·kg<sup>-1</sup> 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 ( $\dot{V}O_{2\max-\text{norm}}$  78.5 ± 1.6 mL·kg<sup>-1</sup>·min<sup>-1</sup>) 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·kg<sup>-1</sup>) 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·kg<sup>-1</sup> (11,19,20). Higher doses (9–12 mg·kg<sup>-1</sup>) 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·kg<sup>-1</sup>) 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.

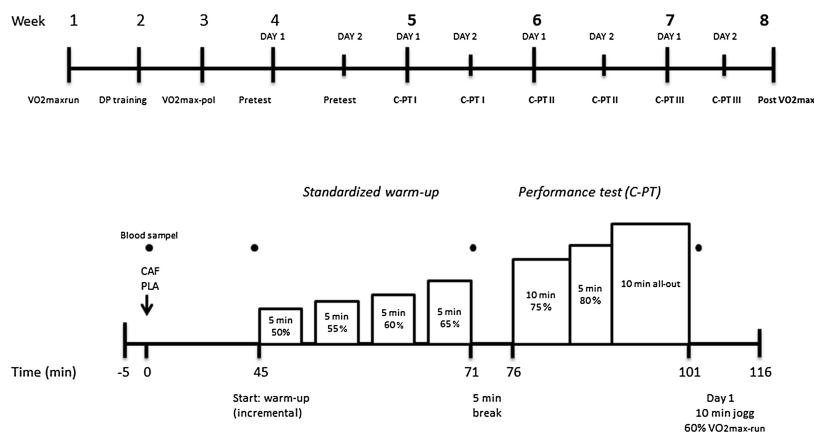
We hypothesized that ingestion of CAF would improve performance in double poling (DP) on day 1, as observed in previous studies. However, because of a higher exercise intensity on day 1, subjects would be more fatigued on day 2, leading to impaired performance in the CAF groups compared with that in 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 ( $\dot{V}O_{2max-run}$ ) (78.5 ± 1.6 mL·kg<sup>-1</sup>·min<sup>-1</sup>), and  $\dot{V}O_{2peak}$  when DP ( $\dot{V}O_{2peak-pol}$ ) (70.5 ± 1.6 mL·kg<sup>-1</sup>·min<sup>-1</sup>). Inclusion criteria were that all subjects had to be male, have a  $\dot{V}O_{2max-run}$  greater than 70 mL·kg<sup>-1</sup>·min<sup>-1</sup>, 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  $\dot{V}O_{2max-run}$  test on a treadmill (Woodway, Weil am Rhein, Germany) and the highest HR was defined as HR<sub>max-run</sub>. 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  $\dot{V}O_{2max-run}$  test was performed with a standardized warm-up consisting of four workloads lasting 5 min (8–11 km·h<sup>-1</sup>) 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·h<sup>-1</sup> before starting the  $\dot{V}O_{2max-run}$  test. The starting speed was 10 km·h<sup>-1</sup>, with a treadmill incline of 10.5°. Each 0.5-min speed was increased with 0.5 km·h<sup>-1</sup> 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  $\dot{V}O_{2max-run}$ : 1) oxygen consumption reached a plateau, meaning  $\dot{V}O_2$  increased less than 1 mL·kg<sup>-1</sup>·min<sup>-1</sup> whereas speed was increased two times 0.5 km·h<sup>-1</sup>, 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 administered and no blood samples were taken.  $\dot{V}O_{2max-run}$ , Training in ThoraxTrainer,  $\dot{V}O_{2peak-pol}$ ; pretest 1, pretest 2, C-PT 1, C-PT 2, and C-PT 3.

greater than 7.0 mM, and 4) RPE  $\geq 19$  on the Borg scale 6–20 (5).  $\dot{V}O_{2\max\text{-run}}$  was based on the average of the two highest measurements. Subjects with  $\dot{V}O_{2\max\text{-run}}$  higher than  $70 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  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  $\text{HR}_{\max\text{-run}}$ . Day 3 subjects performed a  $\dot{V}O_{2\text{peak-pol}}$  test on the poling ergometer, with the highest HR defined as  $\text{HR}_{\max\text{-pol}}$ . Criteria for that  $\dot{V}O_{2\text{peak-pol}}$  were reached were the same as those for  $\dot{V}O_{2\max\text{-run}}$ . 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  $\dot{V}O_{2\text{peak-pol}}$ , 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  $\dot{V}O_{2\text{peak-pol}}$ . 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  $\dot{V}O_{2\max\text{-run}}$ . 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 ( $<150 \text{ mg}$ ), based on a self-reported CAF intake questionnaire.

The subjects arrived at the laboratory at the same time for all tests ( $\pm 15 \text{ min}$ ). 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  $\mu\text{L}$ , 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  $4^\circ\text{C}$  (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  $-80^\circ\text{C}$ .

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  $4.5 \text{ mg}\cdot\text{kg}^{-1}$ ) 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  $4.5 \text{ mg}\cdot\text{kg}^{-1}$  on performance. CAF (Coffeinum; Oslo Apotekproduksjon, Oslo, Norway) was dissolved in a cordial concentrate Fun Light ( $3 \text{ mg}\cdot\text{mL}^{-1}$ ) 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·h<sup>-1</sup>), 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 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

**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

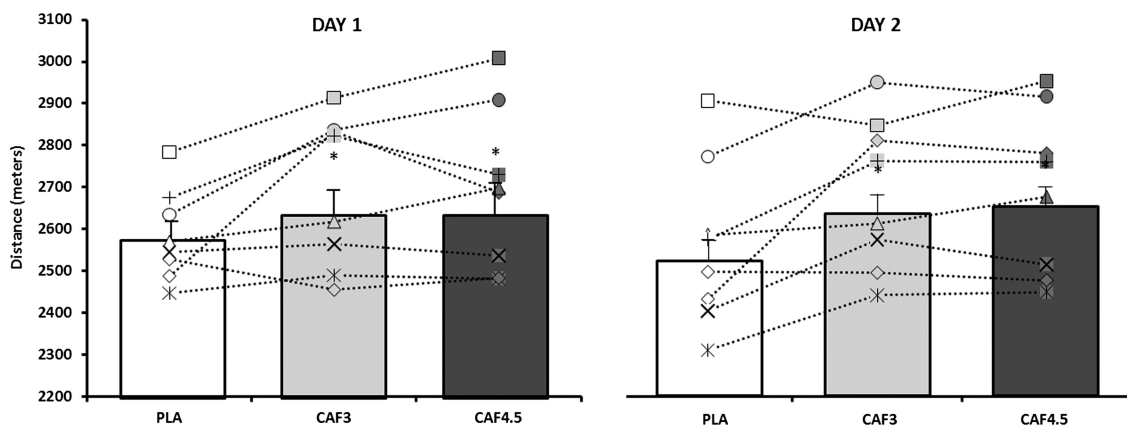


FIGURE 2—Mean and individual distances covered during the 2 d of the 10-min all-out C-PT after consuming PLA, CAF3, or CAF4.5. Values are mean ± SEM. \*Significantly different from PLA ( $P < 0.05$ ).

on any of the two testing days (PLA,  $618 \pm 42$  and  $638 \pm 32$ ; CAF3,  $625 \pm 32$  and  $621 \pm 22$ ; and CAF4.5,  $619 \pm 26$  and  $623 \pm 29$ ). Mean speed was  $15.5 \pm 0.2$  and  $15.4 \pm 0.4 \text{ km}\cdot\text{h}^{-1}$  for PLA days 1 and 2, respectively. After CAF ingestion, mean speed increased to  $16.2 \pm 0.4 \text{ km}\cdot\text{h}^{-1}$  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  $16.1 \pm 0.4 \text{ km}\cdot\text{h}^{-1}$  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  $180 \pm 3$  and  $180 \pm 4$  bpm for PLA. During CAF trials, higher average HR were observed for both CAF3 ( $184 \pm 3$  bpm) and CAF4.5 ( $185 \pm 3$  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  $184 \pm 3$  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  $4.2\% \pm 3.8\%$  and  $4.4\% \pm 3.8\%$ , respectively). On the second testing day, only a tendency was observed for increased  $\dot{V}O_2$  ( $P = 0.12$ ) during CAF trials (difference,  $1.2\% \pm 5.0\%$  and  $1.4\% \pm 5.8\%$ ). Unexpectedly, all subjects were able to reach new  $\dot{V}O_{2\text{peak-pol}}$  values during CAF trials compared with PLA trials, and seven of eight subjects set new  $HR_{\text{max-pol}}$  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 ( $54\text{--}58$  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  $\dot{V}O_{2\text{peak-pol}}$ ) were observed on the first testing day after consuming CAF 3 or  $4.5 \text{ mg}\cdot\text{kg}^{-1}$  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 ( $78 \pm 5$ ,  $82 \pm 5 = \text{“very high”}$ ), CAF3 ( $87 \pm 4$ ,

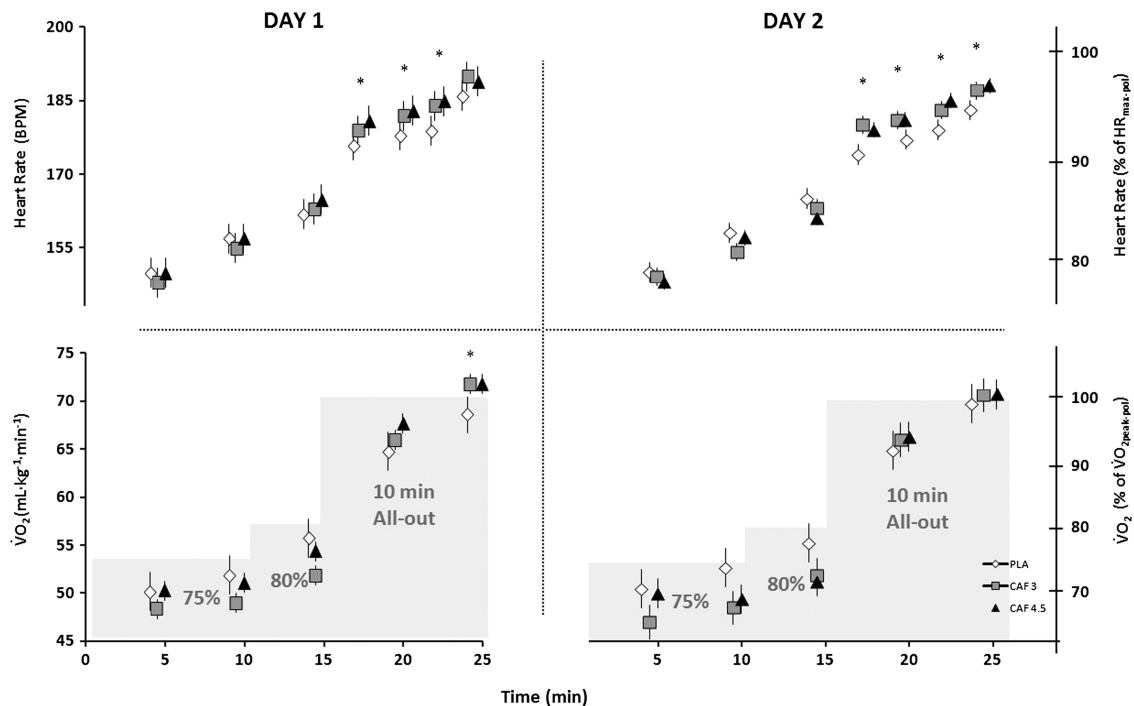


FIGURE 3—HR and  $\dot{V}O_2$  response after consuming PLA, CAF3, and CAF4.5 during the whole performance test 15-min and 10-min all-out C-PT. Values are mean  $\pm$  SEM. \*Significantly different from those in PLA ( $P < 0.05$ ).

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.

Time Point	Variable	Day 1			Day 2		
		PLA	CAF3	CAF4.5	PLA	CAF3	CAF4.5
C-PT finished	Mean speed (km·h <sup>-1</sup> )	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 ( $P < 0.05$ ).

$80 \pm 4$  = “very high”), and CAF4.5 ( $80 \pm 6$ ,  $82 \pm 5$  = “very high”). Subjects also reported similar day forms before and after finishing all tests ( $72$ – $77 \pm 5$  = “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·kg<sup>-1</sup>·min<sup>-1</sup>), 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  $\dot{V}O_{2max-run}$  ( $78.5 \pm 1.6$ ,  $77.0 \pm 1.6$ ) or  $\dot{V}O_{2peak-pol}$  ( $70.5 \pm 1.2$ ,  $69.6 \pm 2.0$ ) 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·kg<sup>-1</sup> 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·kg<sup>-1</sup> leads to similar exercise improvements as those of ingestion of 4.5 mg·kg<sup>-1</sup>. 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·kg<sup>-1</sup>, 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.

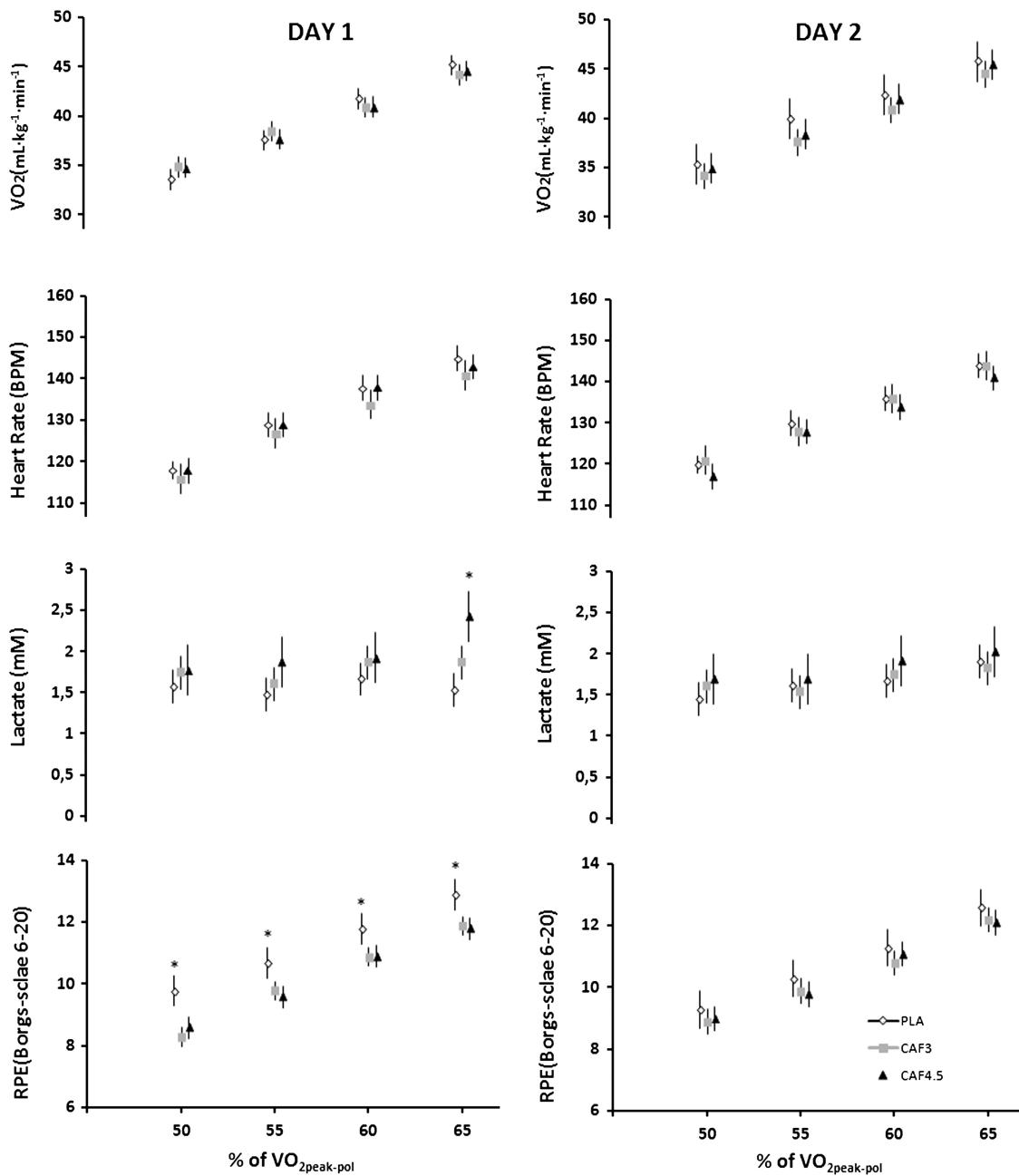


FIGURE 4— $\dot{V}O_2$  uptake, HR, lactate concentration, and RPE as a function of increasing workload during the standardized warm-up (submaximal incremental exercise) after consuming PLA, CAF3, and CAF4.5. Values are mean  $\pm$  SEM. \*Significantly different from PLA ( $P < 0.05$ ).

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  $\mu\text{M}$  (3 mg) and approximately 30  $\mu\text{M}$  (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  $\dot{V}O_{2\text{peak-pol}}$ , and seven of eight subjects, new  $HR_{\text{max-pol}}$  during CAF 10-min all-out C-PT. Posttesting in week 8 of  $\dot{V}O_{2\text{peak-pol}}$  and measurements of  $\dot{V}O_{2\text{peak}}$  during the PLA 10-min all-out C-PT showed no difference from premeasurements. Furthermore, similar results were observed for  $HR_{\text{max-pol}}$ .

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  $\text{mg}\cdot\text{kg}^{-1}$  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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# PAPER III

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

**Stadheim HK**, Nossum E.M, Olsen R, Spencer M, Jensen J.

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HIGHLIGHTED TOPIC | Hypoxia 2015

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

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**Stadheim HK, Nossum EM, Olsen R, Spencer M, Jensen J.** Caffeine improves performance in double poling during acute exposure to 2,000-m altitude. *J Appl Physiol* 119: 1501–1509, 2015. First published October 29, 2015; doi:10.1152/jappphysiol.00509.2015.— There is limited research on the physiological effects of caffeine (CAF) ingestion on exercise performance during acute hypoxia. The aim of the present study was therefore to test the effect of placebo (PLA) and CAF (4.5 mg/kg) on double poling (DP) performance during acute hypoxia. Thirteen male subelite cross-country skiers ( $\dot{V}O_{2\max}$   $72.6 \pm 5.68$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) were included. Performance was assessed as 1) an 8-km cross-country DP time-trial (C-PT), and 2) time until task failure at a set workload equal to ~90% of DP  $\dot{V}O_{2\max}$ . Testing was carried out in a hypobaric chamber, at 800 mbar ( $P_{iO_2}$ : ~125 mmHg) corresponding to ~2,000 m above sea level in a randomized double-blinded, placebo-controlled, cross-over design. CAF improved time to task failure from  $6.10 \pm 1.40$  to  $7.22 \pm 1.30$  min ( $P < 0.05$ ) and velocity the first 4 km ( $P < 0.05$ ) but not overall time usage for the 8-km C-PT. During submaximal exercise subjects reported lower pain in arms and rate of perceived exertion (RPE) following CAF ingestion. Throughout C-PTs similar RPE and pain was shown between treatments. However, higher heart rate was observed during the CAF 8 km ( $187 \pm 7$  vs.  $185 \pm 7$ ;  $P < 0.05$ ) and 90% C-PT ( $185 \pm 7$  vs.  $181 \pm 9$ ) associated with increased ventilation, blood lactate, glucose, adrenaline, decreased pH, and bicarbonate. The present study demonstrates for the first time that CAF ingestion improves DP time to task failure although not consistently time trial performance during acute exposure to altitude. Mechanisms underpinning improvements seem related to reduced pain RPE and increased heart rate during CAF C-PTs.

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

DURING THE 1968 OLYMPIC GAMES in Mexico City at an altitude of 2,240 m sprinters and jumpers set several world records while long distance runners ran markedly slower compared with sea-level results. This launched a scientific interest in understanding mechanisms explaining reduced endurance performance under hypoxic conditions (4, 21, 44).

At sea level the lungs and the pulmonary system normally have no problem fully saturating arterial blood with O<sub>2</sub> (SpO<sub>2</sub>) during rest or high-intensity exercise (3). However, when exposed to hypoxia, the reduction in performance and  $\dot{V}O_{2\max}$  is highly related to pulmonary limitations in saturating hemoglobin while passing alveolar ducts, due to reduction of partial

pressure in the atmosphere (4, 21, 22, 32, 44). This phenomenon, known as the exercise-induced arterial hypoxemia (EIH), is defined as SpO<sub>2</sub> ≤ 92% (44), and despite ventilation ( $\dot{V}_E$ ) increases to prevent EIH during exercise, a greater reduction of SpO<sub>2</sub> in arterial blood is evident during acute hypoxic exercise compared with sea-level conditions (2, 4, 16, 17, 44). The reduction in SpO<sub>2</sub> also triggers a compensatory acceleration of heart rate (HR) during submaximal and maximal exercise to prevent EIH (4, 12, 39, 44). However, a decrease in maximal HR is well established when subjects are exposed to acute hypoxia and may contribute to the observed reduction in  $\dot{V}O_{2\max}$  and exercise performance (2, 4, 25, 30, 39, 44). The reason for the reduction in HR<sub>peak</sub> is not entirely understood, but it is believed to be associated with enhanced parasympathetic neural activity due to decreased signals from skeletal muscles (2, 9).

The extensive research related to the effects of caffeine (CAF) ingestion (3–9 mg/kg) during sea-level testing shows it is beneficial in most sporting conditions for improving endurance performance (10, 14, 41, 42). However, the effects of CAF ingestion on performance during acute hypoxia have so far received little attention. Until now only two studies have addressed the topic at altitudes above 4,300 m under standardized laboratory conditions, whereas the upper limit used in today's elite sports competitions is ~2,000 m (5, 22). Interestingly, one of the most consistent observations associated with performance improvements after CAF ingestion is increased HR (10, 14, 29, 41, 42). The explanation for higher HR following CAF ingestion is increased intensity during time trials and/or sympathetic neural activity explained by higher adrenaline and/or inhibition of adenosine receptors (10, 14, 41, 42). Furthermore, studies have also found the increase in HR and performance capacity to be associated with increased  $\dot{V}O_2$  (1, 27, 35, 41). However, if compromised oxygen saturation limits performance during acute hypoxia, it could be hypothesized that a potential increased HR following CAF ingestion would not necessarily increase  $\dot{V}O_2$  or improve performance as previously observed during sea-level conditions.

The aim of the present study was therefore to test the effect of CAF (4.5 mg/kg) ingestion on DP performance during acute exposure (2 h) to hypoxia corresponding to 2,000 m (800 mbar) in a hypobaric chamber. To investigate the effect of CAF on HR,  $\dot{V}O_2$ , and endurance performance at altitude, an 8-km cross-country skiing double poling (DP) time trial performance test (8-km C-PT) and a time to task failure at a fixed workload (~90% of  $\dot{V}O_{2\text{PEAK-POL-ALT}}$ ; 90% C-PT) was used.

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## MATERIALS AND METHODS

**Subjects.** Thirteen healthy male subelite cross-country skiers gave their written consent to participate after being informed of the purposes of the study and risks involved. Their physical characteristics (means  $\pm$  SD) were age  $21.9 \pm 2.7$ , height  $180.0 \pm 3.7$ , body mass  $77.4 \pm 5.6$ ,  $\dot{V}O_{2\max}$  running at sea level ( $\dot{V}O_{2\max\text{-run}}$ )  $72.6 \pm 5.7$  (ml·kg<sup>-1</sup>·min<sup>-1</sup>),  $\dot{V}O_{2\max}$  DP at sea level ( $\dot{V}O_{2\max\text{-pol}}$ )  $62.9 \pm 6.8$  and  $\dot{V}O_{2\max}$  DP at altitude ( $\dot{V}O_{2\max\text{-pol-alt}}$ )  $53.8 \pm 5.3$  ml·kg<sup>-1</sup>·min<sup>-1</sup>. Inclusion criteria were male,  $\dot{V}O_{2\max\text{-run}}$  above 65 ml·kg<sup>-1</sup>·min<sup>-1</sup>, and training seriously to compete in the Norwegian national cross-country skiing cup in the upcoming season.

**Study design.** The study had a randomized double-blind, placebo-controlled, cross-over design and was evaluated and approved by the Regional Ethics Committee of Southern Norway. The tests and familiarization during the first 4 wk of the study were performed at sea-level conditions at the Norwegian School of Sports Sciences (120-m altitude,  $\sim$ 960 mbar). Testing included  $\dot{V}O_{2\max}$  running (week 1), familiarization DP training (week 2) and DP  $\dot{V}O_{2\max}$  (week 3), a test 8-km C-PT (week 3), and the main 8-km C-PT (week 5). The remaining 5 test wk were carried out during acute (2 h) hypoxia in a hypobaric chamber (Norsk Indervannsteknikk, Haugesund, Norway) and included DP  $\dot{V}O_{2\max}$  (week 5), a pre 8-km C-PT (week 5), two main 8-km C-PTs (weeks 6 and 7) with and without CAF, and two time to task failure tests at fixed workload  $\sim$ 90% of DP  $\dot{V}O_{2\max\text{-pol-alt}}$  (weeks 8 and 9) with and without CAF.

**Experimental procedures.** At sea level subjects the first testing day (day 1) performed a  $\dot{V}O_{2\max\text{-run}}$  test on a treadmill (Woodway, Weil am Rhein, Germany) and the highest HR was defined as HR<sub>max-run</sub>. HR was measured during all tests in the study using a HR monitor (Polar RS 800), with an error of measurement of less than  $\pm 1\%$  as stated by the manufacturer. Oxygen consumption and respiratory exchange ratio (RER) were measured with the Oxycon Pro metabolic system (Jaeger Hochberg). The Oxycon Pro is calibrated each month against the Douglas bag method, and the error of measurement of this ergospirometry measurement is reported to be  $\pm 3\%$ . The equipment for measurement of  $\dot{V}O_2$  was calibrated before tests with mixture gasses with known concentrations of O<sub>2</sub> and CO<sub>2</sub> (14.93% O<sub>2</sub> and 5.99% CO<sub>2</sub>) and normal air ( $\sim$ 20.95% O<sub>2</sub> and 0.039% CO<sub>2</sub>) both at altitude and at sea level. Volume was calibrated manually using a 3-liter pump (Calibration Syringe, Series 5530, Hans Rudolph Instruments). During testing, subjects used a mouth V2 mask (Hans Rudolph Instruments) in combination with a nose bracket. Expired air was sampled through a hose into the mixing chamber (Oxycon Pro) and analyzed with a turbine (Triple V volume transducer). The  $\dot{V}O_{2\max\text{-run}}$  test was performed with a standardized warm-up consisting of four workloads lasting 5 min (8 to 11 km/h) with a 10.5° uphill incline. A 1-min break was given between each workload during which lactate was measured. After the last workload of the warm-up, subjects walked 5 min at 5 km/h before starting the  $\dot{V}O_{2\max\text{-run}}$  test, which was performed as a standardized ramp test. The starting speed for the ramp test was 10 km/h with a treadmill incline of 10.5°. Each half minute speed was increased by 0.5 km/h until subjects were unable to maintain the speed and stepped off the treadmill (voluntary exhaustion). On the basis of the standardized warm-up, a linear regression was done to estimate ending O<sub>2</sub> cost. Results showed subjects were performing supramaximal workloads the last 2–2.5 min and were ending at workloads  $\sim$ 110–115% of reached  $\dot{V}O_{2\max}$ . Furthermore, all 13 subjects had to meet point one and at least two of the three other criteria to obtain  $\dot{V}O_{2\max\text{-run}}$ : 1) oxygen consumption leveled off (plateau), meaning  $\dot{V}O_2$  increased less than 1 ml·kg<sup>-1</sup>·min<sup>-1</sup>, while speed was increased two times 0.5 km/h; 2) RER values were  $>1.10$ ; 3) blood lactate was above 7.0 mmol/l posttesting; and 4) rate of perceived exertion (RPE)  $\geq 19$  on the Borg Scale 6–20 (8).  $\dot{V}O_{2\max\text{-run}}$  was based on the average of the two highest 30-s measurements, and the duration of the test was between 5.5 and 7.5 min. The protocol and criteria for reaching  $\dot{V}O_{2\max}$  differ from some other protocols used for testing of  $\dot{V}O_{2\max}$  (36). Indeed, it

is debatable, therefore, whether all subjects reached  $\dot{V}O_{2\max}$ . However, the fact that the athletes in the study were highly trained and motivated could partially reduce the issue of whether  $\dot{V}O_{2\max}$  was reached. Furthermore, the  $\dot{V}O_{2\max\text{-run}}$  test was only used as an inclusion test; only subjects with  $\dot{V}O_{2\max\text{-run}}$  higher than 65 ml·kg<sup>-1</sup>·min<sup>-1</sup> were included for further participation.

**Day 2** subjects performed 40 min of familiarization DP training on the poling ergometer (Thoraxtrainer Elite) with workloads ranging from  $\sim$ 55 to 85% of their HR<sub>max-run</sub>.

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

**Days 4 and 5** participants completed the pre-8-km C-PT and the 8-km C-PT at sea level, but without supplementation since this has previously been investigated by Stadheim et al. (41). Furthermore, Stadheim et al. showed that a minimum of one habituation trial of at least one 8-km C-PT is required to obtain acceptable reliability [coefficient of variation (%)  $\sim$ 1–2%]. The 8-km C-PT started with a standardized warm-up performed as an incremental test with four 5-min workloads, equivalent to loads corresponding to 50, 55, 60, and 65% of subjects'  $\dot{V}O_{2\max\text{-pol}}$  with a 1-min break between each workload. HR,  $\dot{V}O_2$ , and RER were measured as means between 3 and 4.5 min of each workload. Subjective RPE according to the Borg scale (from 6 to 20), and muscular pain in arms and legs were determined (1–10 point scale) for each workload (8). Following the warm-up, a 5-min break was used for blood sampling and preparation for the 8-km C-PT. During the C-PTs subjects performed the test with the goal of completing the distance in as little time as possible (41). Subjects received the V2 mask and nose bracket  $\sim$ 1.5–2 min before reaching 4 and 8 km for measurement of  $\dot{V}O_2$ .

Altitude and hypoxic testing started in week 5. On day 6 subjects performed the same protocol for testing of  $\dot{V}O_{2\max\text{-pol-alt}}$  as described for sea-level  $\dot{V}O_{2\max\text{-pol}}$  testing. In the hypobaric chamber,  $\dot{V}O_2$  and  $\dot{V}E$  were measured using the V<sub>max29</sub> (Sensormedics), which was calibrated against the Oxycon Pro each week.

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

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

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

**Hypobaric chamber altitude testing.** During all tests in hypoxia, air pressure was reduced to 800-mbar equivalent to  $\sim$ 11.5 psi, or  $\sim$ 590 mmHg, simulating an altitude of  $\sim$ 2,000 m above sea level at 17°C. To ensure maintenance of atmospheric gas concentrations (20.95% O<sub>2</sub> and 0.039% CO<sub>2</sub>) during all trials, concentrations were continuously

measured for both  $\dot{V}_{I\text{CO}_2}$  with the Vaisala GMT222 Carbon Dioxide Transmitter (Vaisala, Stockholm, Sweden) and  $\dot{V}_{I\text{CO}_2}$  with the PMA30 M&C O<sub>2</sub> analyzer (Marseille, France). During the first 2 h (rest) of acute altitude exposure, an ~1 l/min oxygen was added to maintain atmospheric gas concentrations of air. During physical activity oxygen consumption increased, thus additional oxygen was added to maintain stable  $\dot{V}_{I\text{O}_2}$ . On the basis of the pretests, ~3 l/min of extra oxygen was added to cover the enhanced usage of oxygen during physical activity but was adjusted (increased or reduced) according to observed  $\dot{V}_{I\text{O}_2}$  values for each individual hypoxic trail. Three gas scrubbers containing Sofnolime filters and circulating fans worked as CO<sub>2</sub> traps to try and ensure a stable  $\dot{V}_{I\text{CO}_2}$  concentration. However, during the later stages of the C-PTs (~5–10 min) CO<sub>2</sub> production from the subjects exceeded the capacity of CO<sub>2</sub> removal of the three scrubbers. This resulted in an enhanced  $\dot{V}_{I\text{CO}_2}$  concentration of the air inside the chamber with postvalues of CO<sub>2</sub> between 0.05 and ~0.08%. Even though CO<sub>2</sub> concentration increased, it never exceeded 0.08%; these CO<sub>2</sub> values are not considered dangerous for subjects and are unlikely to influence test results. During rest and at sea-level testing,  $\dot{V}_{I\text{CO}_2}$  concentrations were 0.04% as expected. Encouragement was given during all tests by a blinded test leader.

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

**Plasma CAF and catecholamines.** Samples of 200  $\mu$ l plasma were prepared and the subsequent measurements of caffeine and theophylline were taken according to the method previously described in Stadheim et al. (41). Plasma epinephrine and norepinephrine were measured with a Cat Combi Elisa kit (DRG Instruments, Marburg, Germany) according to the manufacturer's instructions.

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

**Thoraxtrainer Elite.** The cross-country DP ergometer used in the study was a Thoraxtrainer Elite (Thoraxtrainer, Holbæk, Denmark). Temperature in the test laboratory was between 16 and 21°C on all test days. Ski poles used during all testing were Swix CT1 (Swix, Lillehammer, Norway) and length standardized to  $85 \pm 2\%$  of subject's height. The ski poles were attached to two sleds that moved independently and were connected to a flywheel that provided resistance. A computer displayed work output (W), km/h, and poling frequency in real time. Resistance in the Thoraxtrainer is generated by

air pressure, and the mean barometric air pressure for PLA and CAF trials averaged  $958 \pm 4$  (sea level) and  $800 \pm 7$  mmHg (altitude), respectively ( $P > 0.05$ ). The Thoraxtrainer Elite was set at level 1 (easiest) of 10 different levels during all testing to optimize technique. For more information about the DP technique and the Thoraxtrainer Elite [see Stadheim et al. (41)].

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

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

**Statistical analysis.** All data are presented as means  $\pm$  SD, and differences in performance during the 8-km and 90% C-PTs were evaluated by a paired *t*-test. A two-way ANOVA for repeated measures was used to elicit differences in  $\dot{V}_{O_2}$ , HR, lactate,  $\text{HCO}_3^-$ , glucose,  $\dot{V}_E$ , muscular pain, and RPE during submaximal workloads between the two treatments. If a significant *f*-ratio was found, a paired *t*-test was used to test differences between treatments on workloads. All data were tested for normal distribution using the Shapiro-Wilk test. Statistical analyses were performed using SPSS, and the level of significance was set at  $P < 0.05$ . Performance data were log transformed to reduce the nonuniformity of error and then back transformed to obtain the percentage difference in the means between the treatment conditions. Precision of estimation was indicated with a 90% confidence interval (26).

## RESULTS

**Comparison of sea-level DP results to acute altitude.**  $\dot{V}_{O_{2\text{max-pol}}}$  was 13.4% lower compared with running. When athletes were exposed to acute altitude,  $\dot{V}_{O_{2\text{max-pol-alt}}}$  was further reduced to 14.5% compared with results at sea level and was associated with a reduction in HR<sub>peak</sub> of 2.2%. This was reflected with subjects using 1.39 min longer to complete the 8-km C-PT at altitude compared with sea level ( $31.6 \pm 1.2$  min), corresponding to an increase in time to complete the test of 5.2% (see Fig. 2). The maintenance of higher velocity during the 8-km C-PT at sea level was associated with higher  $\dot{V}_{O_2}$  and HR (Table 1; see Fig. 2). On average  $\dot{V}_{O_2}$  was 12.5 and 10.5% higher, respectively, while HR was 2.2 and 2.5% higher, respectively, at time points 4 and 8 km (Table 1; see Fig. 2). However, subjects reported similar RPE, obtained similar lactate and glucose values after the 8-km C-PT independent of PLA test conditions (see Fig. 3).

**Performance tests at altitude.** The average time used to complete each kilometer during the 8-km C-PT at altitude showed a progressive reduction in velocity for both treatments from start to finish (Table 2; see Fig. 2), and a nonsignificant difference was observed between treatments ( $P < 0.22$ ). However, a  $0.9 \pm 1.3\%$  (90% confidence interval) improvement was evident in the CAF trial with 9 of the 13 subjects (69%) completing the 8-km C-PT faster (Table 2; see Fig. 2). Indeed, time used to complete the first half of the test (0–4 km) was improved ( $P < 0.05$ ), associated with higher  $\dot{V}_{O_2}$ , HR, and  $\dot{V}_E$

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

Measure	Sea Level Post 8-km C-PT	Altitude 2,000 m Above Sea Level (800 mBar) Post-8-km C-PT				Altitude 2,000 m Above Sea Level (800 mBar) Post 90% of alt. $\dot{V}O_{2max-pol-alt}$ C-PT			
	Placebo	Placebo	Caffeine	Qualitative inference	P values	Placebo	Caffeine	Qualitative inference	P values
Plasma caffeine, $\mu\text{g/ml}$ ( $n = 9$ )	No data	0.7 $\pm$ 0.8	28.1 $\pm$ 7.6*	Very likely	0.01	1.5 $\pm$ 2.7	30.3 $\pm$ 2.5*	Very likely	0.01
Epinephrine, nM ( $n = 13$ )	1.1 $\pm$ 0.7	1.6 $\pm$ 0.9	2.3 $\pm$ 1.6*	Very likely	0.16	1.6 $\pm$ 0.8	2.5 $\pm$ 1.5*	Very likely	0.16
La <sup>-</sup> <sub>Post</sub> , mM	6.3 $\pm$ 1.7	6.9 $\pm$ 1.5	8.2 $\pm$ 1.6*	Very likely	0.03	8.3 $\pm$ 2.1	9.8 $\pm$ 2.1*	Very likely	0.01
HR <sub>peak</sub> , beats/min <sup>-1</sup>	188 $\pm$ 10*	184 $\pm$ 7	187 $\pm$ 7*	Likely	0.03	181 $\pm$ 9‡	185 $\pm$ 7*†	Very likely	0.05
Glucose, mM	No data	7.1 $\pm$ 1.8	8.2 $\pm$ 2.2*	Most likely	0.01	6.1 $\pm$ 0.7	7.0 $\pm$ 0.9*	Most likely	0.01
HCO <sub>3</sub> <sup>-</sup> , mM	No data	16.5 $\pm$ 2.3	13.7 $\pm$ 2.0*	Most likely	0.01	14.5 $\pm$ 1.9	12.9 $\pm$ 1.7*	Most likely	0.01
pH	No data	7.33 $\pm$ 0.05	7.29 $\pm$ 0.05*	Most likely	0.01	7.28 $\pm$ 0.04	7.27 $\pm$ 0.04	Unclear	0.50
Muscular pain arms (1–10)	No Data	7.6 $\pm$ 1.4	7.2 $\pm$ 1.4	Likely	0.38	9.1 $\pm$ 1.4	8.7 $\pm$ 1.8	Likely	0.11
Muscular pain legs (1–10)	No Data	3.9 $\pm$ 2.5	3.7 $\pm$ 2.8	Unclear	0.69	5.5 $\pm$ 2.6	5.5 $\pm$ 2.8	Unclear	0.74
RPE (6–20)	19.5 $\pm$ 0.5	19.7 $\pm$ 0.6	19.4 $\pm$ 0.9	Likely	0.08	18.8 $\pm$ 0.7	18.8 $\pm$ 0.5	Unclear	0.99

Data are given as means  $\pm$  SD. La<sup>-</sup>, blood lactate; HR<sub>peak</sub>, heart rate peak; HCO<sub>3</sub><sup>-</sup>, bicarbonate; RPE, rate of perceived exertion. \* $P < 0.05$ , significant different from placebo between treatments. †Missing values from 1 subject. ‡Missing values from 2 subjects.

at 4 km during the CAF trial compared with PLA (Table 1; Fig. 1). During the second half of the 8-km C-PT (5–8 km) no difference in time usage was evident between treatments, associated with no difference in  $\dot{V}O_2$ , but with higher  $\dot{V}E$  and HR in the CAF trial (Tables 1 and 2; Fig. 1).

During the 90% C-PT subjects improved time to task failure at the fixed workload following CAF consumption compared with PLA ( $P < 0.02$ ). On average, subjects maintained the workload for 1.12 min longer, resulting in a 20.5  $\pm$  13.8% ( $\pm$  90% CL) improvement, and 9 out of 13 subjects (69%) improved time to task failure after CAF consumption. During the 90% C-PT no difference in  $\dot{V}O_2$ ,  $\dot{V}E$ , or HR was observed after 3–4 min between treatments (Table 1; Fig. 2). However, subjects reached higher HR<sub>peak</sub> and  $\dot{V}E$  during CAF testing compared with PLA (Fig. 2).

**Blood values.** Blood concentrations of CAF (plasma), lactate (capillary), adrenaline (plasma), and glucose (capillary) were all higher post both CAF C-PTs compared with PLA (Table 1). In contrast blood bicarbonate (HCO<sub>3</sub><sup>-</sup>) was reduced in both C-PTs, while a significant reduction in pH was only observed after the 8-km C-PT (Table 1).

**Submaximal incremental test.**  $\dot{V}O_2$  and HR linearly increased from the first to the last of the four workloads during all standardized warm-ups, independent of test conditions (Fig. 3). However, higher  $\dot{V}O_2$  was observed at sea level during submaximal intensities due to higher velocity at the workload since acute altitude lead to a reduction in  $\dot{V}O_{2peak-pol-alt}$ , meaning the relative workloads and velocity had to be reduced. Despite this, similar HR and  $\dot{V}E$  were required with lower  $\dot{V}O_2$  at acute hypoxia at the same submaximal percentage of DP  $\dot{V}O_{2peak}$ . Furthermore, no difference was observed independent of test conditions for RPE, blood glucose, or lactate at submaximal exercise.

When exposed to acute hypoxia, no difference was observed among treatments for  $\dot{V}O_2$ ,  $\dot{V}E$ , or HR during submaximal exercise, but CAF ingestion resulted in elevated levels of blood lactate (Fig. 3). Furthermore, CAF ingestion resulted in a significant decrease in HCO<sub>3</sub><sup>-</sup> values after finishing the last workload of the submaximal incremental test, respectively, 25.0  $\pm$  1.0 (PLA) vs. 24.1  $\pm$  1.4 (CAF) 8-km C-PT, and 24.5  $\pm$  1.3 (PLA) vs. 23.4  $\pm$  1.4 (CAF) 90% C-PT. This observation, however, was not reflected in changes in blood pH

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

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

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



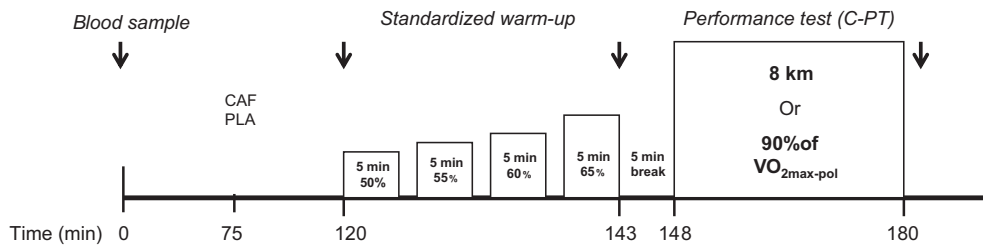


Fig. 1. Experimental design for all performance tests (C-PTs). Before the C-PTs, subjects performed a standardized warm-up (incremental tests) consisting of 4 intensities of a 5-min duration. A similar protocol to the C-PTs was completed at pretests, except that placebo (PLA)/caffeine (CAF) was not administered,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

between treatments either before or after finishing the standardized warm-up, respectively  $7.41 \pm 0.02$  (PLA) vs.  $7.41 \pm 0.01$  (CAF) 8-km C-PT, and  $7.42 \pm 0.02$  (PLA) vs.  $7.42 \pm 0.02$  (CAF) 90% C-PT. No difference in blood glucose was observed between treatments during the incremental testing. RPE was, however, reduced on the last three workloads of the incremental test when subjects consumed CAF dosages compared with that of PLA (Fig. 3). CAF ingestion also reduced perceived muscular pain for the arms during all four workloads before the 8-km C-PT, while this was only observed for the last two workloads before the 90% C-PT. No difference in self-reported muscular pain was observed for the legs between treatments on any tests.

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

## DISCUSSION

The novel finding of the present study is that CAF ingestion improved time to exhaustion by 20.5% during the 90% C-PT for 13 subelite subjects. Subjects reduced time during the first 4 km of the 8-km C-PT, but the 0.9% reduction in time usage for the whole 8-km C-PT was not significant ( $P < 0.22$ ). During all CAF C-PTs, higher HR,  $\dot{V}_E$ , blood lactate, glucose, and epinephrine and lower blood  $\text{HCO}_3^-$  and pH (8-km C-PT) were observed compared with PLA. Furthermore, subjects reported lower RPE and muscular pain in arms during CAF at submaximal intensities.

To the authors’ knowledge we are the first to investigate DP performance during acute exposure to moderate hypoxia (2,000 m). Results show that during sea-level testing subjects reached 13.4% lower DP  $\dot{V}_{O_{2\max}}$  compared with running. These results are comparable to previous studies that have observed that even elite cross-country skiers obtain ~10% lower  $\dot{V}_{O_{2\max}}$  while DP (11, 41, 42). Therefore, although the partial pressure of  $O_2$  was reduced during acute hypoxia

exercise, cardiac output (Q) might not limit DP  $\dot{V}_{O_{2\max}}$  or endurance performance. Nevertheless, a reduction in altitude DP  $\dot{V}_{O_{2\max}}$  (14.5%), performance (5.4%), and  $\text{HR}_{\text{peak}}$  (2.2%) similar to previous studies while cycling or running was observed (4, 12, 23, 44). During the 8-km C-PT, reduction in performance was associated with 12.5 and 10.5% lower  $\dot{V}_{O_{2\max}}$  and 2.4 and 1.7% lower  $\text{HR}_{\text{peak}}$  and mean at 4 and 8 km, respectively. These results indicate DP endurance capacity and performance in acute hypoxia are limited by both supply and extraction, associated with lower HR and  $\dot{V}_{O_2}$  (21, 32, 44).

The major finding in the study was that CAF improved performance during the 90% C-PT, comparable to Fulco et al. (22) who found that CAF improved time to exhaustion during acute exposure to hypoxia while cycling at 4,300 m. Berglund and Hemmingsson (5) have previously reported that CAF improved cross-country skiing performance during time trial testing at an altitude of 2,900 m. In the present study a nonsignificant effect of CAF ingestion was observed for the 8-km DP time trial. However, subjects completed the first 4 km faster and reduced overall time usage with 0.9% with a possible effect with magnitude based statistics. Indeed, it is well documented that CAF improves sea-level exercise performance, and we have previously found that CAF improves DP performance during the 8-km C-PT (14, 28, 29, 41, 42). The improvements following CAF ingestion are linked to the inhibiting of  $A_1$  and  $A_2$  adenosine receptors, reducing RPE and pain sensations due to their involvement and effects on nociception (10a, 15, 18, 24, 33, 41, 42).

In the present study plasma CAF concentration of ~30  $\mu\text{g/ml}$  would partially inhibit  $A_1$  and  $A_2$  adenosine receptor activation (18). Results during CAF submaximal exercise show a reduction in both RPE and muscular pain in arms despite increased blood lactate and reduced blood bicarbonate ( $\text{HCO}_3^-$ ). However, subjects reported maximal effort during both CAF and PLA C-PTs. Indeed, CAF’s ability to lower sensation of pain and RPE may therefore be beneficial for higher performance during the 90% C-PT since the test requires no pacing strategy, as intensity is predetermined. However, higher velocity during the first 4 km of the 8-km C-PT due to lower pain and RPE could result in higher blood lactate and lower  $\text{HCO}_3^-$  and pH leading to intracellular perturbations. Early perturbations during the 8-km C-PT could impair overall performance and may explain why the increased velocity was not sustained. The fact that CAF improved the 90% C-PT but not significantly the 8-km C-PT may indicate that pacing



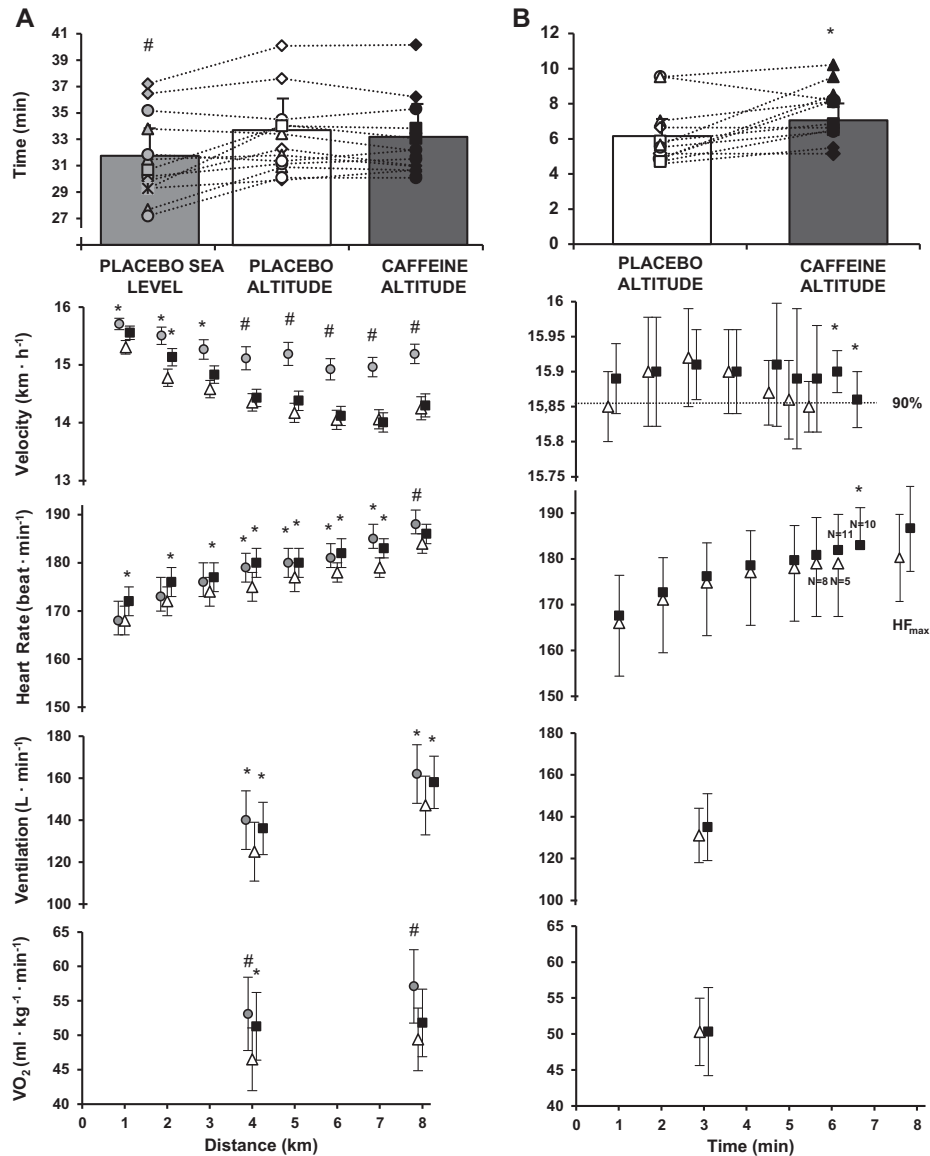


Fig. 2. *A*: time, speed, heart rate,  $\dot{V}_E$ , and  $\dot{V}O_2$  displayed as means during the 8-km C-PT at sea level and 2,000-m altitude during PLA and CAF testing. *B*: time, speed, heart rate,  $\dot{V}_E$ , and  $\dot{V}O_2$  displayed as means during the 90% C-PT at 2,000 m altitude during PLA and CAF trials. Values are listed as means  $\pm$  SD. \*Significant difference between PLA and CAF altitude ( $P < 0.05$ ). #Significant difference between PLA sea level and CAF/PLA altitude ( $P < 0.05$ ). Note:  $\dot{V}O_2$  measurements are missing 5 subjects.

strategy can become inefficient when CAF is ingested at altitude.

Increased exercise duration during the CAF 90% C-PT would require a higher energy production if work efficiency was not improved. Results from the present study show HR,  $\dot{V}_E$ , and  $\dot{V}O_2$  increased similarly during the first part of the 90% C-PT, but higher HR was observed at exhaustion in the CAF

trial. During the standardized warm-up, HR,  $\dot{V}_E$ , and  $\dot{V}O_2$  also increased in a similar measure for both treatments. These results indicate that CAF does not influence cardiac output or work efficiency during submaximal or maximal exercise. Rather, the fact that subjects increased lactate and reduced  $HCO_3^-$  post-CAF C-PTs indicates a larger anaerobic energy contribution.

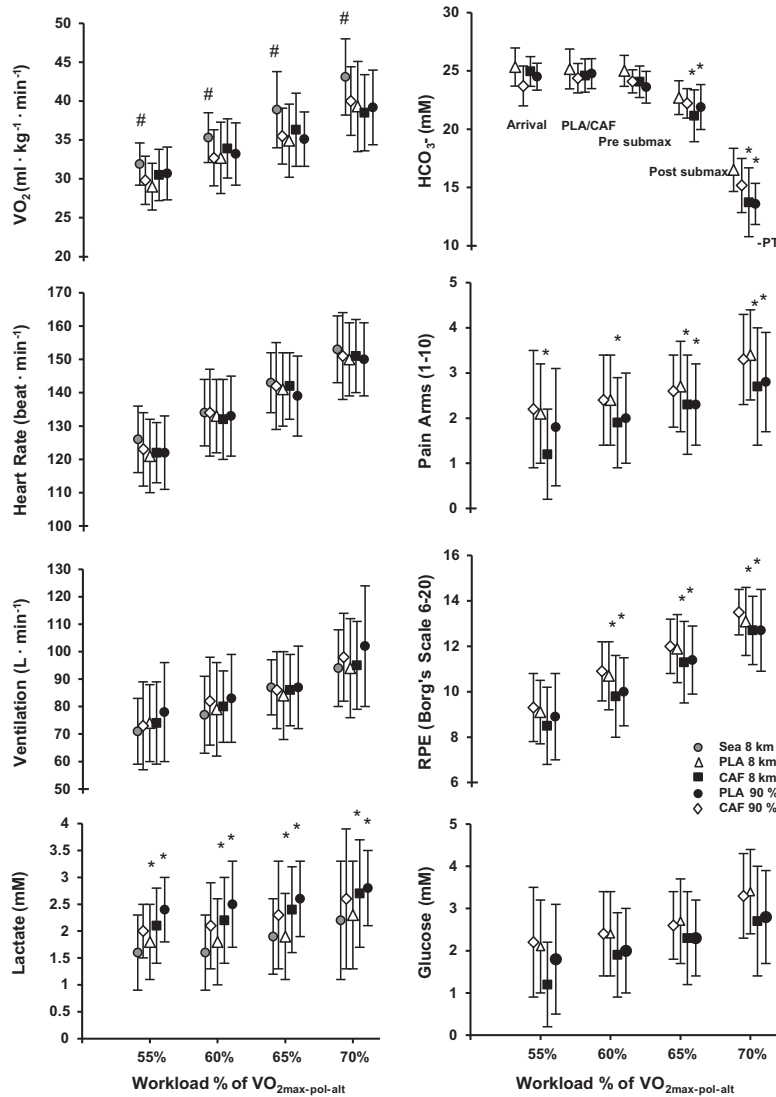


Fig. 3. Physiological and psychological responses during the standardized warm-up during acute hypoxic condition during PLA, CAF, and sea-level submaximal exercise before all C-PTs. Data are given as means  $\pm$  SD. Data on  $\dot{V}O_2$  and  $\dot{V}E$  are missing from 1 subject. \*Significant difference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

Researchers have observed that the acute effects of hypoxia have a minor negative effect on anaerobic capacity (20). An effective way of improving performance following CAF consumption would therefore be to improve the anaerobic energy system by reducing  $\text{HCO}_3^-$  and pH and by increasing lactate production (6, 31, 34). Numerous studies have demonstrated that metabolic acidosis is an important contributing factor to fatigue during prolonged high-intensity exercise (34). During exercise, hydrogen ions produced are transported to the bloodstream and buffered by blood  $\text{HCO}_3^-$  in an attempt to maintain normal pH in exercising muscles to preserve high-intensity performance (6, 34). The increased reduction in  $\text{HCO}_3^-$  during CAF C-PTs would

indicate a larger amount of  $\text{H}^+$  efflux from muscles was buffered by blood  $\text{HCO}_3^-$  possibly preserving favorable intracellular conditions in muscle for high performance. However, improved anaerobic capacity only partly explain improvement during the 90% C-PT. It was therefore interestingly to observe that in contrast to previous acute altitude studies, subjects reached similar HR as in sea-level 8-km C-PT testing during the CAF trials.

In the present study lower  $\text{HR}_{\text{peak}}$  and  $\dot{V}O_2$  were achieved when comparing sea-level and hypoxia 8-km C-PT results. The reduction in HR during acute exposure to hypoxia is believed to be related to enhanced parasympathetic neural activity due to decreased signals from skeletal muscles (2, 9). Fascinat-

ingly, HR was higher and similar to sea-level values during the CAF 8-km time trail at altitude and associated with increased  $\dot{V}O_2$  at 4 km. Increased HR is actually one of the most common observations related to improved sea-level performance after CAF ingestion, and has also been accompanied by higher  $\dot{V}O_2$  (1, 27, 35, 41). The Fick equation states that a higher HR and similar stroke volume increases  $\dot{V}O_2$ , if the A- $\dot{V}O_2$  difference is maintained (7, 38). Yang et al. (45) observed that CAF ingestion had no effect on activity,  $\dot{V}O_2$ , and HR in mice lacking  $A_1$  and  $A_2$  receptors compared with normal wild mice.  $A_1$  receptors are expressed in the human heart where they inhibit adenylyl cyclase (19, 43), and an inhibition following CAF ingestion could increase sympathetic neural activity leading to higher HR and/or maintained contractility qualities of the heart (Q) (18, 42, 45).

In the present study neural activity and Q were not measured. Therefore the effects of the increase in HR on  $O_2$  delivery following CAF ingestion during DP exercise should be interpreted with caution. However, Gonzalez et al. (25) demonstrated that an increase in HR by atrial pacing increased Q,  $\dot{V}O_{2max}$  and performance in hypoxia. During CAF testing, higher HR and  $\dot{V}E$  were observed during C-PTs associated with higher  $\dot{V}O_2$  and velocity in the first 4 km of the CAF 8-km C-PT. The increase in plasma adrenaline and  $\dot{V}E$  might also counteract an increase in vagal nervous drive to the heart due to input from pulmonary stretch receptors (23, 39). It is therefore tempting to suggest that the increase in HR increased  $O_2$  delivery and ATP production in working muscles, thus improving performance quality during CAF C-PTs.

The results from the present study are also of interest for sports performance since CAF has been widely used by elite endurance athletes in competitions since its removal from the World Anti Doping Agency list in 2004 (13). The topic is also important because competitions including the Olympics and world championships are sometimes held at moderate altitude (1500–2,000 m) in sports such as cycling, running, and cross-country skiing. Furthermore, it has been reported that the within-athlete variability in performance times in elite cross-country skiing races is ~1.1–1.4%, and the smallest worthwhile enhancement is 0.3–0.4% (40). The observed improvement of  $0.9 \pm 1.3\%$  during the 8-km C-PT, although not significant ( $P < 0.22$ ), might therefore still have an effect on results in real life competitions.

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

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#### DISCLOSURES

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

#### AUTHOR CONTRIBUTIONS

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

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# PAPER IV

*Caffeine improves exercise performance, maximal oxygen consumption and maximal accumulated oxygen deficit*

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***Caffeine improves exercise performance, maximal oxygen consumption and oxygen deficit***

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Running title: “Effect of caffeine ingestion on  $\dot{V}O_{2max}$ ”

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## ***Abstract***

$\dot{V}O_{2max}$  and the accumulated oxygen deficit ( $O_2$ -deficit) are shown as high interpretations for explaining performance differences between athletes. Caffeine ingestion (CAF) is shown as an effective ergogenic aid where improved performance is often associated with higher ventilation ( $V_E$ ), heart rate (HR), and lactate accumulation (LA-). Higher  $V_E$  and HR could improve conditions for increasing  $\dot{V}O_{2max}$ , while increased LA- indicates a greater  $O_2$ -deficit.

**The aim** of the present study was therefore to test the effect of CAF (4.5 mg · kg<sup>-1</sup>

bodyweight) on time,  $\dot{V}O_{2max}$  and  $O_2$ -deficit during a standardized ramp performance test

**Method:** 23 elite endurance trained male athletes ( $\dot{V}O_{2max}$  76.3±1.4 ml·kg<sup>-1</sup>·min<sup>-1</sup>)

participated in the study performed in a randomized double-blinded, placebo-controlled,

cross-over-design. Performance was assessed as time,  $\dot{V}O_{2max}$  and  $O_2$ -deficit during the

performance test. Oral ingestion of CAF or PLA was consumed 45 min before a standardized

warm-up. **Results:** Following CAF both running time (+19.4 sec, 5.5%),  $\dot{V}O_{2max}$  (+0.9 ml · kg<sup>-1</sup> · min<sup>-1</sup>, 1.2%) and  $O_2$ -deficit ( 6.4 ml · kg<sup>-1</sup>, 10.1%) was significantly improved ( $p<0.05$ ),

associated with higher  $HR_{peak}$ ,  $V_{Epeak}$  and LA-. Furthermore, when the improved running

duration was adjusted for the increase in;  $\dot{V}O_{2max}$ ,  $O_2$ -deficit,  $HR_{peak}$ ,  $V_{Epeak}$  and accumulated

LA- following CAF ingestion time was reduced with 63.5% from 19.4 to 7.1 s. **Conclusion:**

The present study is the first to demonstrate that CAF ingestion can increase  $\dot{V}O_{2max}$  while

running. Improvement in running time seems related with higher  $\dot{V}O_{2max}$ ,  $O_2$ -deficit,  $HR_{max}$ ,

$V_E$  and accumulated LA.

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

## ***Introduction***

Improved exercise performance after caffeine ingestion (CAF) in dosages between 3-9 mg · kg body wt<sup>-1</sup> is well documented (1-4). The mechanisms explaining CAF ergogenic effects are today believed related to the inhabitation of adenosine receptors reducing rate of perceived exertion (RPE) (1-4). During submaximal exercise lower RPE is reported, while during time trial testing (TT) similar RPE is observed despite higher work intensity post CAF (5-9). It therefore seems that subjects increase work intensity during caffeine TT to reach similar level of RPE, and by doing so improve performance. However, a higher work intensity following CAF would require a higher production of ATP, unless work efficiency is improved (10-12).

In exercise science, anaerobic glycolysis for production of ATP is often estimated by measuring the accumulated oxygen deficit (O<sub>2</sub>-deficit) during exercise (13). O<sub>2</sub>-deficit is shown as a remarkable good physiological parameter for explaining performance differences between athletes during high intensive exercise (13-16), and improved performance following CAF have indeed been explained by increased O<sub>2</sub>-deficit during high intensive performance tasks (17;18). However, during exercise of longer duration (4 min <), ATP regeneration is primarily limited by aerobic phosphorylation depend on continuous O<sub>2</sub> availability (10;19), and the effects of CAF on O<sub>2</sub>-deficit during such exercise have not been investigated (17;18).

The highest rate at which O<sub>2</sub> can be taken up and utilized by the human body has been termed the maximal oxygen uptake representing the integrated capacity of the pulmonary, cardiovascular and muscle systems to uptake, transport and utilize O<sub>2</sub> (10;20;21). All though limiting factors, and test protocols for truly reaching  $\dot{V}O_{2max}$  in humans has been a topic of great controversy and discussion since introduced in the 1920s. There is today a broad agreement that  $\dot{V}O_{2max}$  is an important measurement for explaining endurance performance in several sports (12;22-24).

Furthermore, despite the fact inhibition of adenosine receptors following CAF is linked to the reduction of RPE, adenosine receptors are also expressed in the human heart and lungs where inhibition could affect pulmonary and cardiovascular functions. Higher HR and  $V_E$  are actually two of the most consistent observations associated with improved TT following CAF (7;25-27). The *Fick equation* states that a higher HR, with similar stroke volume (SV), should lead to higher cardiac output ( $\dot{Q}_c$ ), consequently increasing oxygen consumption if the arterio-venous difference is unchanged (19;28). Furthermore, pulmonary functions consequently effecting  $V_E$  is shown in several studies to limit oxygen consumption and  $\dot{V}O_{2max}$  due to reduced  $O_2$ -saturation in highly trained endurance athletes (29-31). Indeed though rarely measured, studies have found improved TT performances post CAF to be associated with increase oxygen consumption (9;17;27;32;33). However, a study thoroughly investigating if CAF can increase  $\dot{V}O_{2max}$  have to date not been completed.

The aim of the present study was therefore to investigate the effects of CAF (4.5 mg · kg<sup>-1</sup> bodyweight) ingestion on time,  $\dot{V}O_{2max}$ , and  $O_2$ -deficit during a ramp performance test. We hypothesized that CAF would improve running duration of the ramp test either as a result of higher  $\dot{V}O_{2max}$ , or improved  $O_2$ -deficit.

## ***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 especial approval from REK was not required in order to performed the study as described, and the study was conducted according to the Declaration of Helsinki. Test subjects physical characteristics

(mean $\pm$ SD) were age  $24.0 \pm 1.0$  (yr.), height  $182.1 \pm 1.3$  (cm), weight  $73.5 \pm 1.6$  (kg),  $\dot{V}O_{2\max}$  running ( $\dot{V}O_{2\max}$ )  $75.9 \pm 5.8$  ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Inclusion criteria were that all subjects had to be: male, have  $\dot{V}O_{2\max}$  above  $65 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and that they would train seriously to compete in national or international endurance competitions the upcoming season.

*Experimental Procedures:* The study used a randomized double-blinded, placebo-controlled, test-retest, cross-over-design. Before the main  $\dot{V}O_{2\max}$  performance testing started, each participant performed a pre-test to familiarize with the test procedure, and to verify all subjects had  $\dot{V}O_{2\max}$  above  $65 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . The study had one dropout because of illness after the inclusion test.

*Pre-test:* During the pre-testing all subjects performed a standardized incremental test consisting of four workloads at 7, 8, 9 and 10  $\text{km} \cdot \text{h}^{-1}$  each lasting five minutes. All workloads were performed with a  $10.5^\circ$  uphill incline on the treadmill (Woodway, Weil am Rein, Germany), and one minute break was given between each workload. Oxygen measurements from the four workloads were then used to conduct a linear regression estimating the individual oxygen cost for calculation of  $O_2$ -deficit during the  $\dot{V}O_{2\max}$  performance tests previous described by Medbo et al (13).. The linear regression was also used to calculate individual speeds equal to 55, 60, 65 and 70 % of  $\dot{V}O_{2\max}$  performed as a standardized warm-up (incremental test) before each main  $\dot{V}O_{2\max}$  performance tests. When the standardized incremental test was finished all subjects walked five min at  $5 \text{ km} \cdot \text{h}^{-1}$ , before starting the pre  $\dot{V}O_{2\max}$  test. Starting velocity during all testing was  $10 \text{ km} \cdot \text{h}^{-1}$  with a uphill incline of  $10.5^\circ$  on the treadmill. The  $\dot{V}O_{2\max}$  performance tests was performed as a ramp test where velocity was increased with  $0.5 \text{ km} \cdot \text{h}$  each half minute until subjects were unable to maintain the speed and stepped/jumped off the treadmill. The highest HR and  $V_E$  during the test was defined as  $HR_{\text{peak}}$  and  $V_{E\text{peak}}$ . Requirements for that  $\dot{V}O_{2\max}$  was reached

during all testing was that oxygen consumption reached a plateau, meaning  $\dot{V}O_2$  increased less than  $1 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , while speed was increased two times  $0.5 \text{ km} \cdot \text{h}$ . Additionally RER values should be above 1.10 and post blood LA- above 7.0 mM.  $\dot{V}O_{2\text{max}}$  for each subjects was based on the average of the two highest measurements measured over one whole minute. Subjects with  $\dot{V}O_{2\text{max}}$  higher than  $65 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  during the pre-test were included.

**FIGURE 1 AROUND HERE**

$\dot{V}O_{2\text{max}}$  performance tests: Each subject in total completed four  $\dot{V}O_{2\text{max}}$  performance tests over a two week period. During each week one test was performed with CAF and the other with PLA in a randomized fashion. Before all main  $\dot{V}O_{2\text{max}}$  tests, all subjects measured both resting HR and lung function at arrival, and 30 minutes after consuming either PLA or CAF. The lung function testing consisted of measurements of fraction of exhaled nitric oxide ( $FE_{NO}$ ) and maximum expiratory flow volume. After finishing the lung function testing subjects were given a 10 minute break before starting the standardized warm-up. The warm-up consisted of four workloads from 55-70% of  $\dot{V}O_{2\text{max}}$ , each on lasting 5 minutes, with a one minute break in between were blood glucose and LA- was measured. All workloads of the warm-up were performed with a  $10.5^\circ$  uphill incline on the treadmill (Woodway, Weil am Rein, Germany). During each workload HR,  $\dot{V}O_2$  and RER were measured as means between the 3-4.5 min of each workload. Subjective RPE was evaluated according to the Borg-scale (6 to 20) (34). Following the warm-up, a 5-min break was used for pre blood sampling of LA-, glucose and HR registration and last instructions to subjects were given. The goal for each subject was to run for as long duration as possible during the  $\dot{V}O_{2\text{max}}$  performance tests. Performance was measured as time until task failure during the test. During all testing subjects did not receive information regarding time, velocity or physiological measurements. Requirements for that  $\dot{V}O_{2\text{max}}$  was reached during all testing is described above in the pre-test.

Encouragement was not given during any tests from the blinded test leader to ensure this did not affect performance outcome. After finishing the  $\dot{V}O_{2\max}$  performance tests subjects were given a 5 minute break before they finished the testing that day with post measurements of lung function and filling out questionnaires.

*Measurement and calculation of O<sub>2</sub>-deficit:* During the pre-testing all subjects performed a standardized incremental test consisting of four workloads each lasting five minutes from 7 to 10 km · h<sup>-1</sup>, at an 10.5° uphill incline on the treadmill.  $\dot{V}O_2$ -measurements were carried out between 2.5-4.5 min of each 5 min period, and the mean determined as a subjects oxygen cost for the velocity. After this oxygen cost for the four work loads during sub-maximal exercise was used to make a linear regression estimate oxygen cost during the different velocities of the  $\dot{V}O_{2\max}$  performance tests as previously described by Medbo et al (13). Collection of expired air started 15 seconds before the starting the  $\dot{V}O_{2\max}$  performance tests and continuously until subjects reach task failure (when they step of the treadmill). The difference in estimated oxygen cost for the whole running period, and measured oxygen uptake for the same period was then calculated as subjects O<sub>2</sub>-deficit. In the present study O<sub>2</sub>-deficit was not adjusted for the contribution of the body's oxygen stores to the energy supply. This contribution has been estimated to be ~10% depending on running duration (17).

*Measurement equipment:* During all exercise testing oxygen consumption and RER were measured with a Oxycon Pro metabolic system (Jaeger Hochberg, Germany). Before each individual tests the Oxygen Pro was calibrated with mixture gasses with known amount of O<sub>2</sub> and CO<sub>2</sub> (14.93% O<sub>2</sub> and 5.99 % CO<sub>2</sub>) and normal air (approx 20.90 % O<sub>2</sub> and 0.04 % CO<sub>2</sub>). Volume was calibrated manually using a pump containing 3 liters of volume (Calibration Syringe, Series 5530, Hans Rudolph Inc., MO, USA). During all testing air was collected using a mouth V2-mask (Hans Rudolph Instr., USA) in combination with a nose

bracket. Expired air was led thru a hose into the mixing chamber (Oxycon Pro) and analyzed with a turbine (Triple V volume transducer). Both the hose and V2-mask were always tested for leakage prior to each individual test. Heart rate (HR) was measured using a HR monitor (Polar RS 800, Finland), where the error of measurement stated by the producer is  $\pm 1\%$ .

*Lung function:* Spirometry was measured by maximum expiratory flow volume loops according to European standard (35), and recorded as forced expiratory volume in the first second (FEV<sub>1</sub>), forced vital capacity (FVC) and forced expiratory flow in 50 % of FVC (FEF<sub>50</sub>). Lung function measurement was performed using MasterScreen Pneumo Jeager® (Würzburg, Germany) and reference values are according to Quanjer et al (1993)(35).

*Exhaled nitric oxide (FENO):* FENO was measured by the single breath online technique according to American Thoracic Society (ATS)/ERS guidelines (36). 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·s<sup>-1</sup>. 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. FENO 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 by a certified concentration of NO.

*Pre testing information:* All subjects were informed to only perform light training the last 48 hours before each  $\dot{V}O_{2max}$  performance tests. To minimize variation in pre-exercise glycogen stores, diet and exercises diaries were used to standardize food intake and training

for each subject. The subjects prepared to the tests as prior to a competition, and instructed to follow the same training and diet regime before each test. Subjects had also to refrain from CAF 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 testing days subjects arrived to the laboratory at the same time ( $\pm 15$  min) of the day. The first week the two first tests were performed with a washout period of three days between each test. Before test three a washout period of four days were given so subjects would perform test three and four the following week on the same weekdays as during the first week.

*Capillary samples* for measurements of glucose and LA- were taken from the finger tips punctured by a Saft-T-Pro Plus (Accu-Check, Mannheim, Tyskland). For measurement of blood LA- capillary blood samples were drawn into a 50  $\mu$ l capillary tube and a 20  $\mu$ l pipette was used to draw blood into the analyzer from the 50  $\mu$ l capillary tube. The analyzer was calibrated with a 5.0 mmol  $\cdot$  l<sup>-1</sup> lactate stock solution before each test. Values between 4.95 mmol  $\cdot$  l<sup>-1</sup> and 5.05 mmol  $\cdot$  l<sup>-1</sup> was accepted. Under normal circumstances the uncertainty of measurements are  $\pm 2\%$  with LA- values between 0 and 5 mmol  $\cdot$  l<sup>-1</sup>, and  $\pm 3\%$  for values between 5 and 15 mmol  $\cdot$  l<sup>-1</sup>.

Blood glucose was measured by filling microcuvets (HemoCue Glucose 201) with blood. The microcuvets was placed in a HemoCue Glucose 201<sup>+</sup> (HemoCue Glucose 201<sup>+</sup>, Ängelholm, Sweden) and analyzed. Measurements take 20-60 seconds.

*Treatments in the study* included PLA (vehicle only) and CAF (4.5 mg  $\cdot$  kg<sup>-1</sup>). CAF (Coffeinum, Oslo Apotekerproduksjon, Oslo, Norway) was dissolved in a cordial concentrate Fun Light (3 mg/ml) and was prepared at the laboratory at the Norwegian school of sports sciences. Ingestion of PLA or CAF was done 45 minutes before subjects started the standardized warm-up, meaning after 75 min before the  $\dot{V}O_{2max}$  performance test.



*Questionnaires:* were used to evaluate motivation and "current fitness" using a scale from 1-100 (9). Sleep amount was evaluated by approx. hours of sleep prior to each test. In addition, 30 min post product ingestion, and before leaving the laboratory after each trial subjects answered what product they believed they had received.

*Statistical Analysis:* All data in the study are presented as means  $\pm$  standard , and differences in performance during the 8 km C-PT were evaluated by a paired t-test. 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. 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 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 (37).

## **Results**

*$\dot{V}O_{2max}$  performance tests:* Athletes completed two tests during each of two consecutive weeks following either caffeine or placebo ingestion. Caffeine improved time-to-voluntary exhaustion (performance) in both weeks. In the first week, caffeine increased time to exhaustion by 18 s ( $355 \pm 41$  vs.  $373 \pm 40$  s,  $p < 0.001$ ) compared to placebo, and by 21 s in the second week ( $355 \pm 44$  vs.  $376 \pm 45$  s,  $p < 0.001$ ), the average effect being 19.4 s (5.45%;  $P < 0.001$ ; Figure 1A). Time to exhaustion was highly reproducible, with no statistical difference between the two trials with placebo ( $p = 0.78$ ), or with caffeine ( $p = 0.74$ ); this was reflected in high intra-class correlations of coefficient (ICC) of 0.90 and 0.94, respectively.

Mean maximal oxygen uptake increased by  $0.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (1.2%;  $p < 0.003$ ) after caffeine ingestion compared to placebo. ICC values for  $\dot{V}O_2$  were  $\geq 0.95$  for both conditions. The  $O_2$ -kinetics was similar between CAF and PLA until the last minute where higher  $\dot{V}O_{2\text{max}}$  was reached with CAF ( $76.7 \pm 6.0$  vs  $75.8 \pm 5.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). The higher  $\dot{V}O_{2\text{max}}$  after CAF ingestion contributed to the longer running time at the performance test, since statistical adjustment for  $\dot{V}O_{2\text{max}}$  reduced the CAF effect in running time from 19.4 s to 15.4 s.

**FIGURE 2 AROUND HERE**

HR and  $V_E$  during the performance test developed similarly after PLA or CAF during the first part of the test; however, higher maximal HR and  $V_E$  values were reached during the last minute of the test after CAF ingestion compared to PLA. Specifically,  $HR_{\text{peak}}$  increased from  $191 \pm 8$  to  $193 \pm 9 \text{ beats} \cdot \text{min}^{-1}$  during the  $\dot{V}O_{2\text{max}}$  test under the influence of CAF compared to PLA ( $p < 0.001$ ), and maximal ventilation ( $V_{E\text{peak}}$ ) increased from  $187.8 \pm 17.8$  (PLA) to  $192.2 \pm 15.3 \text{ l} \cdot \text{min}^{-1}$  after caffeine ingestion ( $p < 0.001$ ) (Table 1). The increase in  $\dot{V}O_{2\text{max}}$  after caffeine ingestion was attenuated to  $0.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $p < 0.001$ ) after adjustment for the increase in  $HR_{\text{peak}}$ . Importantly, when  $\dot{V}O_{2\text{max}}$  was adjusted for  $V_{E\text{peak}}$  the effects of caffeine on  $\dot{V}O_{2\text{max}}$  decreased by about 50%, and the effect was no longer significant ( $p = 0.11$ ). Despite higher  $V_{E\text{peak}}$  breathing frequency (BF) was not significantly higher after CAF ingestion ( $60 \pm 7$  vs  $59 \pm 9 \text{ breaths} \cdot \text{min}^{-1}$ ,  $p < 0.07$ ; Figure 3). When running duration was adjusted for  $\dot{V}O_{2\text{max}}$ ,  $V_{E\text{peak}}$  and  $HR_{\text{peak}}$  there was still a 11.7 s ( $p < 0.001$ ) improvement in running duration after CAF compared to PLA, to which most likely anaerobic processes would have contributed.

**FIGURE 3 AROUND HERE**

The accumulated oxygen deficit during the  $\dot{V}O_{2\text{max}}$  performance test increased from  $63.1 \pm 18.2$  in PLA to  $69.5 \pm 17.5 \text{ ml} \cdot \text{kg}^{-1}$  with CAF ingestion ( $p < 0.02$ ). Blood lactate values

where higher post CAF compared to PLA ( $8.54 \pm 1.02$  vs  $7.94 \text{ mM} \pm 1.06$ ;  $p < 0.001$ ) (Table 1). Calculations showed that the anaerobic processes ( $O_2$ -deficit) covered  $14.7 \pm 3.1$  and  $15.0 \pm 2.7$  % of total  $O_2$ -cost in placebo and caffeine trials. When running duration at the performance test was adjusted for both  $O_2$ -deficit and LA- concentration, the effect of CAF was reduced from 19.4 to 13.2 s ( $p < 0.001$ ). With additional adjustment for  $\dot{V}O_{2\text{max}}$  this effect was reduced to 8.0 s but still significant ( $p < 0.001$ ), and with further adjustment for  $V_{\text{Epeak}}$  and  $HR_{\text{peak}}$  this effect was reduced to 7.1 s but still significant ( $p = 0.003$ ). For measurements of  $O_2$ -deficit, the ICC was 0.61 and 0.64, respectively for PLA and CAF trials; for LA-, ICC values were 0.46 and 0.39. Plasma glucose after the performance tests were also higher in caffeine compared to placebo trials ( $7.9 \pm 1.1$  vs  $7.3 \pm 0.9 \text{ mM}$ ;  $p < 0.001$ ). The highest RER during the performance test was independent of test conditions ( $1.11 \pm 0.04$  and  $1.11 \pm 0.04$  for placebo and caffeine, respectively).

**TABLE 1 AROUND HERE**

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

**TABLE 2 AROUND HERE**

*Lung function measurements* performed at arrival, 30 minutes post PLA/CAF ingestion, and post  $\dot{V}O_{2max}$  performance test showed no difference between CAF and PLA trials for FENO, FEV1, FVC or FEF (Table 3).

**TABLE 3 AROUND HERE**

*Questionnaires* showed no difference between CAF and PLA trials regarding “current fitness” and motivation. Prior to the test subjects reported motivation of:  $77 \pm 14$ ,  $79 \pm 16$  (PLA), and  $76 \pm 14$ ,  $76 \pm 14$  (CAF) before tests (75 = high/very high), and  $79 \pm 17$ ,  $82 \pm 12$  (PLA), and  $79 \pm 14$ ,  $81 \pm 14$  (CAF) after the tests. Ratings pre testing of form of the day were  $62 \pm 11$ ,  $62 \pm 13$  (PLA), and  $61 \pm 13$ ,  $63 \pm 12$  (CAF) (65 = high) (25) before, and post  $62 \pm 11$ ,  $65 \pm 14$  (PLA), and  $67 \pm 15$ ,  $79 \pm 14$  (CAF). Furthermore, the questionnaires revealed that subjects were unable to sense which product they received during the different trials with 50% answering uncertain. Of the subjects that answered that they knew the treatment (caffeine or placebo), about ~50% guessed wrong product both pre and post testing independent of treatment ingestion. Amount of sleep (hours), training, intake of food and liquid intake did not differ before tests, and showed that subjects had followed instructions given regarding training, food, liquid and CAF consumption the last 48 h prior to each C-PT.

## **Discussion**

In the present study CAF improved running duration by 5.5% during the  $\dot{V}O_{2max}$  performance test, with as much as 91.3% of participants improving performance. The high reproducibility for running duration during both placebo and caffeine trials was reflected in strong ICC of 0.90 and 0.94, indicating CAF was an effective stimulate for improving running duration during the  $\dot{V}O_{2max}$  performance tests. To the authors knowledge the present study is the first to observed CAF increases  $\dot{V}O_{2max}$  (+1.2%), but improved running duration was also associated with increased  $O_2$ -deficit (+10.1%). Specifically, 69.6% of participant in the study

improved  $\dot{V}O_{2max}$ , and 72.7%  $O_2$ -deficit following CAF. Results indicate improved performance,  $\dot{V}O_{2max}$  and  $O_2$ -deficit following CAF was associated with subjects reaching higher  $HR_{peak}$  (+1.2%),  $V_{Epeak}$  (+2.3%) and LA- (+6.3%).

Several studies have found CAF to be an effective stimulant improving exercise performance despite usage of different muscle groups and test protocols (5;8;9;25;26). The mechanisms explaining improvements following CAF seems multifunctional, but most likely related to the inhibition of adenosine receptors reducing RPE (1;2;4). In the present study CAF resulted in a reduction in RPE during the standardized warm-up (1;25). The caffeine ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) dosage ingested in the present study would lead to peak plasma caffeine concentrations of 25~35  $\mu\text{M}$  reducing adenosine receptor activation, possibly explaining reduction in RPE during submaximal exercise (5;9;25). This means that CAF might have allowed higher discomfort during the  $\dot{V}O_{2max}$  performance test, and the authors therefore speculate that in order to reach maximal RPE subjects increased running duration. Nevertheless, an increased exercise duration following CAF would require a higher production of ATP either by aerobic or anaerobic processes, unless work efficiency was improved (10-12). Results from the present study indicate no change in work economy following CAF since HR,  $V_E$  and  $\dot{V}O_2$  increased in a similar linear fashion independent of treatment ingestion during submaximal (Table 2) and maximal exercise (Figure 3). There was actually a tendency for higher  $\dot{V}O_2$  measurements ( $p < 0.077$ ) following CAF during submaximal which would indicate reduced work economy and reduced performance.

Inhibition of adenosine receptors could also affect pulmonary and cardiovascular functions since they are expressed in the human heart and lungs (38). Specifically, an inhibition of the  $A_1$ -receptor in the lungs is observed leading to a bronchial dilation, by which pulmonary functions for gas exchange during exercise could be improved (10;38). Studies

have found CAF to be a strong ventilatory stimulant, increasing the sensitivity of both peripheral chemoreceptors in untrained subjects as well as  $V_E$  during exercise for highly trained endurance athletes (5;30). In the present study no change in lung function (FENO, FEV1, FVC or FEF) pre or post performance test was observed between treatments. However, during maximal exercise higher  $V_E$  was observed following CAF, despite similar BF as the placebo trail (Table 1). The increase in  $V_{Epeak}$  during the caffeine  $\dot{V}O_{2max}$  performance tests could have improved conditions for  $O_2$  saturation (29;31). Several studies have found that maximal  $V_E$  indeed can limit maximal oxygen consumption for highly trained athletes during exercise (29-31). This is perhaps even more evident during hypoxic testing where pulmonary functions and  $O_2$  saturation directly represents a limiting factor for  $\dot{V}O_{2max}$  and performance (5;39). Furthermore, when adjusted for  $V_{Epeak}$  the effects of CAF on  $\dot{V}O_{2max}$  decreased by about 50%, and the effect was no longer significant ( $p=0.11$ ).

Inhibition of  $A_1$  receptors in the heart following CAF could also affect cardiovascular functions (9;38;40). Mice lacking  $A_1$  and  $A_2$  receptors is observed to have no effect of CAF on HR or activity compared to normal wild mice (40). It is also reported that an increase in  $V_E$  would counteract to increased vagal nervous drive to the heart due to input from pulmonary stretch receptors (41;42). The “classical view” of  $\dot{V}O_{2max}$  is that maximal rates of  $O_2$  utilization and production of ATP in skeletal muscles are limited by cardiovascular functions (10;43). It is estimated that 70-85% of limitations in  $\dot{V}O_{2max}$  during sea level testing are linked to  $\dot{Q}_c$ , meaning that in most situations  $\dot{V}O_{2max}$  is primarily limited by the  $\dot{Q}_c$  (10;44). Supporting this assumption is the observation that elite endurance athletes have higher  $\dot{V}O_{2max}$  primarily due to higher  $\dot{Q}_c$  (19;45). The increased  $HR_{peak}$  following CAF during the  $\dot{V}O_{2max}$  performance could therefore increased  $\dot{Q}_c$ , consequently increasing oxygen consumption, if

the arterio-venous difference and SV were maintained (19;28). In the present study, statistical analyses suggest that increased  $HR_{peak}$  explained 0.2 ml · kg · min of the increase in  $\dot{V}O_{2max}$ .

In the present study neural activity, SV, arterio-venous difference and  $\dot{Q}_c$  was not measured, so the effects of the increased  $HR_{peak}$  and  $V_{Epeak}$  on  $O_2$  delivery following CAF ingestion while running should be interpreted with caution. Still, during both pre and the two main PLA tests subjects were unable to reach as high  $HR_{peak}$ ,  $V_{Epeak}$  and  $\dot{V}O_{2max}$  as during CAF testing (Table 1). This was reflected in very high ICC values for  $\dot{V}O_{2max}$  of 0.95 during both PLA and CAF trails. Furthermore, the observation that the increase in  $\dot{V}O_{2max}$  after CAF was attenuated to 0.7 ml · kg<sup>-1</sup> · min<sup>-1</sup> (p<0.001) after adjustment for the increase in  $HR_{peak}$ , and further more with ~50%, after adjustment for  $V_{Epeak}$ , the changes in HR and  $V_E$  seem important for explaining increased  $\dot{V}O_{2max}$  following CAF.

It could be speculated that subjects increased  $\dot{V}O_{2max}$  during caffeine trials due to the fact that they were able to increase running duration, meaning being unable to reach the famous *plateau* concept. Contradicting to this is the observation that when comparing the last 30 s of the placebo trial vs the same time point during the caffeine trial, there is already here a tendency (p< 0.12) for increased oxygen consumption (Figure 3). Certainly, the combined increase in  $\dot{V}O_2$ ,  $V_{Epeak}$  and  $HR_{peak}$  reached during caffeine trails would improved conditions for exercising muscles to extract oxygen and when running duration was adjusted for  $\dot{V}O_{2max}$ ,  $V_{Epeak}$  and  $HR_{peak}$  running duration was reduced form 19.4 to 11.7 s (p<0.001). The remaining improvements after CAF compared to PLA, we therefore speculated anaerobic processes would explain.

In the present study anaerobic processes ( $O_2$ -deficit) accounted for 14.7±3.1 and 15.0±2.7 % of total  $O_2$ -cost during the  $\dot{V}O_{2max}$  performance test in placebo and caffeine  $\dot{V}O_{2max}$

trials, respectively. Studies have suggested that the total amount of anaerobic energy produced during a TT are fixed (46). However, it has been observed CAF ingestion can promote the quantity of anaerobic energy produced during high-intensity exercise (17;46). Specifically, Doherty (1998) observed CAF ingestion (5 mg· kg body wt<sup>-1</sup>) improved running duration during a short turn exercise (~2-3 min) for 9 highly trained male athletes ( $\dot{V}O_{2max} \geq 60$ ). Performance was measured as running until exhaustion at a velocity of ~125% of  $\dot{V}O_{2max}$  on a treadmill with an uphill incline of 10.5%. The improvements were mainly explained due to a 11.1% improvement in O<sub>2</sub>-deficit (17). These results are very comparable to results in the present study both due to methodical design, CAF and the observed improvement in O<sub>2</sub>-deficit (10.1%). However, our data also shows that CAF increases O<sub>2</sub>-deficit during longer exercise tasks (6-6.5 min). Furthermore, statistical calculations show that when running duration was adjusted for both O<sub>2</sub>-deficit and LA<sup>-</sup>, the effect of CAF ingestion was reduced by 31 % from 19.4 to 13.2 s (p<0.001). Based on results from the present study the increase in both O<sub>2</sub>-deficit and LA<sup>-</sup> therefore seems important contributing factors for the improved running duration during the  $\dot{V}O_{2max}$  performance test.

## ***Conclusion***

The present study shows for the first time that CAF resulted in higher  $\dot{V}O_{2max}$  while performing a standardized  $\dot{V}O_{2max}$  performance test. The increase in  $\dot{V}O_{2max}$  and O<sub>2</sub>-deficit seem important for explaining the 5.5 % longer running duration during the  $\dot{V}O_{2max}$  performance test. Results indicate higher  $\dot{V}O_{2max}$  came as a result of increased HR<sub>peak</sub> and V<sub>Epeak</sub>, while the larger O<sub>2</sub>-deficit seems associated with the increased LA<sup>-</sup> during CAF exposure compared to PLA. Furthermore, when the improved running duration was adjusted for the increase in;  $\dot{V}O_{2max}$ , O<sub>2</sub>-deficit, HR<sub>peak</sub>, V<sub>Epeak</sub> and accumulated LA<sup>-</sup> following CAF ingestion time was reduced with 63.5% from 19.4 to 7.1 s.



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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  $\dot{V}O_{2\max}$  test for one subject. **B:** The bottom figure shows the test procedure for all  $\dot{V}O_{2\max}$  performance tests. Prior to the  $\dot{V}O_{2\max}$  test, subjects performed a standardized warm-up (incremental test) consisting of four intensities all lasting five minutes.

**Figure 2:** **A)** Individual and mean performance: duration,  $\dot{V}O_{2\max}$  and  $O_2$ -deficit obtain during the  $\dot{V}O_{2\max}$  performance tests after PLA (open symbols) or CAF (filled symbols). **B)** Percent change in running duration,  $\dot{V}O_{2\max}$  and  $O_2$ -deficit following CAF consumption compared to PLA for each subject. Values are listed as means  $\pm$  SD. \* Significant different from PLA trials ( $p < 0.05$ )

**Figure 3:** **A)** 30 sec measurements for  $\dot{V}O_2$ , HR,  $V_E$ , BF and RER during PLA (open symbols) and CAF (filled symbols)  $\dot{V}O_{2\max}$  performance tests **B)** The last 120 seconds for each individual shown as mean for the group for  $\dot{V}O_2$ , HR,  $V_E$ , BF and RER. Values are listed as means  $\pm$  SD. \* Significant different from PLA trials ( $p < 0.05$ ).

**Table 1:** Data from the pre-test and four  $\dot{V}O_{2max}$  performance tests following PLA or CAF consumption.

	PLA		CAF		PLA		CAF		P-Value	% Dif
	Pre-Test	Test 1	Test 2	Test 1	Test 2	Mean	Mean			
Time (min:sec)	5:59±00:49	5:55±0:45	5:55±0:45	6:12±0:42*	6:16±0:45*	5:53±0:41	6:13±0:23*	6:13±0:23*	< 0.01	5.5
$\dot{V}O_{2max}$ (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	75.9±6.2	75.7±5.7	75.7±5.7	76.6±5.9*	76.8±6.4*	75.8±5.6	76.7±6.0*	76.7±6.0*	< 0.018	1.2
ΣO <sub>2</sub> Deficit (ml · kg <sup>-1</sup> )	No Data	64.1±19.3	64.1±19.3	69.9±20.2	70.4±17.9	63.1±18.2	69.5±17.5*	69.5±17.5*	< 0.02	10.1
V <sub>Epeak</sub> (L · min <sup>-1</sup> )	193.8±17.0	189.3±18.4	185.8±18.3	193.2±17.6*	191.2±14.2*	187.3±17.8	192.2±15.3.2*	192.2±15.3.2*	< 0.001	2.3
BF <sub>peak</sub> (breaths · min <sup>-1</sup> )	59±8	58±9	58±9	60±9	59±7	59±9	60±7	60±7	<0.07	3.4
RER	1.10±0.04	1.10±0.23	1.11±0.22	1.11±0.24	1.11±0.23	1.11±0.04	1.11±0.04	1.11±0.04	<0.78	0.4
HF <sub>peak</sub> (beats · min <sup>-1</sup> )	192±9	192±6	191±8	194±8*	193±7*	191±8	193±9*	193±9*	< 0.01	1.1
HF <sub>pre</sub> (beats · min <sup>-1</sup> )	No Data	113±12	109±9	113±15	113±13	111±12	113±14	113±14	< 0.11	1.8
L-Apre (mmo/L)	No Data	0.86±0.28	0.79±0.27	1.12±0.21*	1.06±0.33*	0.82±0.26	1.09±0.31*	1.09±0.31*	< 0.01	32.9
L-A post (mmo/L)	8.34±1.33	7.90±1.05	8.00±1.13	8.21±1.14*	8.65±0.94*	7.94±1.06	8.54±1.02*	8.54±1.02*	< 0.01	7.0
GLU pre (mmo/L)	No Data	5.2±0.5	5.1±0.4	5.3±0.5	5.3±0.5	5.1±0.4	5.2±0.8	5.2±0.8	< 0.61	2.0
GLU post (mmo/L)	No Data	7.4±0.8	7.2±0.9	7.8±0.7*	8.0±0.9*	7.3±0.9	7.9±1.1*	7.9±1.1*	< 0.01	8.2

Values are listed as means ± SD. \* Significant difference different from PLA (p<0.05). # Significant difference different from PLA (p<0.10).

**Table 2:** Data from submaximal incremental testing (standardized warm-up) after PLA or CAF consumption.

	WORKLOAD % OF $\dot{V}O_{2max}$											
	55%		60%		65%		70%		75%		80%	
	PLA	CAF	PLA	CAF	PLA	CAF	PLA	CAF	PLA	CAF	PLA	CAF
$\dot{V}O_2$ (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	40.5±3.4	41.0±3.3	44.8±3.7	45.1±3.8	49.3±4.2	49.6±4.1	53.5±4.7	53.7±4.2				
HR (beats · min <sup>-1</sup> )	131±8	129±9	141±9	140±9	151±9	151±9	160±9	160±9				
LA <sup>-</sup> (mmo/L) <sup>†</sup>	1.00±0.20	1.20±0.25*	0.91±0.35	1.1±0.30*	1.06±0.40	1.28±0.40*	1.43±0.43	1.67±0.46*				
Borg (6-20) <sup>†</sup>	8.8±1.2	8.6±1.3	10.2±1.1	10.0±1.3	11.8±1.1	11.5±1.0*	13.3±1.1	12.9±1.1*				
$V_E$ (L · min <sup>-1</sup> ) <sup>†</sup>	70.4±3.2	74.3±3.5*	79.8±3.3	84.0±3.5*	89.2±3.6	92.9±3.9*	98.6±4.0	101.9±4.0*				
BF (breath · min <sup>-1</sup> )	28±6	28±6	32±7	31±6	34±7	34±7	36±8	36±8				

Values are listed as means ± SD. \* Significant difference between PLA and CAF (p<0.05). † Treatment effect of CAF ingestion (p<0.05)

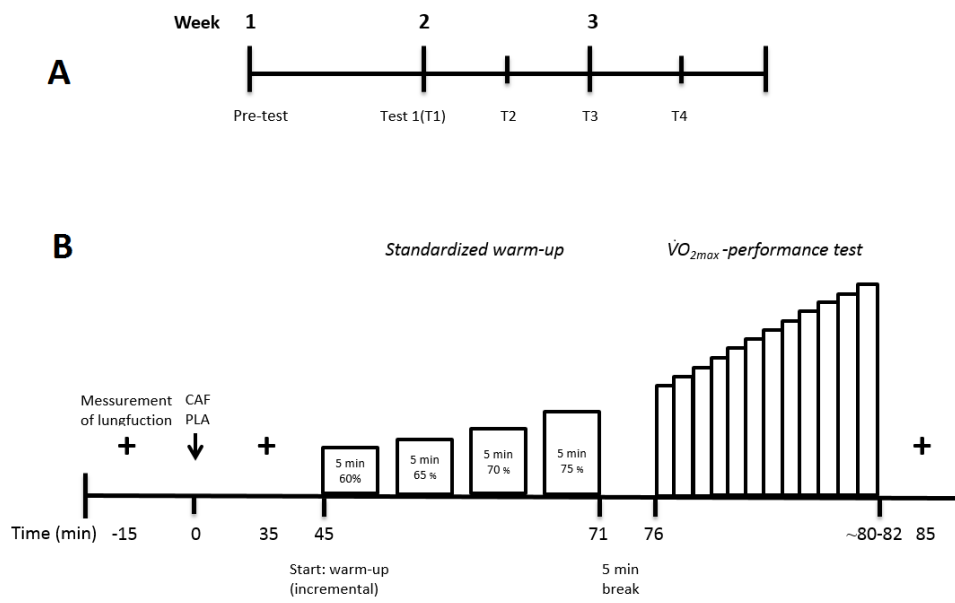
**Table 3:** Mean lung function measurements at: arrival, 35 min post CAF/PLA ingestion and post  $\dot{V}O_{2max}$  performance tests after PLA or CAF consumption.

	ARRIVAL			30 MIN POST CAF/PLA INGESTION			POST $\dot{V}O_{2max}$ PERFORMANCE TEST		
	PLA	CAF	P-value	PLA	CAF	P-value	PLA	CAF	P-value
FEV <sub>0</sub>	27.9±25.1	26.0±27.1	0.22	29.0±26.9	27.1±30.1	0.45	21.0±17.8	20.4±20.1	0.57
FEV <sub>1</sub>	5.0±0.5	5.0±0.6	0.19	5.0±0.5	5.0±0.7	0.15	5.2±0.6	5.2±0.7	0.31
FVC	6.1±0.6	6.1±0.7	0.73	6.2±0.8	6.0±0.7	0.13	6.0±0.7	6.0±0.7	0.68
FEF <sub>50</sub>	5.9±1.4	5.9±1.4	0.67	5.9±1.3	5.9±1.4	0.49	6.3±1.5	6.3±1.5	0.20

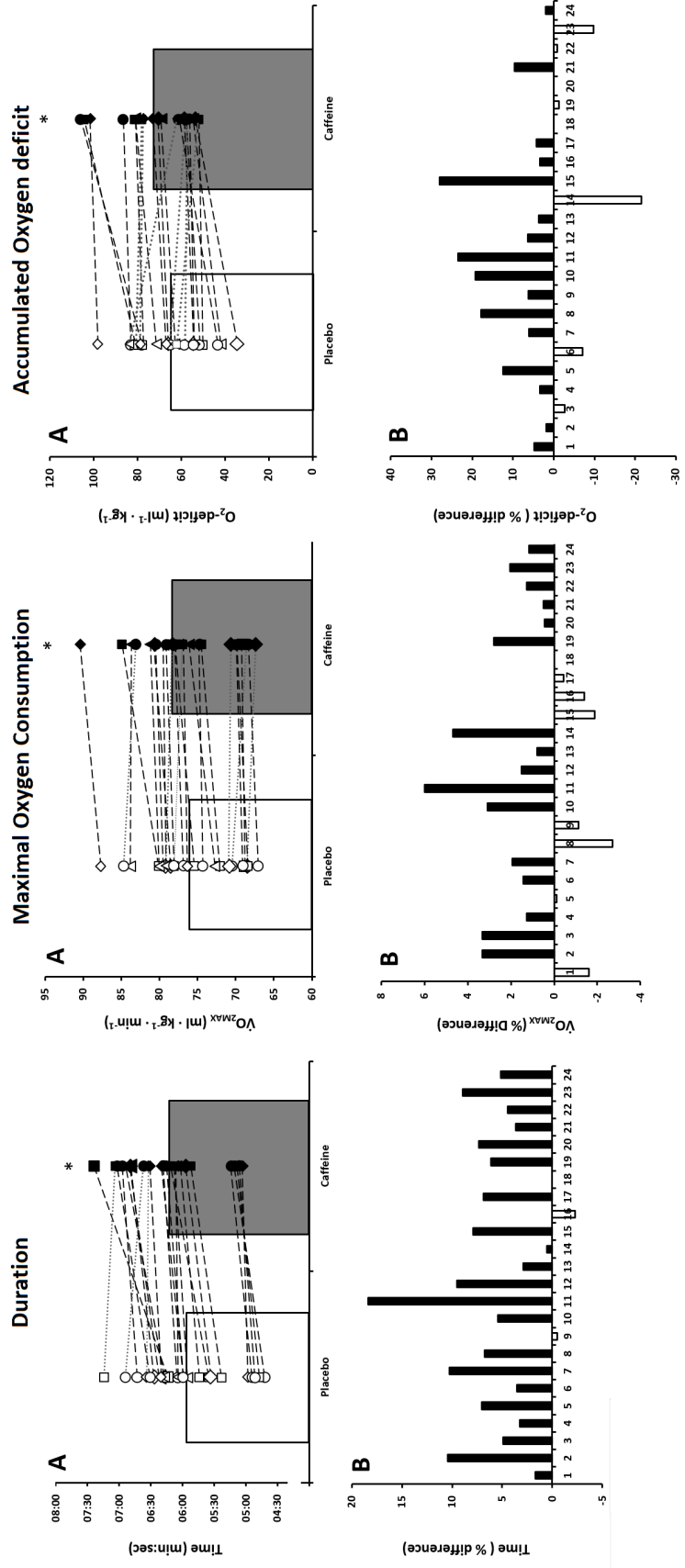
Values are listed as means ± SD. \* Significant difference between sea level (p<0.05). # Significant difference different from PLA (p<0.10).



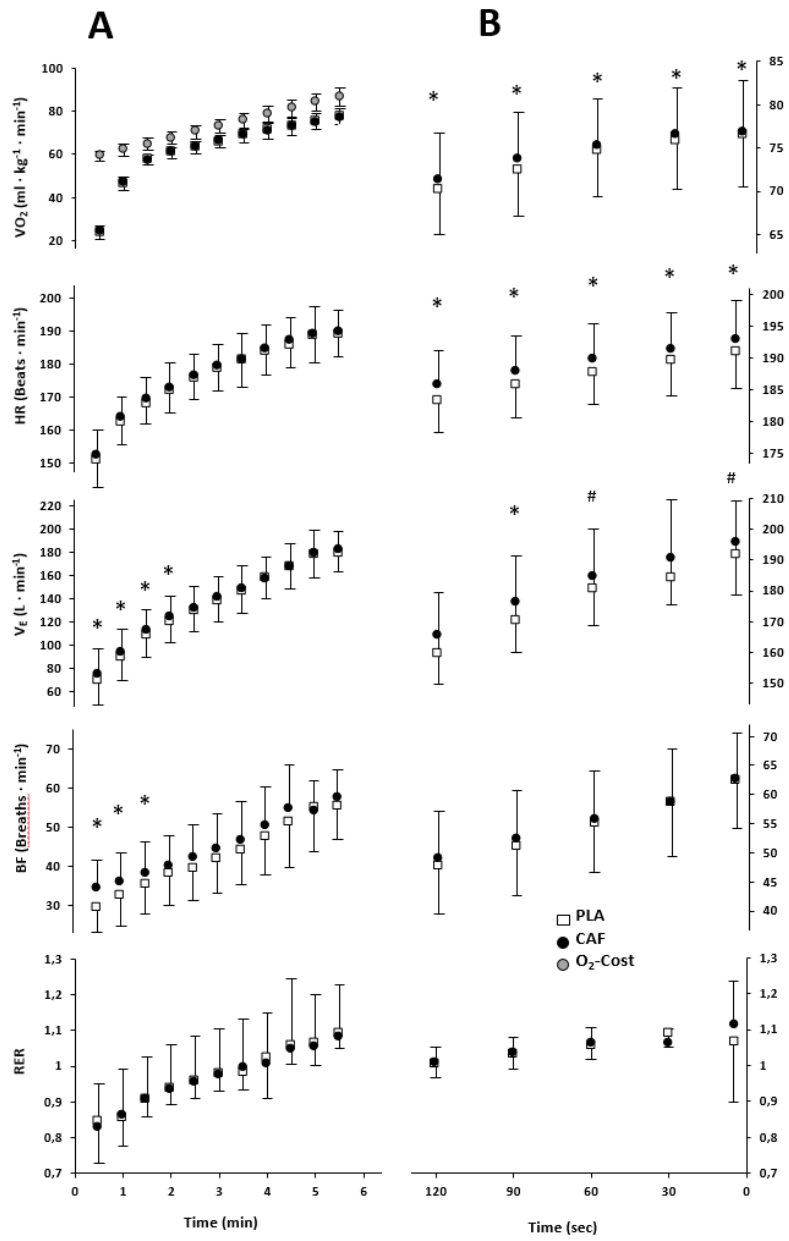
**FIGURE 1**



**FIGURE 2**



**FIGURE 3**



# PAPER V

*Caffeine increases prolonged running performance and counter movement jump height*

**H.K. Stadheim** and J. Jensen

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## **Caffeine increases prolonged running performance and counter movement jump height**

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## ***Abstract***

**Introduction:** Caffeine (CAF) intake is known to improve endurance performance, as well as counter movement jump (CMJ) height. However, whether improved neuromuscular function, as judged by improved CMJ height, contributes to prolonged running performance and lower rate of perceived exertion (RPE) is unknown. **The purpose** of the present study was therefore to test the effect of CAF ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) ingestion on CMJ height and RPE during running until voluntary exhaustion at 80% of  $\dot{V}O_{2\text{max}}$ . **Methods:** 11 male sub-elite endurance athletes ( $\dot{V}O_{2\text{max}} 71.0 \pm 5.6 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) were included in the study, which used a randomized, double-blinded, placebo-controlled, cross-over design. **Results:** CAF ingestion improved time until voluntary exhaustion by 8:40 min (10.0%). RPE was lower and CMJ heights were higher during the performance test following CAF ingestion compared to placebo. However, CMJ did not decline during running until exhaustion in either placebo (PLA) or CAF trials.  $\dot{V}O_2$ , heart rate (HR), substrate oxidation, respiratory exchange ratio, blood lactate, and glucose were similar between treatments. At exhaustion, HR and blood lactate were higher in CAF compared to PLA. The longer running time to exhaustion after CAF ingestion was also associated with higher plasma creatine kinase activity, but plasma troponin T was similar. **Conclusion:** The present study demonstrated for the first time that a longer running time to exhaustion at 80% of  $\dot{V}O_{2\text{max}}$  following CAF ingestion was associated with higher CMJ height and lower RPE.

**Keywords:** Exercise performance, rate of perceived exertion, substrate oxidation, work economy and oxygen consumption

## Introduction

Caffeine (CAF) is commonly ingested by endurance athletes to improve exercise performance (Graham, 2001; Del, Munoz, & Munoz-Guerra, 2011). The mechanisms explaining the ergogenic effects of CAF (3-6 mg · kg<sup>-1</sup>) ingestion during exhausting exercise were initially thought to be related to a lower respiratory exchange ratio (RER), and increased free fatty acids in the bloodstream (Ivy, Costill, Fink, & Lower, 1979). Since elite runners maintain intensities of ~80% of  $\dot{V}O_{2max}$  during half-marathon and marathon competitions, sparing of glycogen following CAF ingestion could be beneficial for performance outcomes by avoiding glycogen depletion (Joyner & Coyle, 2008; Coetzer et al., 1993; Abbiss & Laursen, 2005). However, several studies have failed to observe glycogen sparing following CAF ingestion, both when measured as RER during steady state exercise (Graham & Spriet, 1991), and as glycogen content in muscle biopsies post prolonged exercise (Graham, Helge, MacLean, Kiens, & Richter, 2000). During prolonged endurance exercise, such as a half marathon, RPE increases due to fatigue (Rustad et al., 2016; Abbiss et al., 2005; Fobes, 1989). A reduction in RPE as observed following CAF ingestion would therefore be favourable for sustaining a given workload for a longer period of time, and could explain performance improvements (Stadheim, Myhr, Olsen, Spencer, & Jensen, 2015; Stadheim et al., 2013; Stadheim, Spencer, Olsen, & Jensen, 2014; Doherty & Smith, 2005; Gonglach, Ade, Bemben, Larson, & Black, 2016; Graham, 2001).

During prolonged competitions, several factors have been shown to be important in maintaining high performance (Joyner et al., 2008; Coetzer et al., 1993; Abbiss et al., 2005). Despite the development of exhaustion being a complex phenomenon, exhaustion represents the inability to sustain further exercise at a required power or workload, and is considered to be largely dependent on two separate processes (Millet & Lepers, 2004; Froyd, Millet, &



Noakes, 2013). Peripheral fatigue occurring in the muscle itself (including depletion of carbohydrates and contractile functions, and/or the build-up of end products of metabolism), and central fatigue reducing the initiation or transmission of electrical activity in motor neurons (Froyd et al., 2013; Abbiss et al., 2005). It is generally accepted that fatigue during short term exercise is primarily caused by metabolic or muscular functions (Millet et al., 2004). During prolonged exercise, the aetiology of fatigue is more complex, but neuromuscular fatigue is often defined and quantified by measurements of changes in maximal voluntary contraction (MVC) shown to be reduced during prolonged running exercise (Froyd et al., 2013; Millet et al., 2004; Overgaard et al., 2004). Several methods are used to assess muscle function during exercise and exhaustion (Froyd et al., 2013). The measurement of counter movement jumping (CMJ) heights is popular and practical method, which has shown a close relationship with muscular functions and MVC measurements (Rouis et al., 2015; Young, Cormack, & Crichton, 2011; Driss, Lambertz, Rouis, Jaafar, & Vandewalle, 2015). It has recently been suggested that augmented strength and motor-unit recruitment, rather than a sparing of glycogen or reductions in effort, may underlie CAF's ergogenic effect on endurance exercise (Black, Waddell, & Gonglach, 2015; Graham, 2001; Warren, Park, Maresca, McKibans, & Millard-Stafford, 2010).

Several studies have found that CAF ingestion can increase power production (Warren et al., 2010; Black et al., 2015; Beck et al., 2006). Specifically, CAF ingestion is observed to improve MVC as well as CMJ height (Black et al., 2015; Lopes, Aubier, Jardim, Aranda, & Macklem, 1983; Bloms, Fitzgerald, Short, & Whitehead, 2015; Graham, 2001). Increased power through strength training is also reported to improve running performance (Guglielmo, Greco, & Denadai, 2009; Paavolainen, Hakkinen, Hamalainen, Nummela, & Rusko, 1999; Warren et al., 2010). Improved or the maintenance of power following CAF ingestion during prolonged exercise tasks could therefore very well postpone the development of fatigue,

reduce RPE and improve performance. However, improved neuromuscular function as judged by improved CMJ heights during prolonged running exercise following CAF ingestion have not been performed to verify this assumption.

The aim of the present study was therefore to investigate the effects of CAF (4.5 mg · kg<sup>-1</sup>) ingestion on CMJ and RPE during running until voluntary exhaustion at 80% of  $\dot{V}O_{2max}$ .

## ***Materials and Methods***

*Participants:* Eleven healthy male sub-elite endurance-trained athletes 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; 2013/1243) concluding that especial approval from REK was not required in order to performed the study as described, and the study was conducted according to the Declaration of Helsinki. Subjects physical characteristics (mean ± SD) were age 23.9 ± 2.8 years, height 181.9 ± 3.0 cm, weight 76.6 ± 7.1 kg,  $\dot{V}O_{2max}$  running ( $\dot{V}O_{2max}$ ) 71.0 ± 5.6 mL · kg<sup>-1</sup> · min<sup>-1</sup>. Inclusion criteria were: male;  $\dot{V}O_{2max} \leq 65$  mL · kg<sup>-1</sup> · min<sup>-1</sup>; and training in excess of 500 hours a year, where running was a large part of the total training volume.

*Study design:* The study had a randomized, double-blinded, placebo-controlled, cross-over design. All tests were performed at the Norwegian School of Sports Sciences (120 m altitude) from September through November in 2014 and 2015. Treatments in the study included CAF (4.5 mg · kg body wt<sup>-1</sup>) and placebo (PLA) (vehicle only). Caffeine (Coffeinum, Oslo Apotekerproduksjon, Oslo, Norway) was dissolved in Fun Light cordial concentrate (3 mg/mL) and was prepared by the lead researcher. Before all performance tests, participants underwent a  $\dot{V}O_{2max}$  test to establish that they had a level above 65 mL · kg<sup>-1</sup> · min<sup>-1</sup>, and to calculate running velocity during the performance tests (80%  $\dot{V}O_{2max}$ ).

*Experimental Procedures:* On the first test day ("day 1") participants performed a  $\dot{V}O_{2\max}$  test while running on a treadmill (Woodway, Weil am Rein, Germany) and the highest heart rate (HR) was defined as  $HR_{\max}$ . During all testing HR was measured using a HR monitor (Polar RS 800, Kempele, Finland), with an error of measurement stated by the manufacturer as  $\pm 1\%$ . Oxygen consumption and RER were measured during all tests with an Oxycon Pro metabolic system (Jaeger, Hochberg, Germany) and air was collected using a mouth V2-mask (Hans Rudolph Instr., Shawnee, KS, USA) in combination with a nose bracket. The error of measurement of this ergospirometry measurement system is reported to be  $\pm 3\%$  by the manufacturer (Stadheim et al., 2014; Stadheim et al., 2013). The  $\dot{V}O_{2\max}$  test was performed with a standardized warm-up consisting of four workloads lasting 5 min (8 to 11  $\text{km} \cdot \text{h}^{-1}$ ) with a  $5.3^\circ$  uphill incline. A 1 min break was given between each workload, during which lactate ( $LA^-$ ) was measured. After the last workload of the warm-up, subjects walked for 5 min at 5  $\text{km} \cdot \text{h}^{-1}$ , before starting the  $\dot{V}O_{2\max}$  test. Starting speed was set at 10  $\text{km} \cdot \text{h}^{-1}$  with a treadmill uphill incline of  $10.5^\circ$ . Every half minute, speed was increased by 0.5  $\text{km} \cdot \text{h}^{-1}$  until subjects were unable to maintain the speed, thus reaching voluntary exhaustion. All 11 subjects had to meet criterion 1, and at least two of the three other following criteria: 1) oxygen consumption levelled off, meaning  $\dot{V}O_2$  increased less than  $1 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , while speed was increased twice by 0.5  $\text{km} \cdot \text{h}^{-1}$ ; 2) RER values were above 1.10; 3) blood lactate was above 6.0 mmol/L after the test; and 4)  $RPE \geq 19$  on the Borg Scale 6-20.  $\dot{V}O_{2\max}$  was based on the average of the two highest 30-second measurements. Subjects with  $\dot{V}O_{2\max}$  higher than  $65 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  were included in the study.

*Instructions to test subjects:* All subjects were instructed to perform only light training (and no strength training) in the 48 h before each test. Subjects were also asked to refrain from any CAF consumption during the 48 h before each test. Only five subjects in the study had an

intake of CAF products on a daily basis (< 150 mg), while the remaining test subjects did not consume CAF on a daily basis. Subjects were also instructed to prepare for the two main tests as they would prior to a competition, and to follow the same training and diet regimes before both tests. Diet and exercise diaries were therefore kept by participants to ensure instructions given had been followed. On each main testing day subjects arrived at the laboratory in a fasted state, at the same time ( $\pm 15$  min) and on the same day of the week. For each main test, the first blood samples were drawn before subjects performed two CMJs. After this a standardized breakfast was eaten to minimize variation in pre-exercise glycogen stores. The standardized breakfast was a meal consisting of 2.0g of carbohydrates and 0.25g of protein  $\cdot$  kg body wt<sup>-1</sup>. The meal consisted of three pieces of bread with Nutella, and the remaining amount of carbohydrate and protein was given as a sports drink mixed in 0.5 L of water. The standardized meal was also consumed in order to ensure stable RER measurements during testing. Consumption of CAF or PLA was done 90 min after the standardized breakfast, and 30 min post this ingestion the second measurement of CMJ was performed. During performance testing water was offered throughout the whole performance test to ensure participants were hydrated. A 7 day washout period was provided between each of the two main performance tests.

*Main Performance tests:* On "Day 2" and "Day 3" subjects performed the two running performance tests until voluntary exhaustion at a work load/velocity equal to 80% of  $\dot{V}O_{2max}$  with a 5.3° uphill incline on the treadmill. When starting the main performance tests, subjects completed a standardized warm-up protocol lasting 8 min (Fig.1). The warm-up was performed as a consistent incremental test as part of the first 20 min interval. The standardized warm-up consisted of four 2-min workloads, with a velocity equal to ~60, ~65, ~70 and ~75% of subjects'  $\dot{V}O_{2max}$ . The standardized warm-up was performed to avoid early intracellular perturbations affecting running performance. The running performance test was built up as an interval test, with sets lasting 20 min at 80% of  $\dot{V}O_{2max}$  (except the first 8 min of the first set) and breaks

between each set. Immediately after finishing a 20 min set, subjects performed two CMJs on a force platform. A 1 min break was given between the first, third and fifth sets, while a 3 min break was given between the second and fourth sets (Fig 1). The reason for the 3 min breaks was that during this period a venous blood sample was taken from the cephalic vein after finishing the CMJs. During all testing, HR,  $\dot{V}O_2$  and RER were measured as means between minutes 3-5, 8-10 and 17-19 in each interval set. Subjective ratings of perceived exertion (RPE) according to the Borg Scale (from 6 to 20) were determined for each interval set at the time points 5,10 and 20 min. Capillary blood samples for measurement of both glucose and lactate (LA-) were taken while running, at 7.5 and 15 min in each set. Capillary blood samples were taken from the fingertip, and measurement of glucose was done using a cuvette (HemoCue glucose 201<sup>+</sup>; Ängelholm, Sweden) and LA- analysis was conducted using a YSI 1500 Sport lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Encouragement was given during the performance test until subjects rated the test workload at 18 or higher on the Borg Scale. After this no encouragement was given by the researchers. During performance tests, subjects could see the remaining time for each interval until they reported 18 on the Borg Scale. After this time point, all information regarding time was removed to blind test subjects between trials.

*FIGURE 1 AROUND HERE*

Counter movement jumps (CMJs) in the present study were performed on a force platform from Biomekanikk AS (Oslo, Norway). The force platform uses a sandwich construction with one Vetek VZ563YH-200 kg load cell (Hantverksvägen 15. 76493 VÄDDÖ, Sweden) in each corner to calculate force. Calculation of CMJ heights was done using the impulse method (Linthorne & NP, 2001). The software (Biojump, Oslo, Norway) samples force data with a frequency of 2000 Hz, using a Butterworth low pass filter with a

cut-off frequency of 120 Hz. CMJ tests were performed on arrival, post ingestion (30 min), after each 20 min running period and at exhaustion (Fig 1). Before each CMJ, the subject's body weight was measured on the force platform. CMJs were performed starting from standing with straight legs and beginning with a counter movement down to a knee angle of 90 degrees. The hands were held on the hips during the jump to avoid any effect of arm-swing. The subjects were instructed to always take off and land in the same position, marked with tape on the force platform. A valid jump required that the landing was done with straight legs (Fig 1). Each subject performed two CMJs and the average of the two jumps was used as the subject's CMJ height for that time point. If one of the two CMJs was not completed as instructed above, a third CMJ was performed to ensure a valid result. The two jumps that were closest in height were then used to calculate the subject's CMJ height at that time point. The time between subjects stepping off the treadmill and performing the first CMJ was on average  $12\pm 2$  sec and the time to the second jump was on average  $17\pm 2$  sec.

*Blood samples:* All blood samples for measurements of creatine kinase (CK) activity and troponin T were drawn from the subjects' median cubital vein using a BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). A 7 ml blood sample was drawn for all blood samples and placed in tubes containing EGTA/glutathione [20  $\mu$ l 0.2 M glutathione and 0.2 M EGTA per ml blood] for analysis of adrenaline, noradrenaline, and caffeine. Blood samples were immediately placed in ice water and centrifuged at 2500 rpm for 10 min at 4 °C (Heraeus Megafuge 16R centrifuge, Thermo Electron, Osterode am Harz, Germany). Thereafter, plasma was divided into three Eppendorf tubes (Microtube Superspin, VWR International, West Chester, PA, USA) and frozen at -80°C. Analyses of CK and Troponin T from blood samples were conducted by Radium Hospitalet (Ullernchaussen 70, 0379 Oslo). The enzymatic photometry method was used for analyses of CK and Troponin T by electrochemiluminescence immunoassay.

For each capillary sample a finger prick was performed using a Saft-T-Pro Plus (Accu-Check, Mannheim, Germany) for measurements of glucose or LA-. For measurement of blood LA-, capillary blood samples were drawn into a 50  $\mu\text{l}$  capillary tube and a 20  $\mu\text{l}$  pipette was used to draw blood into the analyzer from the 50  $\mu\text{l}$  capillary tube. The analyzer was calibrated with a 5.0  $\text{mmol} \cdot \text{l}^{-1}$  lactate stock solution before each test and between the submaximal workloads and main tests. Values between 4.95  $\text{mmol} \cdot \text{l}^{-1}$  and 5.05  $\text{mmol} \cdot \text{l}^{-1}$  were accepted. Under normal circumstances the errors of measurement are  $\pm 2\%$  for blood LA- values between 0 and 5  $\text{mmol} \cdot \text{l}^{-1}$  and  $\pm 3\%$  for values between 5 and 15  $\text{mmol} \cdot \text{l}^{-1}$ . Blood glucose measurements were carried out as previously described by Stadheim et al (2013) (Stadheim et al., 2013).

*Calculations of carbohydrate and fat oxidation* were done for each 20 min interval, based on RER and  $\dot{V}\text{O}_2$  values for each period as outlined by Peronnet and Massicotte (Peronnet & Massicotte, 1991). Questionnaires were used to evaluate motivation, "current fitness" and sleep quality using a scale from 1-100 (Stadheim et al., 2014).

*Statistical Analysis:* All data are presented as means  $\pm$  standard deviation (SD), and differences in performance during running until voluntary exhaustion were evaluated using a paired t-test. A two-way ANOVA for repeated measures was used to elicit differences for time or treatment effects for CMJ, HR, LA-,  $\dot{V}\text{O}_2$ , glucose, and RPE during testing. If a significant f-ratio was found, a paired t-test was used to test differences between treatments at specific time points. All data were tested for normal distribution using the Shapiro-Wilk test. Statistical analyses were performed using SPSS (IBM, New York, NY, USA), and the level of significance was set at  $p < 0.05$ . Performance data were log-transformed to reduce the non-uniformity of error. Precision of estimation was indicated with 90% confidence limits.

## Results

*Running performance:* CAF ingestion increased participants time until voluntary exhaustion with 8:40 min, resulting in a 10.0% improvement, and 10 out of the 11 subjects (90.9%) improved their time to task failure after CAF consumption (Fig 2). Specifically, running durations for PLA and CAF trials were 75:20±14:28 and 83:44±12:23 min ( $P<0.05$ ), respectively. During both tests RPE, HR and  $\dot{V}O_2$  increased gradually from start to finish, independent of product ingestion (Fig 3). However, RPE was lower during CAF trials compared to PLA after the first 7 minutes of running until voluntary exhaustion. However, at voluntary exhaustion no difference was observed in RPE between treatments. The RER values showed a progressive decline as an effect of increased duration for both treatments (Fig 3). However, no difference was observed in carbohydrate or fat oxidation between PLA and CAF trials. Blood glucose values were stable and did not change during running until voluntary exhaustion for either of the two treatments (Fig 4). The increase in blood LA- values was similar between treatments during the first 67 min of the performance test. However, higher blood LA- accumulation was observed at voluntary exhaustion in the CAF trial compared to PLA (Fig 4).

*FIGURE 2 AND 3 AROUND HERE*

*CMJ heights:* At arrival, no difference was observed between treatments for CMJs ( $p<0.91$ ). At 30 min post CAF ingestion there was a tendency to higher CMJs compared to PLA ( $p<0.11$ ). During the performance test, CMJ heights were significantly higher for CAF compared to PLA (Fig 4). The CMJ heights for PLA and CAF trials respectively were: 20 min - 30.2±3.3, 32.0±3.1 cm; 40 min - 30.6±4.0, 32.6±2.3; 60 min - 30.2±4.3, 32.3±3.0; and at voluntary exhaustion - 30.4±3.9, 32.0±3.3 cm (Figure 4;  $P<0.05$ ). Body weight measured prior to all CMJs showed a progressive reduction during the tests, with no difference between



treatments. Despite consuming water throughout the whole test, subjects' bodyweights reduced by  $1.4 \pm 1.8$  kg (approx 1.9%) and  $1.7 \pm 2.2$  kg (approx 2.2%) during the PLA and CAF trials from start to voluntary exhaustion (Table 1).

*FIGURE 4 AND 5 AROUND HERE*

CK and troponin T were higher for both PLA and CAF trials at voluntary exhaustion than at arrival (Table 1). However, during the CAF trials significantly higher CK values were observed at voluntary exhaustion compared to PLA. Measurements 24 h post performance testing showed no difference between PLA and CAF for either CK or Troponin T despite the increased running duration in the CAF trial.

*TABLE 1 AROUND HERE*

*Other results:* No differences were observed between groups regarding responses to questionnaires, including “current fitness”, motivation, amount of sleep (hours) or eating patterns before the different treatments. There was also no difference between participants consuming CAF on a daily basis (test subjects 2, 5, 6, 7 and 8) and non-consumers in percent improvement during the performance test following CAF ingestion. Furthermore, the questionnaires revealed that participants were unable to sense which product they received during the different trials, and had followed instructions regarding training, food, liquid and CAF consumption in the 48 h prior to each C-PT.

## **Discussion**

The novel finding in the present study was that CAF ingestion ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) increased CMJ during the prolonged running test, which may have contributed to lower RPE and improved time until voluntary exhaustion at 80% of  $\dot{V}O_{2\text{max}}$ . No difference was however observed for HR,  $\dot{V}O_2$ , LA-, glucose, RER, fat or carbohydrate oxidation between the

different treatments. Blood levels of CK and troponin T increased with increased running duration for both PLA and CAF trials, but higher CK was observed at voluntary exhaustion for the CAF trial compared to PLA.

It has previously been observed CAF ingestion can improve performance when running at 70% of  $\dot{V}O_{2max}$  until exhaustion, in agreement with the data from the present study (Ivy et al., 1979; Essig, Costill, & Vanhandel, 1980; Graham, 2001). Unfortunately studies testing the effects of CAF ingestion at higher sport specific intensities (80-85% of  $\dot{V}O_{2max}$ ) have found subjects included were unable to maintain the intensity for more than a short period of time (~15 min) (Beck, Housh, Malek, Mielke, & Hendrix, 2008; Candow, Kleisinger, Grenier, & Dorsch, 2009; Joyner et al., 2008; Coetzer et al., 1993). The subjects included in the present study were highly trained endurance athletes ( $\dot{V}O_{2max}$  ~70 mL · kg<sup>-1</sup> · min<sup>-1</sup>), running a minimum of 65 min at 80% of  $\dot{V}O_{2max}$  before reaching voluntary exhaustion during both PLA (75:20±14:28) and CAF (83:44±12:23) trials. The present study is therefore one of the first to demonstrate that CAF ingestion can improve prolonged running performance for highly trained athletes while maintaining a sport-specific intensity until voluntary exhaustion (Fig 2 and 3) (Joyner et al., 2008; Coetzer et al., 1993; Abbiss et al., 2005) .

Based on the available literature, the actions of CAF ingestion in improving running performance seem multi-functional (Stadheim et al., 2013; Stadheim et al., 2014; Stadheim et al., 2015; Graham, 2001; Doherty et al., 2005; Black et al., 2015; Warren et al., 2010). Initial studies suggested that improvements were related to the observation of lower RER, and increased free fatty acids in the blood stream (Ivy et al., 1979; Essig et al., 1980). In the present study no differences in RER values were observed as an effect of prolonged running performance between PLA and CAF trials. This was also the case for oxidation of fat and carbohydrates during the performance test. The stable, high glucose values, showing no

difference between PLA and CAF performances, also indicate that no subjects became hypoglycaemic before reaching voluntary exhaustion. It is therefore unlikely that glycogen sparing, as suggested in the classic study of cyclists by Costill et al. (1978) would give support or explanation for the improved running duration after CAF ingestion in the present study (Ivy et al., 1979; Essig et al., 1980).

During athletic competitions, the ability to sustain high RPE is important for endurance performance (Stadheim et al., 2013; Stadheim et al., 2015; Rustad et al., 2016; Gonglach et al., 2016; Doherty et al., 2005). Several studies have concluded that CAF's ergogenic potential is related to the reduction of RPE due to the inhibition of adenosine receptors (Doherty et al., 2005; Bazzucchi, Felici, Montini, Figura, & Sacchetti, 2011; Graham, 2001; Gonglach et al., 2016). Adenosine receptors are expressed in most tissues of the human body, and the CAF dosage used in the present study would lead to peak plasma CAF concentrations of ~ 30  $\mu\text{M}$  (Stadheim et al., 2014; Stadheim et al., 2015). A plasma concentration of ~ 30  $\mu\text{M}$  CAF would reduce adenosine receptor activation, and possibly lead to the condition termed hypoalgesia (Stadheim et al., 2015; Stadheim et al., 2014; Gonglach et al., 2016; Black et al., 2015). The hypoalgesic effects of CAF ingestion might have allowed subjects to tolerate higher levels of discomfort, consequently improving running duration. During prolonged exercise tasks RPE increases as an effect of fatigue (Rustad et al., 2016). In the present study, RPE increased throughout both trials, but was lower during the CAF trial compared to PLA. At exhaustion, similar RPE ratings were observed in both treatments, leading to the assumption that in order to reach similar levels of RPE, subjects increased their running duration following CAF ingestion (Stadheim et al., 2015; Stadheim et al., 2013; Stadheim et al., 2014; Doherty et al., 2005; Gonglach et al., 2016). The higher blood LA- accumulation and CK values only observed at exhaustion for the CAF trial fit well with this assumption. However, it has been suggested that augmented strength and motor-unit recruitment, rather than reductions in effort, may

underlie CAF's ergogenic effect on endurance exercise (Black et al., 2015; Gonglach et al., 2016; Warren et al., 2010).

To the authors' knowledge, we are the first to measure CMJ during prolonged running exercise. In the present study, no difference in CMJ heights was observed at arrival ( $p < 0.91$ ). However, 30 min post CAF ingestion a tendency was already evident for increased CMJ compared to PLA ( $p < 0.11$ ). During prolonged running, CAF ingestion resulted in consistently higher CMJs. It was, however, surprising that CMJ did not decline for either treatment while running until exhaustion, since several studies have observed that prolonged running is associated with reduced power (Overgaard et al., 2004). This is possibly explained by the time taken before the CMJs were performed after finishing an interval set (12 and 17 sec), as highlighted by Froyd et al. (Froyd et al., 2013). In support of our findings, Bloms et al. reported CAF ( $5 \text{ mg} \cdot \text{kg}^{-1}$ ) increased CMJ height, associated with increased peak force in 25 collegiate athletes (Bloms et al., 2015). Studies have reported a close relationship between CMJ heights, force development and MVC (Rouis et al., 2015; Young et al., 2011; Driss et al., 2015), and several studies have shown CAF can improve MVC and maximal power (Black et al., 2015; Lopes et al., 1983; Graham, 2001). The mechanisms explaining improved MVC and CMJ following CAF ingestion are speculated to be related to either a direct effect on the muscle (e.g., maintaining electrolyte homeostasis or enhancing sarcoplasmic reticulum  $\text{Ca}^{2+}$  release) or on the CNS (e.g. increasing motor unit recruitment) (Kalmar, 2005; Graham, 2001; Kalmar & Cafarelli, 2006; Black et al., 2015; Gonglach et al., 2016). CAF is reported to increase cortical, spinal neuron excitability and motor unit recruitment, which may explain the increased MVC, CMJ and reduction in RPE (Black et al., 2015; Kalmar et al., 2006). Specifically, Black et al. found that CAF ingestion ( $5 \text{ mg} \cdot \text{kg}^{-1}$ ) resulted in an increase in MVC and in motor unit recruitment, associated with performance improvements due to a reduction in RPE (Black et al., 2015). The improved CMJ height following CAF ingestion in the present study could

therefore very well have contributed to the reduction in RPE and improved performance. Likewise, strength training and increased power have been reported to improve running efficiency and exercise performance (Guglielmo et al., 2009; Black et al., 2015; Paavolainen et al., 1999). However, the  $O_2$ -cost and HR were similar in PLA and CAF trials, negating the suggestion that increased running economy can explain improved performance. Furthermore, measurements of subjects' body weights before each CMJ indicate that the reduction in body weight (~2%, most likely due to loss of water in sweat) was similar between treatments, and probably did not affect performance outcome or CMJ measurements.

Higher exercise intensity or longer duration as observed in the present study after CAF ingestion has been associated with greater muscular damage (Shing et al., 2007; Scharhag, George, Shave, Urhausen, & Kindermann, 2008). CK and troponin T have been used in several studies as markers of muscular damage during exercise (Scharhag et al., 2008), and increased plasma concentrations have been identified in ultra-distance marathon running, double poling and strength training (Stadheim et al., 2014; Scharhag et al., 2008). During running it was observed that CAF ingestion resulted in increased oxidative stress markers (IL-6 and IL-10) in blood after a 15 km running competition compared to PLA (Tauler et al., 2013). In the present study both CK and troponin T values increased during the running test independent of treatment conditions, but the 10% increase in running duration following CAF ingestion was associated with increased CK values indicating larger muscular damage (Scharhag et al., 2008; Tauler et al., 2013; Shing et al., 2007; Stadheim et al., 2014).

Results from the present study are of great interest for sports performance since CAF is legal to use during endurance sports competitions. It was removed from the World Anti-Doping Agency list of prohibited substances in 2004 (Chester & Wojek, 2008), and the improvement after CAF ingestion at an intensity (80% of  $\dot{V}O_{2max}$ ) and duration (65-75 min) typically

maintained during a half-marathon competition display CAF might be an effective ergogenic drug for improving performance in elite runners during competitions (Joyner et al., 2008; Coetzer et al., 1993; Abbiss et al., 2005). A criticism of the present study is of course that during a competition subjects have to pace themselves and not run at a set velocity until voluntary exhaustion. However in order to investigate the effects of CAF ingestion on CMJ height during prolonged running it was important for the study to ensure that subjects performed the same amount of work during each 20 min interval.

## ***Conclusion***

The present study demonstrated that CAF ingestion ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) improved running until voluntary exhaustion at 80% of  $\dot{V}O_{2\text{max}}$  associated with increased CMJ heights and reduced RPE. No difference was observed for work economy, HR,  $\dot{V}O_2$ , LA-, glucose, RER, fat or carbohydrate oxidation between the two treatments. However higher LA- and HR were observed at CAF exhaustion compared to PLA. Blood levels of CK and troponin T were elevated at exhaustion, and CK was higher in CAF than in PLA. The authors speculate that the improvements in running performance following CAF ingestion results from both improved neuromuscular function, assessed from higher CMJ heights, and reduced RPE.

## **Acknowledgements**

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## *Perspectives*

Despite several studies observing CAF ingestion can reduce RPE, increase power (MVC and CMJ height) and exercise performance, the present study is the first to investigate the effects of CAF ingestion on muscular function and power during prolonged running exercise. Based on our results CAF ingestion improved neuromuscular function, and power as judged by improved CMJ heights following CAF ingestion. We also speculate that the improved CMJ heights might have contributed to the reduction in RPE during the CAF trial compared to PLA. No difference was observed for HR,  $\dot{V}O_2$ , glucose, RER, fat or carbohydrate oxidation between the two treatments during exercise, negating that improved work economy or a sparing of glycogen explain exercise improvements. Blood levels of CK and troponin T were elevated at exhaustion, and CK was higher in CAF than in PLA indicating the 10% increase in running duration following CAF ingestion increased muscular damage. The authors speculate that improvements in running performance following CAF ingestion was therefore a result of both improved neuromuscular function, assessed from higher CMJ heights, and reduced RPE.

In addition, there are to date insufficient studies that have specifically investigated the effects of CAF ingestion on endurance capacity for top trained athletes with high  $\dot{V}O_{2max}$  ( $\sim 70 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Results from the present study are therefore important since it gives information on psychological (RPE) and physiological ( $\dot{V}O_2$ , HR,  $\dot{V}O_2$ , LA-, glucose, Troponin T, CK, RER, fat and carbohydrate oxidation) responses following CAF ingestion ( $4.5 \text{ mg} \cdot \text{kg}^{-1}$ ) during a sport specific (80 % of  $\dot{V}O_{2max}$ ) prolonged running performance.

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## Legends

**Figure 1:** Experimental design. Top panel (A) shows the 3 weeks of testing. The bottom panel (B) shows the test procedure for all performance tests in weeks 2 and 3, with an illustration of how each CMJ was performed.

**Figure 2:** Mean and individual participant time to exhaustion during the running test. PLA (open) or CAF (filled). Mean values are listed as means  $\pm$  SD. \* Significant difference from PLA trials ( $p < 0.05$ ).

**Figure 3:**  $\dot{V}O_2$ , HR, RER, utilization of CHO, fat and RPE displayed as means during the performance test. Values are listed as means  $\pm$  SD. \* Significant difference between PLA and CAF ( $p < 0.05$ ).

**Figure 4:** LA- and glucose values displayed as means during the performance test. Values are listed as means  $\pm$  SD. \* Significant difference between PLA and CAF ( $p < 0.05$ ).

**Figure 5:** CMJ heights displayed as means during the performance test. Values are listed as means  $\pm$  SD. \* Significant difference between PLA and CAF ( $p < 0.05$ ).

**Table 1:** Plasma CK, troponin T and body weight for each 20 min interval during PLA and CAF testing.

When	Creatine K (U/L)			Troponin T (ng/L)			Body weight (kg)		
	PLA	CAF		PLA	CAF		PLA	CAF	
Arrival	235±139	278±142		7.8±3.1	7.0±1.9		75.3±7.9 (-1.7%)	75.8±7.8 (-1.2%)	
Pre	218±119	279±127		6.8±2.4	7.3±3.1		76.7±7.6 (100%)	77.1±7.3 (100%)	
20 min		No Measurements					76.4±7.8 <sup>#</sup> (-0.4%)	76.9±7.1 (-0.2%)	
40 min	294±162 <sup>#</sup>	374±201 <sup>#</sup>		9.7±4.0 <sup>#</sup>	9.3±3.2 <sup>#</sup>		76.0±7.7 <sup>#</sup> (-0.9%)	76.4±7.2 <sup>#</sup> (-0.9%)	
60 min		No Measurements					75.6±7.7 <sup>#</sup> (-1.4%)	75.9±7.2 <sup>#</sup> (-1.6%)	
Finish	342±178 <sup>#</sup>	436±221 <sup>#*</sup>		15.5±6.1 <sup>#</sup>	16.9±6.5 <sup>#</sup>		75.3±7.5 <sup>#</sup> (-1.9%)	75.4±7.2 <sup>#</sup> (-2.2%)	
24h post testing	450±406 <sup>#</sup>	450±224 <sup>#</sup>		15.4±9.0	17.1±10.3				No Measurements

Values are listed as means ± SD. \* Significant difference from PLA (p<0.05). # Significant difference from previous 20 min measurement (p<0.05)

Figure I

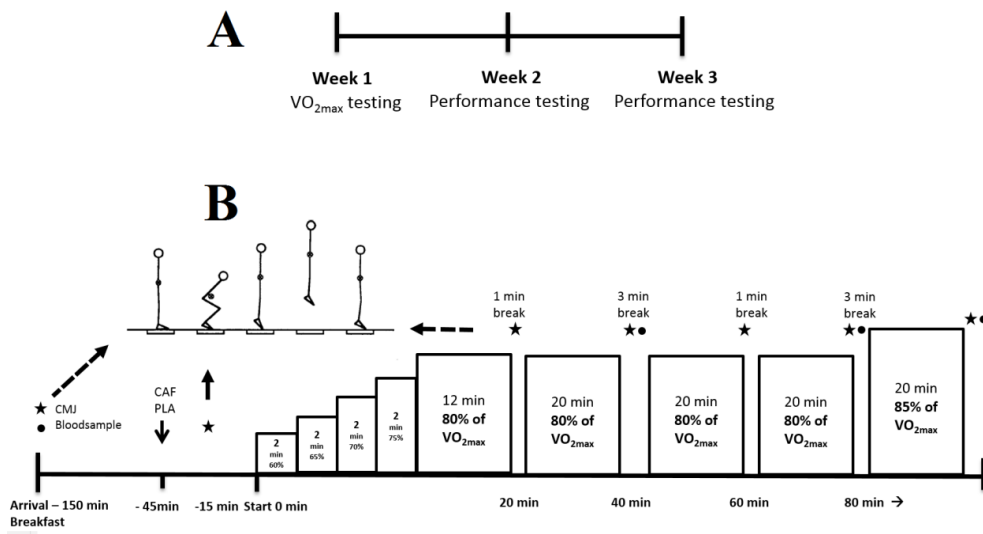


Figure II

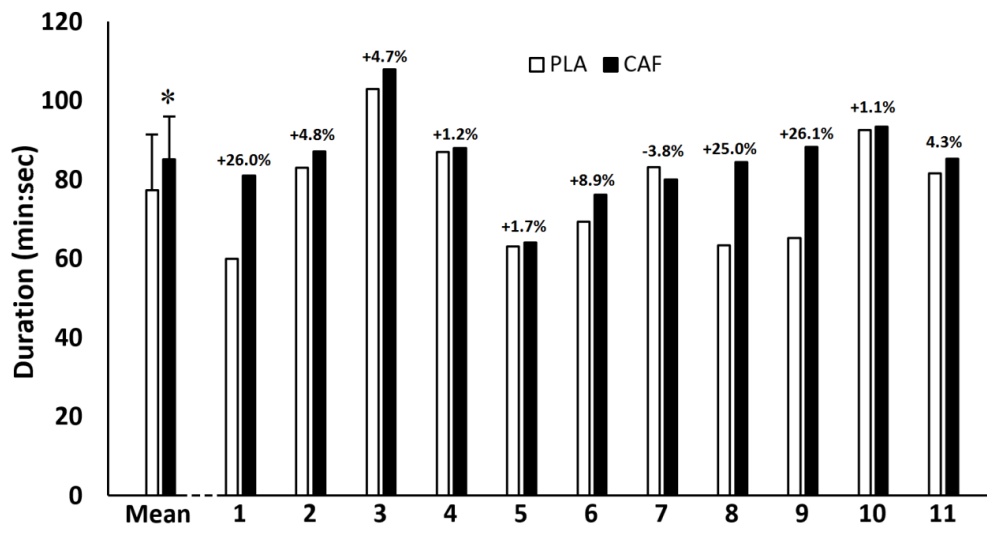




Figure III

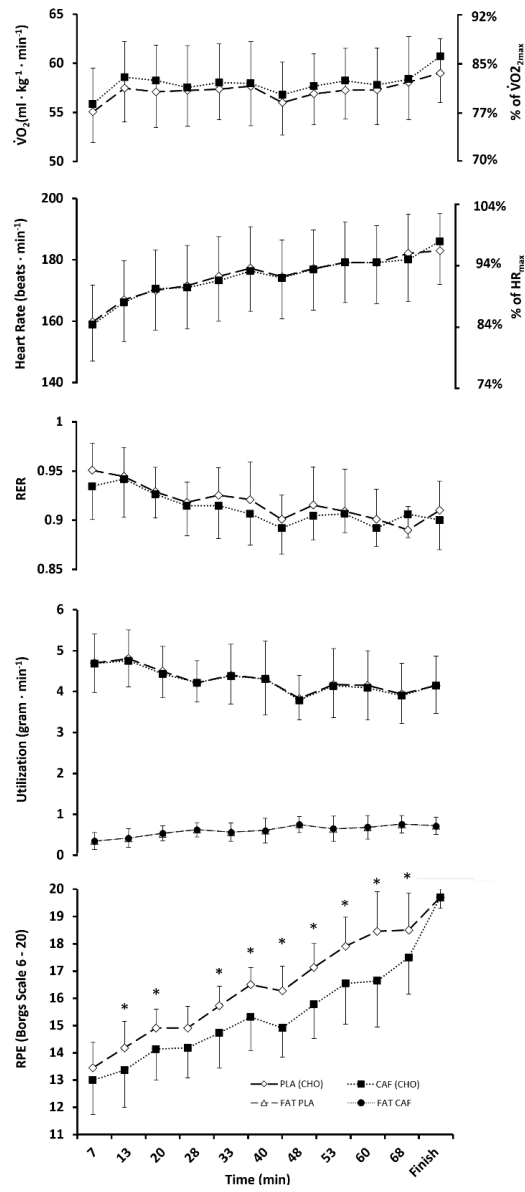


Figure IV

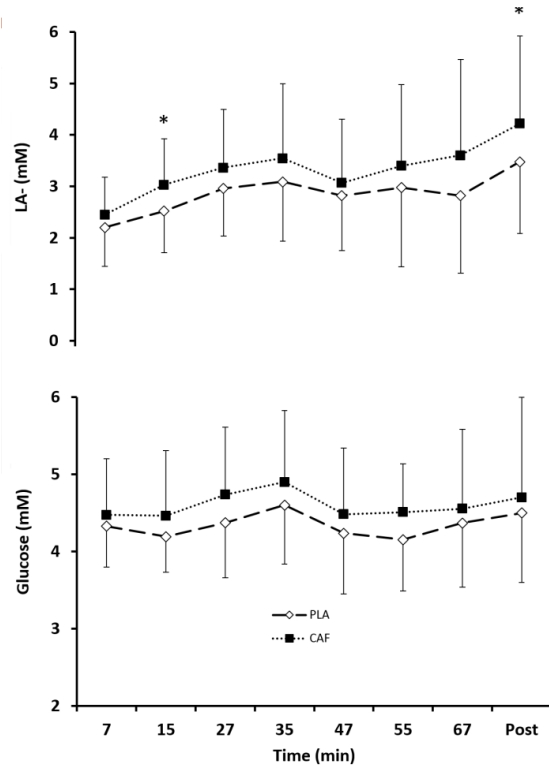
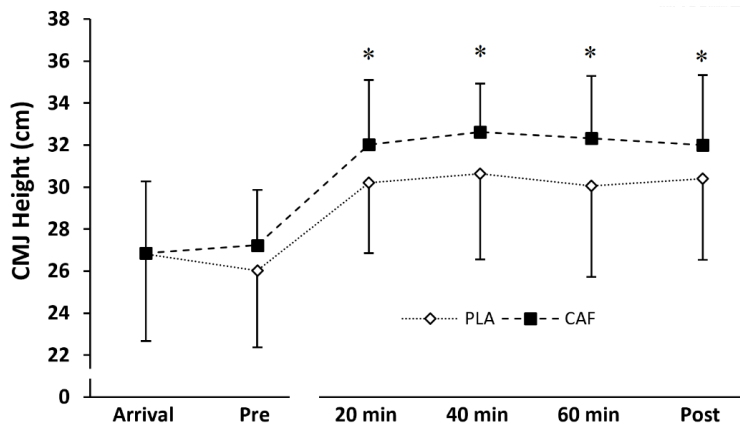


Figure V



# **APPENDIX I**

*Table of Time Trail improvements following caffeine ingestion*



**Appendix I.** Studies researching the effects of CAF ingestion and TT performance showing improvements between 2000 and 2016.

Study	Test exercise	VO <sub>2max</sub>	CAF Dosage (mg <sup>-1</sup> · kg <sup>-1</sup> )	Test method	Endurance Performance	HR	VO <sub>2</sub>	RPE/ Pain	Lactate	Adrenaline
Gonglach et al. 2016	13 cyclists <sup>M</sup>	-	5	TT Cycling at 5 in pain level	↑	↑	↑	↔	↑	NM
Glaister et al. 2015	14 cyclists <sup>F</sup>	52.3	5	20 km TT	↑	↑	↔ (†)	↔	↑	NM
Paton 2014	20 cyclists <sup>M</sup>	62.7	3-4	20 km TT	↑	↑	↔	↔	↑	NM
Brotolotti et al. 2014	13 cyclists <sup>M</sup>	-	6	20 km TT	↑	↑	NM	↔	NM	NM
Pitchford et al. 2014	9 cyclists <sup>M</sup>	64.4	3	60 min TT	↑	↑	↔ (†)	↔	↑	↑
Astorino et al. 2012	13 cyclists <sup>M</sup>	57.5	5	10 km TT	↑	↑	NM	↔	NM	NM
Desbrow et al. 2012	16 cyclists <sup>M</sup>	60.4	3 or 6	75% of VO <sub>2peak</sub> 60 min + TT	↑	↑	NM	↔	NM	NM
Irwin et al. 2011	12 cyclists <sup>M</sup>	63.7	1.5 and 3	60 min TT	↑	↑	NM	↔	↑	NM
Ivy et al. 2009	12 cyclists <sup>M/F</sup>	54.9	2.5-3.0	40 min TT	↑	↑	NM	↔	↑	↑
McNaughton et al. 2008	8 cyclists <sup>M</sup>	65.0	6	60 min TT	↑	↔	NM	NM	↑	NM
Walker et al. 2008	9 cyclists <sup>M</sup>	71.2	6	90 min 70 % of VO <sub>2max</sub> + TT 30 min	↑	NM	NM	NM	↑	↑
Cureton et al. 2007	16 cyclists <sup>M</sup>	60.5	5	120 min 60/75 % av VO <sub>2max</sub> + 15 min TT	↑	NM	↑	↓	↑	NM
Bridge & Jones 2006	8 runners <sup>M</sup>	-	3	8 km race running	↑	↑	NM	↔	↑	NM
Cox et al. 2002	12 cyclists <sup>M</sup>	66.4	6	2 hours at 70 % av VO <sub>2max</sub> + TT (30 min)	↑	NM	NM	↓	↑	NM
Bruce et al. 2000	8 rowers <sup>M</sup>	4.7 L	6 and 9	60 min pre-exercise - TT- 2000 m	↑	↔	↔	↔	↑	NM

↑ = Improvement with ingestion of caffeine (<0.05) (†) = Improvement with ingestion of caffeine (<0.10) ↔ = No difference NM = No data/ Not measured or reported F= Female, M= Male  
 \*Studies conducted between 2000 and 2016. Ingestion of CAF was between 3-6 mg x kg<sup>-1</sup> approximately one hour prior to the test. The performance test was completed as a TT with highly trained subjects (VO<sub>2max</sub>, male ~60 ml · kg<sup>-1</sup> · min<sup>-1</sup> or higher). The study measured/reported two or more of the following parameters: HR, VO<sub>2</sub>, RPE, lactate or adrenaline.



# **APPENDIX II**

*Table of sub-maximal exercise and physiological responses following caffeine ingestion.*





**Appendix II:** Summary of randomly selected studies investigating the effects of CAF ingestion during submaximal exercise, and time until exhaustion.

Study	Subjects	Dosage (mg <sup>-1</sup> x kg <sup>-1</sup> )	% of VO <sub>2max</sub>	Mode	Performance	HR	RER	FFA	Catecholamines	RPE	LA-	Glucose
Cruz et al. (2015)	8	6	73	Cycling	↑	↔	↓	NM	↔	↔	↔	↔
Doherty et al. (2004)		5	30-130	Cycle	↑	↔	NM	NM	NM	↓	↑	NM
Doherty et al. (2002)	14	5	125	Running	↑	NM	NM	NM	↔	↓	↔	↔
Bell et al. (2002)	21	5	50 & 80	Cycle	↑	↔ 50% ↑ at 80%	↔	↔	NM	↓	↑	↑
Greer et al. (2000)	8	6	80-85	Cycle	↑	↔	↔	↔	Adrenaline ↑ Noradrenaline ↔	NM	↑	↑
Greer et al. (2000)	8	6	70	Cycle	↑	↔	↔	↑	Adrenaline ↑ Noradrenaline ↔	NM	↔	↔
Graham et al. (2000)	9	6	70	Cycle	↑	↔	↔	↑	Adrenaline ↑	NM	↑	↑
Daniels et al. (1998)	10	6	65	Cycle	↑	↔	NM	NM	NM	NM	NM	NM
Bell et al. (1998)	8	5	85	Cycle	↑	↔	↔	↑	Adrenaline ↑	↓	↑	↔
Cole et al. (1996)	10	6	30, 50 and 70	Cycle	↑	↔	↔	↑	NM	↓	NM	↔
Casal et al. (1985)	9	6	75	Running	↑	↔	↔	↑	NM	NM	↑	↔
Costill et al. (1978)	9	330 mg	80	Cycle	↑	↔	↓	↑	Adrenaline ↑	↓	↔	↔

↑ Increased after caffeine intake ↔ No difference between placebo and caffeine values ↓ Decreased after CAF intake NM = NO data/ Not measured or reported

\*Inclusion criteria for studies are: They used CAF dosages between 3-6 mg x kg<sup>-1</sup> approximately one hour prior to the test. The performance test was completed as time until exhaustion, where subjects

performed the same workload. Highly trained subjects (VO<sub>2max</sub> male ~60 ml · kg<sup>-1</sup> · min<sup>-1</sup> or higher). The study measured/reported two or more of the following parameters: HR, VO<sub>2</sub>,

RPE, lactate or adrenaline.



# **APPENDIX III**

*Regional Ethics Committee approval studies I-V*





# UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

Professor Jørgen Jensen  
Norges idrettshøgskole  
Sognsveien 220  
P.O. Box 4014 Ullevål Stadion  
0806 Oslo

Regional komité for medisinsk og helsefaglig  
forskningsetikk Sør-Øst D (REK Sør-Øst D)  
Postboks 1130 Blindern  
NO-0318 Oslo

Telefon: 22 85 05 93

Telefaks: 22 85 05 90

E-post: [i.m.middelthon@medisin.uio.no](mailto:i.m.middelthon@medisin.uio.no)

Nettadresse: <http://helseforskning.etikkom.no>

Dato: 12.07.10

Deres ref.:

Vår ref.: 2010/1525-1

KOPI

## 2010/1525-1 Effekt av koffein på prestasjonen i simulert skiløp

Forskningsansvarlig: Norges Idrettshøgskole

Prosjektleder: Jørgen Jensen

*Formålet med studien er å undersøke om inntak av koffein en time før start av en 15 km test i stakeergometer vil øke prestasjonsevnen. Det skal inkluderes 16 godt trente mannlige langrennsutøvere. Det søkes om opprettelse av en spesifikk forskningsbiobank i forbindelse med studien, hvor blodprøvene skal oppbevares. Det skal totalt tas 16 blodprøver av hver forsøksperson.*

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional forskningsetisk komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst D) i møtet 17.06.2010. Søknaden er vurdert i henhold til lov av 20. juni 2008 nr. 44, om medisinsk og helsefaglig forskning (helseforskningsloven), med tilhørende forskrift om organisering av medisinsk og helsefaglig forskning av 1. juli 2009 nr 0955.

### Komiteens vurdering

Komiteen registrerer at det i denne studien skal tas svært mange blodprøver av forskningsdeltakerne. Det henstilles til prosjektleder å vurdere om antall blodprøver kan reduseres.

Komiteen ønsker en tilbakemelding på om det i dette tilfellet søkes om opprettelse av en generell forskningsbiobank.

### Vedtak

Med hjemmel i helseforskningsloven § 10, jfr. forskningsetikkloven § 4 godkjenner komiteen at prosjektet gjennomføres i samsvar med det som framgår av søknaden.

REK godkjenner opprettelse av forskningsbiobank ” NIH-JJ-Caffeine1-2010”. Melding om godkjenningen er sendt Biobankregisteret.

- Ansvarshavende er professor Jørgen Jensen.
- Materiale som inngår i forskningsbiobanken er blodprøver.

- Bruk av det humant biologiske materialet kan bare skje etter prosjektdeltakernes samtykke og er begrenset til hva som fremgår av informasjonsskrivet.
- Forskningsbiobankens varighet er satt til 31.12.2012.

Dersom forskningsbiobanken opphører, nedlegges eller overtas av andre, skal det søkes REK om tillatelse, jfr. helseforskningsloven § 30.

Godkjenningen av prosjektet gjelder til 31.12.2012. Av dokumentasjonshensyn skal opplysningene og det humant biologiske materialet likevel bevares inntil 31.12.2017. Opplysningene og det humant biologiske materialet skal deretter slettes eller anonymiseres, senest innen 30.06.2018.

Opplysningene skal lagres avidentifisert, det vil si adskilt i en nøkkel- og en opplysningsfil.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren».

Prosjektet skal sende sluttmelding til sør-øst D på fastsatt skjema senest 30.06.2013.

Tillatelsen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden og protokollen, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK. Vi gjør oppmerksom på at hvis endringene er "vesentlige", må prosjektleder sende ny søknad, eller REK kan pålegge at det sendes ny søknad.

Vi ber om at alle henvendelser sendes inn via vår saksportal:

<http://helseforskning.etikkom.no> eller på e-post til: [post@helseforskning.etikkom.no](mailto:post@helseforskning.etikkom.no)

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen,

Stein A. Evensen (sign.)  
prof. dr.med.  
leder

  
Arvid Grønlie Olsen  
julist  
fungerende komitésekretær

Kopi til: Norges Idrettshøgskole, ved øverste adm. ledelse  
Biobankregisteret

Norges idrettshøgskole  
Hans Trankjær Andresen  
Postboks 4014 Ullevål Stadion  
0806 OSLO  
Norge

Offl § 13 første ledd, jf fvl § 13 første ledd nr 2, jf lml

Deres ref.	Dato	Vår ref.	Arkivkode	Seksjon/Saksbehandler
	25-10-2010	10/17625-9	343.1	Preklinisk utprøving/ Maria Almløf

#### **KOFFEIN - KLINISK UTPRØVING - EUDRACT NR. 2010-022582-95**

Vi viser til korrespondanse i ovennevnte sak, senest Deres brev datert 2010-10-14. Vi har følgende kommentarer.

#### **Farmasøytisk/kjemisk dokumentasjon**

Vi har ingen innvendinger.

#### **Protokoll**

- Dato for protokoll stemmer ikke med dato i EudraCT-skjema.
- Det er oppgitt signaturfelt for Hans Christian Stadheim, men denne signaturen mangler.
- EudraCT-skjema angir at kun menn skal inkluderes i studien. Dette fremkommer ikke i protokollen. Dokumentene bør harmoniseres på dette punktet.

#### **Pasientinformasjon datert 2010-10-14**

Informasjonen om forsikring er ikke korrekt. Statens Legemiddelverk forsikrer ikke pasienter i kliniske studier, slik informasjonen kan gi inntrykk av. Vi anbefaler at dere heller benytter teksten "Du er forsikret i henhold til Lov om produktansvar i Legemiddelforsikringen".

#### **Legemiddelmerking**

- Etiketten skal påføres batchnr. eller annet id-nummer som identifiserer produksjonsprosessen.
- Forutsatt at utprøvningspreparatet utelukkende skal administreres ved testlokalet, det vil si at forsøkspersonene ikke vil ha mulighet til å ta det med utenfor disse lokalene, vil vi ikke kreve at utprøvningspreparatet merkes "Oppbevares utilgjengelig for barn".

Ovennevnte kommentarer er ikke til hinder for at studien kan starte opp, men vi ber om at disse punktene rettes opp før studien starter.

*Brev stiles til Statens legemiddelverk. Vennligst oppgi vår referanse*

Statens legemiddelverk  
Sven Oftedals vei 6, 0950 Oslo

Tlf.: 22 89 77 00  
Fax: 22 89 77 99

[www.legemiddelverket.no](http://www.legemiddelverket.no)  
[post@legemiddelverket.no](mailto:post@legemiddelverket.no)

Kto. 7694 05 00903  
Org.nr. 974 761 122



**Konklusjon**

Studien kan starte når ovennevnte punkter er rettet opp. Oppdaterte dokumenter oversendes Legemiddelverket til vår informasjon.

Med vennlig hilsen  
STATENS LEGEMIDDELVERK

Ingvild Aaløkken  
Leder, seksjon for preklinikk og klinisk utprøving

Maria Almløf  
Forsker

Kopi: REK-Sør-Øst



**KOFFEIN - KLINISK UTPRØVING - EUDRACT NR. 2010-022582-95**

Jeg vil personlig takke Legemiddelverket for en grundig og imøtekommende prosess i utarbeidingen av søknad i studiet "Coffein and X-country polling performance".

De siste tilbakemeldingene er nå blitt utbedret som følgende:

**Protokoll**

- I. Datoen er nå den same mellom Protokoll og EudraCT-skjema.
- II. Hans Kristian Stadheim har nå signert protokollen.
- III. EudraCT-skjema og Protokoll er harmonisert og inneholder nå informasjon om at kun menn skal inkluderes i studien.

**Pasientinformasjon datert 2010-11-2**

Informasjonen om forsikring er nå korrekt, og forandret til "Du er forsikret i henhold til Lov om produktansvar i Legemiddelforsikringen".

**Legemiddelmerking**

Etiketten er nå utbedret med id-nummer som identifiserer produksjonsprosessen. Produktet skal oppbevares kun på testlokalet, men har uansett fått påført "Oppbevares utilgjengelig for barn" (vedlegg 2 i protokoll).

Alle nye forandringer er markert med **grønn** skrift.

*Norges Idrettshøgskole*

*Oslo, Norge 8.11.2010*

*Mvh. Hans Kristian Stadheim*

*Hans Kristian Stadheim*

.....

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<b>Region:</b> REK sør-øst	<b>Saksbehandler:</b> Tone Gangnæs	<b>Telefon:</b> 22845520	<b>Vår dato:</b> 10.11.2011	<b>Vår referanse:</b> 2011/1683/REK sør-øst B
			<b>Deres dato:</b> 28.10.2011	<b>Deres referanse:</b>

Vår referanse må oppgis ved alle henvendelser

Hans Kristian Stadheim  
Norges Idrettshøgskole

### 2011/1683 b Koffein inntak og prestasjonsevne i langrenn

Vi viser til mottatt tilbakemelding på komiteens utsettende vedtak av 21. oktober 2011. Komiteen har gitt komiteens leder fullmakt til å ta stilling til godkjenning av prosjektet etter mottatt svar.

Forskningsansvarlig: Norges Idrettshøgskole  
Prosjektleder: Hans Kristian Stadheim

Komiteens leder finner at tilbakemeldingen som er gitt på komiteens merknader til risiko, beredskap og revisjon av informasjonsskrivet er tilfredsstillende besvart og at prosjektet således kan godkjennes. Det tas til orientering at søknaden ikke behøver forelegges Statens Legemiddelverk.

Komiteen godkjenner at prosjektet gjennomføres i samsvar med det som framgår av søknaden. Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden og med etterfølgende dokumentasjon datert 26.10.2011 og i samsvar med de bestemmelser som følger av helseforskningsloven med forskrifter.

Dersom det skal gjøres flere endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kapittel 2, og Helsedirektoratets veileder for «Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse og omsorgssektoren». Personidentifiserbare data slettes straks det ikke lenger er behov for dem og senest ved prosjektets avslutning.

Godkjenningen gjelder til 01.06.2012. Prosjektet skal sende sluttmelding på eget skjema, se helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Vi ber om at alle henvendelser sendes inn via vår saksportal: <http://helseforskning.etikkom.no> eller på e-post til: [post@helseforskning.etikkom.no](mailto:post@helseforskning.etikkom.no)

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Stein Opjordsmoen Ilnes (sign.)

---

**Besøksadresse:**  
Gullhaug torg 4 A,  
Nydalen, 0484 Oslo

**Telefon:** 22845511  
**E-post:**  
[post@helseforskning.etikkom.no](mailto:post@helseforskning.etikkom.no)

**Web:**  
[www.helseforskning.etikkom.no](http://www.helseforskning.etikkom.no)

All post og e-post som inngår i saksbehandlingen, bes adressert til REK sør-øst og ikke til enkelte personer

Kindly address all mail and e-mails to the Regional Ethics Committee, REK sør-øst, not to individual staff

Professor  
Komiteleder

Tone Gangnæs  
Seniorrådgiver

**Kopi til:** [jorgen.jensen@nih.no](mailto:jorgen.jensen@nih.no)

---

<b>Region:</b> REK sør-øst	<b>Saksbehandler:</b> Tone Gangnæs	<b>Telefon:</b> 22845520	<b>Vår dato:</b> 28.06.2012	<b>Vår referanse:</b> 2012/964/REK sør-øst A
			<b>Deres dato:</b> 22.05.2012	<b>Deres referanse:</b>

Vår referanse må oppgis ved alle henvendelser

Jørgen Jensen  
Norges idrettshøgskole  
Sognsveien 220  
P.O. Box 4014 Ullevål Stadion  
0806 Oslo

### **2012/964 A Effekt av koffein på prestasjon ved akutt hypoksi**

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk i møtet 14.06.2012.

**Forskningsansvarlig:** Norges idrettshøgskole  
**Prosjektleder:** Jørgen Jensen

#### **Prosjektomtale**

*Studien er en masterstudie hvor formålet er å studere i hvilken grad koffein har påvirkning på prestasjon i forbindelse med høyde. Prosjektet vil studere om inntak av koffein kan øke prestasjon ved akutt hypoksi og hvilke fysiologiske parametre som blir påvirket av inntatt koffein i forbindelse med akutt hypoksi. Deltakerne skal innta enten koffein tilsvarende fire kopper kaffe eller placebo, og så arbeide ved 85 % av maks. inntil utmattelse. Det vil bli benyttet lavtrykkstank for å stimulere høyde. Antall deltakere vil være 15-20 personer som rekrutteres blant personer som driver utholdenhetsidrett og studenter ved Norges idrettshøgskole. Deltakelse bygger på informert samtykke.*

#### **Komiteens vurdering**

Komiteen finner at formålet med prosjektet er å øke prestasjonsevnen hos friske veltrente personer og at det er idrettsforskning som kan gjennomføres uten godkjenning fra REK.

#### **Vedtak**

Etter søknaden fremstår prosjektet som idrettsforskning og ikke som et medisinsk og helsefaglig forskningsprosjekt. Det faller derfor utenfor helseforskningslovens virkeområde, jf. helseforskningsloven § 2 og kan gjennomføres uten godkjenning fra REK.

Vi gjør oppmerksom på at den forskningsansvarlige institusjon er ansvarlig for at personopplysningene behandles forsvarlig og lovlig i henhold til personopplysningsloven og personopplysningsforskriftens bestemmelser, og må vurdere om prosjektet må forelegges lokalt personvernombud.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jf. helseforskningsloven § 10, 3 ledd og forvaltningsloven § 28. En eventuell klage sendes til REK Sørøst A. Klagefristen er tre uker fra mottak av dette brevet, jf. forvaltningsloven § 29.

Vi ber om at alle henvendelser sendes inn via vår saksportal: <http://helseforskning.etikkom.no> eller på e-post til: [post@helseforskning.etikkom.no](mailto:post@helseforskning.etikkom.no).

---

**Besøksadresse:**

Gullhaug torg 4A, Nydalen,  
0484 Oslo

**Telefon:** 22845511

**E-post:** [post@helseforskning.etikkom.no](mailto:post@helseforskning.etikkom.no)  
**Web:** <http://helseforskning.etikkom.no/>

All post og e-post som inngår i saksbehandlingen, bes adressert til REK sør-øst og ikke til enkelte personer

Kindly address all mail and e-mails to the Regional Ethics Committee, REK sør-øst, not to individual staff

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Gunnar Nicolaysen  
professor dr. med.  
komiteleder

Tone Gangnæs  
seniorrådgiver

**Kopi til:**      Forskningsansvarlig ved kontaktperson: [turid.sjostedt@nih.no](mailto:turid.sjostedt@nih.no)

---

<b>Region:</b> REK sør-øst	<b>Saksbehandler:</b> Tone Gangnæs	<b>Telefon:</b> 22845520	<b>Vår dato:</b> 02.02.2012	<b>Vår referanse:</b> 2011/2454/REK sør-øst B
			<b>Deres dato:</b> 13.12.2011	<b>Deres referanse:</b>

Vår referanse må oppgis ved alle henvendelser

Hans Kristian Stadheim  
Norges Idrettshøgskole  
Grefsenveien 51  
0485 Oslo

### **2011/2454 B Effekten av koffein på maksimalt oksygenopptak**

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk i møtet 11.01.2012.

**Forskningsansvarlig:** Norges Idrettshøgskole  
**Prosjektleder:** Hans Kristian Stadheim

#### **Prosjektomtale (revidert av REK):**

*Formålet med studien er å teste effekten av koffein på VO<sub>2</sub>maks (maksimalt oksygenopptak). Det skal inkluderes 20 deltakere i studien. Forsøkspersoner vil være elever ved Norges Idrettshøgskole og personer som driver med utholdenhetsidrett, og som skal innta enten koffein eller placebo for å se hvordan dette påvirker deres oksygenopptak. I tillegg til fysiske tester skal deltakerne besvare et spørreskjema og det skal tas blodprøver til analyse. Deltakelse er basert på informert samtykke.*

#### **Komiteens vurdering**

Slik prosjektet er beskrevet i søknad og forskningsprotokoll, så har komiteen ingen innvendinger til at prosjektet gjennomføres.

#### **Vedtak**

Komiteen godkjenner at prosjektet gjennomføres, jf helseforskningsloven § 10.

Godkjenningen er gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden, og i samsvar med de bestemmelser som følger av helseforskningsloven med forskrift.

Godkjenning gjelder til 01.01.2014.

Forskningsprosjektets data skal oppbevares forsvarlig, se personopplysningsforskriften kap 2 og Helsedirektoratets veileder for "Personvern og informasjonssikkerhet i forskningsprosjekter innenfor helse- og omsorgssektoren".

Opplysningene skal ikke oppbevares lenger enn det som er nødvendig for å gjennomføre prosjektet, deretter skal opplysningene anonymiseres eller slettes.

Dersom det skal gjøres endringer i prosjektet i forhold til de opplysninger som er gitt i søknaden, må prosjektleder sende endringsmelding til REK.

---

**Besøksadresse:**  
Gullhaug torg 4 A,  
Nydalens, 0484 Oslo

**Telefon:** 22845511  
**E-post:**  
post@helseforskning.etikkom.no

**Web:**  
www.etikkom.no

All post og e-post som inngår i saksbehandlingen, bes adressert til REK sør-øst og ikke til enkelte personer

Kindly address all mail and e-mails to the Regional Ethics Committee, REK sør-øst, not to individual staff

Prosjektet skal sende sluttmelding på eget skjema, senest et halvt år etter prosjektslutt, jf helseforskningsloven §12.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komite for medisin og helsefag, jf helseforskningsloven § 10, 3. ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør øst B. Klagefristen er tre uker fra mottak av dette brevet, jf forvaltningsloven § 29.

Vi ber om at alle henvendelser sendes inn via vår saksportal: <http://helseforskning.etikkom.no> eller på e-post til: [post@helseforskning.etikkom.no](mailto:post@helseforskning.etikkom.no).

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Stein Opjordsmoen Ilnes (sign.)  
professor dr. med.  
komiteleder

Tone Gangnæs  
seniorrådgiver

**Kopi til:** Norges Idrettshøgskole: [jorgen.jensen@nih.no](mailto:jorgen.jensen@nih.no), [jorgen.jensen@nih.no](mailto:jorgen.jensen@nih.no)



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<b>Region:</b> REK sør-øst	<b>Saksbehandler:</b> Jakob Elster	<b>Telefon:</b> 22845530	<b>Vår dato:</b> 18.09.2013	<b>Vår referanse:</b> 2013/1243 REK sør-øst B
			<b>Deres dato:</b> 25.06.2013	<b>Deres referanse:</b>

Vår referanse må oppgis ved alle henvendelser

Til Hans Kristian Stadheim

### **2013/1243 Effekt av koffein på kraftutvikling som følge av et langvarig fysisk arbeid**

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 23.08.2013. Vurderingen er gjort med hjemmel i helseforskningsloven § 10, jf. forskningsetikklovens § 4.

**Forskningsansvarlig:** Norges Idrettshøgskole  
**Prosjektleder:** Hans Kristian Stadheim

#### **Prosjektomtale**

*Prosjektet skal studere effekten av koffein på idrettslig prestasjonsevne. Bakgrunnen for studien er at til tross for at flere studier har vist at koffein har en positiv effekt på prestasjonsevnen, er det fortsatt usikkert hva årsakene til denne effekten er. Det virker å være en sammenheng at etter et koffein inntak på mellom 3-6 mg x kg-1 kroppsvekt kan utøvere klare å holde en høyere arbeidsintensitet. Nyere studier har også observert at koffein kan forbedre både rekruttering av motoriske enheter og kraftutvikling under fysisk aktivitet. Skulle dette være tilfellet ville den forbedrede kontraktiliteten i de arbeidene musklene kunne forklare en av grunnene til hvordan prestasjonsevnen er forbedret som følge av koffein inntak. På bakgrunn av dette ønsker studien å studere: Kan inntak av koffein (4 mg x kg-1) vedlikeholde/øke kraftutviklingen i benene, pre og post et submaksimalt arbeid tilsvarende 75% av VO2max løp som varer i en time.*

*Studien er en randomisert, cross-over design-studie, der forskningsdeltakerne får enten koffein eller placebo i forkant av en prestasjonstest. 15 forskningsdeltakere skal inkluderes i studien.*

#### **Komiteens vurdering**

Slik komiteen forstår prosjektets formål, er det å studere hvordan koffein kan påvirke idrettslig prestasjonsevne. Komiteen anser at dette ikke dreier seg om forskning som har som formål å fremskaffe ny kunnskap om helse og sykdom. Prosjektet faller dermed utenfor helseforskningslovens virkeområde. For å gjennomføre prosjekter av denne typen, trenges det ingen særskilt godkjenning fra REK.

Komiteen er klar over at lignende prosjekter har blitt vurdert til å falle innenfor helseforskningslovens virkeområde. Komiteen anser imidlertid at det er gode grunner for å endre praksis på dette området.

#### **Vedtak**

Prosjektet faller utenfor komiteens mandat, jf. helseforskningslovens § 2.

#### **Klageadgang**

Du kan klage på komiteens vedtak, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst B. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst B, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Komiteens avgjørelse var enstemmig.

Vi ber om at alle henvendelser sendes inn med korrekt skjema via vår saksportal:  
<http://helseforskning.etikkom.no>. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post  
til: [post@helseforskning.etikkom.no](mailto:post@helseforskning.etikkom.no).

Med vennlig hilsen

Grete Dyb  
førsteamanuensis dr. med.  
leder REK sør-øst B

Jakob Elster  
Seniorrådgiver

**Kopi til:** *Professor Jørgen Jensen, Norges Idrettshøgskole*



# **APPENDIX IV**

*Caffeine information sheet, Health form and  
Questionnaires used during testing*



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## SAMMENDRAG AV FARMASØYTISK OG KJEMISK DOKUMENTASJON

### UTPRØVINGSPREPARAT

Navn: Coffeinum 0,15 (MM)  
Kodenavn: Anr. 09L046/2  
Legemiddelform og styrke(r): 3 mg koffein/ml.  
Virkestoff: koffein

### PLACEBO-PREPARAT

Navn: Funlight Granateple  
Legemiddelform: Vanlig saft

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#### S.1 GENERELL INFORMASJON:

Kjemisk navn: Koffein (0,15 MM)  
Strukturformel:  $C_8H_{10}N_4O_2$

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#### S.2 TILVIRKNING:

**Produksjon av koffeindrikke og placebo:**  
Norges Idrettshøgskole Sognsveien 220 Oslo

**Koffein:**  
Apotekerproduksjon AS, Karihaugveien 22, 1086 Oslo  
Tilvirkingskode: Tilvirker id: 9109 – Tillatelseskode: 10/11925-1

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#### S.3 KARAKTERISERING:

Fra produsent: Apotekerproduksjon Produksjon AS 22, 1086 Oslo  
**Kvalitet/Kodex: Ph.Eur**

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#### S.4 KONTROLL AV VIRKESTOFF:

Apotekerproduksjon Produksjon AS 22, 1086 Oslo  
**Kvalitet/Kodex: Ph.Eur**

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#### S.5 REFERANSEMATERIALE ELLER STANDARDER:

Apotekerproduksjon Produksjon AS 22, 1086 Oslo  
**Kvalitet/Kodex: Ph.Eur**

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**S.6 EMBALLASJE:**

Plastbeholder 250 ml

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**S.7 STABILITET:**

Holdbarhetsdata/diskusjon: Retest dato 05-2013

Forslag til holdbarhetstid og oppbevaringsbetingelser: Retestdato 05-2013

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**P.1 BESKRIVELSE OG SAMMENSETNING AV PREPARAT:**

Styrke			Referanse/ standard
Substansnavn	Mengde	Mengde	
Koffein	4 mg/kg kroppsvekt		
Funlight Granateple	2 ml/kg kroppsvekt		
<b>Total mengde: 140- 200ml</b>			

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**P.2 FARMASØYTISK UTVIKLING**

*IKKE AKTUELT- FERDIGPRODUSERT*

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**P.4 KONTROLL AV HJELPESTOFFER**

Ikke aktuell. Fun Light fra butikken.

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**P.5 KONTROLL AV PREPARAT**

Frigis på bakgrunn av arbeidsseddel.

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**P.6 REFERANSEMATERIALE ELLER STANDARDER:**

Ikke aktuell. Fun Light fra butikken.

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**P.7 EMBALLASJE**

Emballasjetype: Plastflasker

Pakningsstørrelse(r): 250 ml flasker

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**P.8      HOLDBARHET OG OPPBEVARINGSBETINGELSER**

Holdbarhetsdata/diskusjon

Holdbarhet: 7 dager etter produksjon  
Oppbevaring: Kjølig/romtemperatur.

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**A.2      PREPARATETS SIKKERHET MED HENSYN PÅ SMITTESTOFFER**

Risikovurdering, Ansees som liten

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**SAMMENLIGNINGSPREPARAT / PLACEBO-PREPARAT**

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**SAMMENSETNING:**

<b>Styrke</b>			<b>Referanse/ standard</b>
<b>Substansnavn</b>	<b>Mengde</b>	<b>Mengde</b>	
Funlight Granateple	2 ml x kg <sup>-1</sup> kroppsvekt		
<b>Total mengde fra : 140-200ml</b>			

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**TILVIRKNING**

Fun Light fra butikken.  
Prosessvalidering/prosesskontroller:  
Visuell kontroll av løsning

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**SPESIFIKASJONER**

Funlight Granateple, Produsert av Stabburet AS, Postboks 711, 1411 Kolbotn



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**EMBALLASJE:**

Plastbeholder

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**HOLDBARHET OG OPPBEVARINGSBETINGELSER:**

Forslag til holdbarhetstid og oppbevaringsbetingelser

Dato: 3 måneder etter produksjon.

Oppbevaring: Romtemperatur

---

**UNDERSKRIFT:**

Dateres og underskrives av den(de) som er ansvarlig for dokumentets innhold

Punkt s3-s5 er bekreftet av Apotekerproduksjon AS

Punkt P1-P8, A2 og punkt under placeboproduksjon er bekretet av Martin Berg, ved Ullevål  
Universitetssykehus Apotkerproduksjon.

*Hans Kristian Stadheim* - 18.06.2013

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**KOMMENTARER OG KONKLUSJON**

# SIKKERHETSDATABLAD

## KOFFEIN

### 1. Identifikasjon av stoffet / produktet og av selskapet / foretaket

Utgitt dato	01.06.2007
Revisjon	20.05.2010
Kjemikaliets navn	KOFFEIN
Kjemisk navn	Coffeinum
Artikkelnr.	310872,262873,305680,305664
CAS-nr.	58-08-2
Formel	C8H10N4O2
Kjemikaliets bruksområde	Legemiddel: ATC-kode: N06B: psykostimulantia.

### Produsent

Firmanavn	APOTEKPRODUKSJON AS
Postadresse	Karihaugveien 22
Postnr.	1086
Poststed	OSLO
Land	Norge
Telefon	21 60 87 00
Telefaks	21 60 87 10
E-post	tln@apotekproduksjon.no

### 2. Fareidentifikasjon

Klassifisering	Xn; R22
Klassifisering CLP	Acute tox. 4; H301
Farebeskrivelse	Farlig ved svelging

### 3. Sammensetning /opplysning om innholdsstoffer

Komponentnavn	Identifikasjon	Klassifisering	Innhold
koffein	CAS-nr.: 58-08-2	Xn; R22	100
Kolonneforklaring	CAS-nr. = Chemical Abstracts Service; EU (Einecs- eller Elincnummer) = European inventory of Existing Commercial Chemical Substances; Ingrediensnavn = Navn iflg. stoffliste (stoffer som ikke står i stofflisten må oversettes hvis mulig). Innhold oppgitt i; %, %vkt/vkt, %vol/vkt, %vol/vol, mg/m3, ppb, ppm, vekt%, vol%		
FH/FB/FM	T+ = Meget giftig, T = Giftig, C = Etsende, Xn = Helseskadelig, Xi = Irriterende, E = Eksplosiv, O = Oksiderende, F+ = Ekstremt brannfarlig, F = Meget brannfarlig, N = Miljøskadelig.		
Komponentkommentarer	Inneholder 100% koffein. En kopp kaffe inneholder 100 - 150 milligram koffein.		

### 4. Førstehjelpstiltak

Generelt	Den skadede bringes bort fra eksponeringskilden, og det gis vanlig førstehjelp. Ved mistanke om alvorlig skade og/eller forgiftning: Transport til legehjelp snarest.
Hudkontakt	Vask de tilsølte områder godt med såpe og vann.
Øyekontakt	Skyll straks med store mengder vann og kontakt lege. Øynene skylles i minst 15 minutter, - også under evt. transport til lege / sykehus.
Svelging	Gi straks rikelig drikke og fremkall brekninger. Gi aktivt kull. Kontakt lege.

Informasjon til helsepersonell	Toksisk nivå: Plasmakonsentrasjon > 15 milligram /L Dødelig nivå: Plasmakonsentrasjon > 80 milligram /L
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## 5. Tiltak ved brannslukning

Brann- og eksplosjonsfarer	Koffein er brennbart, men ikke brannfarlig. Støveksplasjon kan forekomme.
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## 6. Tiltak ved utilsiktet utslipp

Metoder til opprydding og rengjøring	Destrueres ved brenning.
--------------------------------------	--------------------------

## 7. Håndtering og lagring

Oppbevaring	Oppbevares i godt lukket beholder.
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## 8. Eksponeringskontroll / personlig verneutstyr

### Eksponeringskontroll

Begrensning av eksponering på arbeidsplassen	Sørg for god ventilasjon, vann lett tilgjengelig (nøddusj) og mulighet for øyespyling.
Åndedrettsvern	Unngå innånding av støv. Ved høye støvkonsentrasjoner: Maske med støvfilter P2.
Håndvern	Vurder bruk av hansker ved arbeid med større mengder
Øyevern	Ved risiko for direkte kontakt eller sprut skal ansiktsbeskyttelse benyttes.

## 9. Fysiske og kjemiske egenskaper

Tilstandsform	Føreligger som 0,15mm krystallinsk pulver og som granulat.
Farge	Hvitt.
Smeltepunkt/smeltepunktintervall	234-239 °C
pH (bruksløsning)	pH = ca 6 i 1% løsning

### Andre fysiske og kjemiske egenskaper

Fysiske og kjemiske egenskaper	Løslighet: 1:60 i vann, 1:1 i kokende vann. 1:130 i etanol, 1:7 i kloroform. Løselig i fortynnede syrer. Molvekt: 194,2 Sublimerer ved: ca 180 °C
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## 10. Stabilitet og reaktivitet

Forhold som skal unngås	Ingen særlige forholdsregler.
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## 11. Toksikologisk informasjon

### Toksikologisk informasjon

Oral toksisitet	LD50 rotte: 192 mg/kg
Andre toksikologiske data	LDLo, menneske, peroralt: 0,2 - 1,0 gram / kg kroppsvekt

### Øvrige helsefareopplysninger

Generelt	Det er individuelle forskjeller i følsomhet for koffein.
Innånding	Innånding av større mengder støv vil gi slimhinneirritasjon i nese og svelg.
Øyekontakt	Irritasjon.
Svelging	Mindre mengder virker stimulerende på aktivitet i hjerne, hjerte, muskler, lever og nyrer. Større mengder kan gi hodepine, kvalme, irritasjon av mageslimhinnen, og søvnproblemer. Svelging av 1 gram vil videre forårsake rastløshet, uro, oppstemthet, muskelskjelvinger, øresus, hetetokter, hjertebank, hjerterytmeforstyrrelser, rask, overflattisk pust, kramper.

Dødelig dose: mellom 5 og 50 gram koffein.

## 12. Miljøopplysninger

### Øvrige miljøopplysninger

Persistens og nedbrytbarhet Koffein er biologisk nedbrytbart.

## 13. Fjerning av kjemikalieavfall

Avfallskode EAL 7153 Medisnavfall  
Egnede metoder til fjerning av kjemikallet Destrueres ved forbrenning.

## 14. Transportinformasjon

Andre relevante opplysninger Klassifiseres ikke som farlig gods.

## 15. Opplysninger om lover og forskrifter

### Faresymbol



Helseskadelig

R-setninger R22 - Farlig ved svelging.  
S-setninger S-2 Oppbevares utilgjengelig for barn.

### FAREPIKTOGRAMMER (CLP)



Signalord Fare  
Faresetninger H301 Giftig ved svelging.  
Sikkerhetssetninger P309 + P311 Ved eksponering eller ubehag: Kontakt GIFTINFORMASJONEN eller lege.  
Referanser (Lover/Forskrifter) Lov om legemidler m. v. av 4. desember 1992 med tilhørende forskrifter.

## 16. Andre opplysninger

Liste over relevante R-setninger (i seksjon 2 og 3). R22 Farlig ved svelging.  
Ansvarlig for Sikkerhetsdatablad APOTEKPRODUKSJON AS

Etternavn:	Fornavn:	Født:
Studentadresse:		
Hjemmeadresse:		
Tlf.: E-mailadresse:		
Idrettsbakgrunn (angi idrettsgrener og omtrent hvor mange timer du trener pr. uke):		

### **EGENERKLÆRING FOR FORSØKSPERSONER**

Takk for at du vurderer å delta som forsøksperson ved Norges idrettshøgskole! Før du kan delta, må vi imidlertid kartlegge om din deltakelse kan medføre noen form for helserisiko. Vær snill å lese gjennom alle spørsmålene nøye og svar ærlig ved å krysse av for JA eller NEI. Hvis du er i tvil, bør du be om å få snakke med legen som er ansvarlig for forsøket.

Hvis du krysser av for JA på ett eller flere av disse spørsmålene, må du gjennomgå en legeundersøkelse før forsøksstart. Ved enkelte typer forsøk vil du uansett bli innkalt til legeundersøkelse.

Spørsmål	Ja	Nei
1. Kjenner du til at du har en hjertesykdom? 2. Hender det du får brystmerter i hvile eller i forbindelse med fysisk aktivitet? 3. Kjenner du til at du har høyt blodtrykk? 4. Bruker du for tiden medisiner for høyt blodtrykk eller hjertesykdom (f.eks. vandrivende tabletter)? 5. Har noen av dine foreldre, søsken eller barn fått hjerteinfarkt eller dødd plutselig (før fylte 55 år for menn og 65 for kvinner)? 6. Røyker du? 8. Har du besvimt i løpet av de siste 6 måneder? 9. Hender det du mister balansen på grunn av svimmelhet? 10. Har du sukkersyke (diabetes)? 11. Kjenner du til <u>noen annen grunn</u> til at din deltakelse i prosjektet kan medføre helse- eller skaderisiko? 12. Har du opplev ubehag som: Hjertebank, kvalme, urolig mage eller magesmerter ved inntak av kaffe, cola eller redbull? 13. Har du magesår? 14. Har du tyreotoksikose?		

Gi beskjed straks dersom din helsesituasjon forandrer seg fra nå og til undersøkelsen er ferdig, f.eks. ved at du blir forkjølet, får feber, eller blir gravid.

\_\_\_\_\_

Sted – dato

\_\_\_\_\_

Underskrift



# Spørreskjema

(Du setter et X ved ditt svar )

1. Hvilket produkt tror du at du har fått i dag ?

Koffein

Usikker

Placebo

2. Om du har svart koffein eller placebo, hvorfor tror du dette, og hvor sikker er du 0-100% ?

.....

3. Hvordan er din dagform (tall) og motivasjon (tall) for å prestere i dag (se skala side 2) ?

Dagsform:.....Motivasjon:.....

4. Hvor godt har du sovet i natt (se linje og sett **X**), samt svar på spørsmål under:

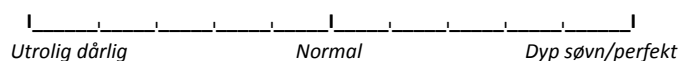
Når la du deg for å sove i går kveld **Kl:** .....

Hvor lenge ca tok det før du sovnet ..... timer.....minutt

Hvor mange ganger våknet du **Antall:** .....

Hvor lenge sov du til / når stod du opp ca **KL:** .....

Kvalitet på søvn:





## Dagsform og motivasjon

100	Perfekt
95	Svært bra
90	Svært bra
85	Veldig bra
80	Veldig bra
75	Veldig bra
70	Bra
65	Bra
60	Bra
55	Moderat
50	Moderat
45	Moderat
40	Dårlig
35	Dårlig
30	Dårlig
25	Veldig Dårlig
20	Veldig Dårlig
15	Veldig Dårlig
10	Forferdelig
5	Forferdelig
0	Forferdelig

**OBSS!!! FYLLES UT ETTER GJENNOMFØRT TEST**

5. Følte du noe uvanlig ubehag under testen ?      **Ja**       **Nei**

Svarer du nei her gå til spm nr 9.

6. Om **ja**, noen av følgende ubehag/smerte?

**Hodepine**       **Magesmerter**       **Rastløshet**       **Kvalme**   
**Hjertesmerter**       **Kramper**       **Synsforvrringer**

**Annet:**.....  
.....  
.....

7. Har du hatt noen av disse ubehagene/smertene tidligere ved fysiske anstrengelser?

**Ja**       **Nei**

8. Om **ja**, hvilken av de ovenfor?

.....  
.....

**\*OBS: Spm 9-13 skal ikke fylles ut etter første prestasjonstest, kun resterende 3 tester.**

9. Har du følt noe ubehag/smerte etter du kom hjem etter første prestasjonstest?

**Ja**       **Nei**

Svarer du nei her gå til spm nr 14.

10. Om **ja**, noen av følgende ubehag/smerte?

**Hodepine**       **Magesmerter**       **Rastløshet**       **Kvalme**   
**Hjertesmerter**       **Kramper**       **Synsforvrringer**

**Annet:**.....  
.....  
.....

11. Har du hatt noen av disse ubehagene/smertene tidligere etter en fysisk anstrengelse?

**Ja**       **Nei**

12. Om **ja**, hvilken av de ovenfor?

.....  
.....  
.....

13. Skulle det være noe annet du ønsker å utdype/bemerke av smerte/ubehag fyll ut dette her:

.....  
.....  
.....

14. Hvilket produkt tror du at du har fått ?

Koffein

Usikker

Placebo

15. Om du har svart koffein eller placebo, hvorfor tror du dette og hvor sikker er du 0-100% ?

.....

16. Hvordan var din dagsform og motivasjon for å prestere i dag (se skala) ?

Dagsform:.....Motivasjon:.....

### Dagsform og motivasjon

100	Perfekt
95	Svært bra
90	Svært bra
85	Veldig bra
80	Veldig bra
75	Veldig bra
70	Bra
65	Bra
60	Bra
55	Moderat
50	Moderat
45	Moderat
40	Dårlig
35	Dårlig
30	Dårlig
25	Veldig Dårlig
20	Veldig Dårlig
15	Veldig Dårlig
10	Forferdelig
5	Forferdelig
0	Forferdelig

## Ærneringskjema

Hva har du spist de 24 timene ?

Matttype (navn)	Mengde (stk/gram)	Når (tt:min)	Bemerkning
<i>Eksempel: Frokost 3 brødskiver med hvit ost og smør</i>	<i>3 stk</i>	<i>8:00</i>	<i>X</i>

Væske inntak siste 24 timer ?

Væsketype (navn)	Mengde (Dl/glass/kopp)	Når (tt:min)	Bemerkning
<i>Eksempel: Melk</i>	<i>2 glass</i>	<i>8:00</i>	<i>Frokost</i>

Trening siste 48 timene

Treningstype (navn)	Tid (tt:mm)	Når (tt:min)	Bevegelsesform/dato







