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Press

Institute of Sport Sciences and Physiotherapy, Faculty of Medicine, University of Tartu, Tartu, Estonia

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In Papers I, II and III Silva Suvi had primary responsibility for study design development, recruiting the participants, conducting measurements, data analysis, and writing the manuscripts.

LIST OF ABBREVIATIONS

ALDO	aldosterone
BM	body mass
CAF	caffeine
CFF	critical flicker frequency
CIT	sodium citrate
CNS	central nervous system
CORT	cortisol
DE	dehydrating cycling exercise
GH	growth hormone
HR	heart rate
$NaHCO_3$	sodium bicarbonate
PLC	placebo
PRL	prolactin
PV	plasma volume
RH	relative humidity
RPE	ratings of perceived exertion
RPF	ratings of perceived fatigue
SOsm	serum osmolality
subjCAF	confidence percentage of receiving caffeine
T_b	mean body temperature
T_c	core body temperature
T_{gr}	temperature gradient
TS	thermal sensation
T_{sk}	mean skin temperature
TT	time-trial
UOsm	urine osmolality
USG	urine specific gravity
VAS	visual analog scale
VAS_A	visual analog scale for perceived arousal
VAS_M	visual analog scale for mood
VO_2max	maximal oxygen uptake
VO_2peak	peak oxygen uptake

1. INTRODUCTION

Endurance and ultra-endurance sports are becoming more and more popular, which are reflected by the increasing number of participants in different sporting events every year. People run marathons, ultramarathons, do triathlons and 100 km paddling marathons. At the same time different level athletes are looking for ways to optimize their performance by training and nutrition (Jeukendrup, 2011). In addition to athletes, military personnel are also trying to find ways to train or operate better on missions. However, there is discrepancy between the term „endurance ability“ in the sport and military contexts. In sports it is important to cover a certain distance with maximum speed or to perform as much work as possible in a certain time. In military context, it is more important to work with constant intensity for a very long time, often even until exhaustion and simultaneously dealing with sleep depriviation. These aspects of endurance ability could be affected by various dietary supplements.

Caffeine is probably the most popular legal work-enhancing supplement on the market, but until 2004 it was prohibited by the rules of WADA (Burke et al., 2006). Caffeine in its different forms is nowadays again widely used by endurance athletes and military personnel, because of its ability to enhance performance (Goldstein et al., 2010) and decrease somnolence (Adan et al., 2008). Different aspects of caffeine intake have been extensively studied, however, mainly in temperate environmental conditions. There is still lack of data on the ergogenic effect of caffeine in high-temperature environment.

Sodium citrate as a dietary supplement is not equally well known with caffeine, but it has some specific properties that could make it useful in sports. For example, sodium citrate increases plasma volume (Timpmann et al., 2012) and extracellular buffering capacity (Requena et al., 2005). The latter feature is more important in high-intensity activities, where large acid-balance disturbances occur. However, plasma volume is an important parameter reflecting the water status of the body. Dehydration is known to impair endurance performance (Cheuvront et al., 2010; Cheuvront and Kenefick, 2014) and should therefore be avoided, although during long-duration competitions or tours it is often impossible. Because of this, more attention should be paid to effective rehydration supporting recovery of plasma volume between training sessions or competition stages. Numerous studies about ingestion of sodium citrate have shown ergogenic effects mostly on short-term high-intensity exercises in temperate environment (Burke et al., 2006; Carr et al., 2011). Similarly to caffeine, there is lack of data on the ergogenic effect of sodium citrate in high-temperature environment.

In conlusion, there is abundant information about dietary supplements, especially about caffeine, but obvious deficit of studies conducted in high-temperature environment. Knowing that remarkable decrements in performance may occur in warm compared with temperate environment (Tatterson et al., 2000), some dietary supplements could also affect the organism differently than they do in temperate conditions. Therefore, the aim of the current thesis was to

determine the effects of ingesting two dietary supplements, caffeine and sodium citrate, on endurance ability in recreationally active participants performing in the heat. This study will hopefully provide useful advice to athletes, coaches and military personnel who train/compete or operate in warm climates.

2. LITERATURE REVIEW

2.1. Physiological and nutritional determinants of endurance ability

Major physiological determinants of endurance ability are maximal oxygen uptake (VO_2max), lactate threshold and exercise economy (Joyner and Coyle, 2008; Midgley et al., 2007; Pate and Branch, 1992). All these factors can be significantly improved by systematic endurance training, but marked inter-individual variability occurs in the magnitude of improvements. For example, Bouchard et al. (2011) engaged 473 sedentary adults into standardized 22-week continuous endurance training program and reported a mean increase in VO_2max of 0.4 $L \cdot min^{-1}$, whereas in 7% of participants the gain was 0.1 $L \cdot min^{-1}$ or less and 8% of participants showed improvement of 0.7 $L \cdot min^{-1}$ or more. The results of recent meta-analysis reveal that similar mean increase in VO_2max (0.5 $L \cdot min^{-1}$) could be achieved much faster (within 6–13 weeks) if interval training or combined interval and continuous training programs are employed (Bacon et al., 2013).

In previously sedentary participants, relative increases in VO_2max in the range of 15–20% occur over 5.5 months of continuous endurance training (Skinner et al., 2001). An individual's highest attainable VO_2max that may exceed his or her baseline level up to 50% is usually reached within 18–24 months of systematic endurance training (Hoffman, 2002; Wilmore and Costill, 2004). After occurrence of plateau in VO_2max, continuing training still increases lactate threshold and exercise economy. Based on these training adaptations, endurance ability can be markedly improved over many years without further increases in VO_2max. For example, between 1992 and 2003 Paula Radcliffe's VO_2max remained stable at approximately 70 $ml \cdot min^{-1} \cdot kg^{-1}$, but her lactate threshold and running economy increased by 15% and 24%, respectively (Jones, 2006).

In addition to VO_2max, lactate threshold and exercise economy that can be improved through systematic training, there are other factors, including nutrition and hydration, that influence endurance ability in a more acute manner. Modern scientific understanding about nutritional factors influencing endurance ability is largely based on studies of Scandinavian exercise physiologists who introduced needle biopsy method for investigation of the impact of exercise and nutrition on human skeletal muscle structure and function in the 1960s. One of their classical studies convincingly demonstrated strong positive association between food carbohydrate content, muscle glycogen concentration and endurance capacity (Bergström et al., 1967). They also were the first to show that if glucose is infused continuously during endurance exercise, muscle glycogen use is significantly lower if no glucose is administered (Bergström and Hultman, 1967). American researchers demonstrated that oral ingestion of carbohydrates during prolonged exercise could improve endurance capacity (Coyle et al.,

1983; Ivy et al., 1983) and endurance performance (Murray et al., 1989; Neufer et al., 1987). Many studies conducted in the 1990s revealed that peak rates of exogenous carbohydrate oxidation during prolonged exercise are approximately 1g·min^{-1} (for review, see Jeukendrup and Jentjens, 2000). However, Jentjens et al. (2004) demonstrated that combined ingestion of carbohydrates possessing different intestinal transport mechanisms (glucose and fructose) may increase exogenous carbohydrate oxidation rates to the level of approximately 1.3 g·min^{-1}. Later Currell and Jeukendrup (2008) reported a marked performance benefit with ingestion of glucose and fructose compared with glucose alone in cyclists during exercise of approximately 3-h duration.

Carter et al. (2004) were the first to demonstrate that rinsing the mouth with a carbohydrate solution significantly improved performance during approximately 1 h cycling time-trial (TT) even when their endurance-trained participants did not swallow the carbohydrate. Pottier et al. (2010) reported that rinsing the mouth with, but not ingesting the carbohydrate-electrolyte solution (6% carbohydrates), resulted in improved performance during similar cycling TT in trained triathletes. Generally, carbohydrate mouth rinse is considered to improve endurance performance during exercise of moderate (65–75% VO_2 max) to high (>75% VO_2max) intensity and during relatively short durations (∼1 h) (Jeukendrup, 2014; Jeukendrup and Chambers, 2010; Stellingwerff and Cox, 2014), and the positive effect seems to be accentuated when muscle and liver glycogen stores are reduced (Ataide-Silva et al., 2014; Rollo and Williams, 2011). However, recent findings suggest that in high-temperature environments the ergogenic effect of carbohydrate mouth rinse may disappear (Chryssanthopoulos et al., 2018; Watson et al., 2014).

Whole body sweating rates during exercise vary widely and may reach from the common range of 0.5–1.5 L·h^{-1} in temperate environments (Baker et al., 2016) to values as high as 2.5–4 L·h^{-1} in the heat (Baker et al., 2016; Sawka and Montain, 2000; Taylor and Machado-Moreira, 2013). As athletes usually do not drink enough to compensate water loss, they dehydrate during prolonged exercise (Atkinson et al., 2003), while the magnitude of their acute dehydration is reflected in body mass (BM) loss (Baker et al., 2009). Literature suggests that dehydration in the magnitude of ≥ 2% of BM impairs endurance exercise performance (Cheuvront et al., 2010; Cheuvront and Kenefick, 2014). Recent meta-analysis revealed that the ergogenic effect of fluid ingestion during endurance cycling depends on the intensity and duration of exercise (Holland et al., 2017). Compared with no fluid intake, the average performance improvements of 2.1% and 3.2% might occur when fluid is consumed in a volume sufficient to limit the loss of BM below 2% during moderate-intensity cycling exercise of > 1 h to ≤ 2 h and > 2 h duration, respectively. During high-intensity cycling task of ≤ 1 h duration, fluid consumption compared with no fluid intake, despite preventing ≥ 2% loss of BM, decreases performance by 2.5% on average (Holland et al., 2017). However, the meta-analysis of Holland et al. (2017) involved only one study where participants performed 1 h cycling TT in the heat. The authors of this study reported that compared with no fluid intake,

the ingestion of water in volume replacing 100% of sweat losses had no effect, positive or negative, on performance (Kay and Marino, 2003). In previously dehydrated participants, Arnaoutis et al. (2012) demonstrated that ingestion of a small amount of water (25 mL every 5 min), compared with mouth rinse or no fluid manipulation, significantly increased endurance capacity in cycling test at 75% of maximum power output in the heat.

Sports foods and supplements possessing proved property to acutely improve endurance ability are sports drinks, sports gels, sports bars, energy drinks, caffeine (CAF), dietary nitrate, and during ultra-endurance activities, electrolyte replacements (Maughan et al., 2018). Some, but not convincing, evidence suggest that acute ingestion of sodium bicarbonate ($NaHCO_3$), or sodium citrate (CIT) may enhance endurance ability (Carr et al., 2011).

2.2. Impact of high environmental temperature on endurance ability

Remarkable decrements in physical performance may occur in a warm environment compared to temperate climatic conditions (Galloway and Maughan, 1997; Tatterson et al., 2000). In laboratory experiments mostly two different methods are used for evaluating endurance ability: 1) participants work with constant intensity until exhaustion (endurance capacity); 2) participants cover a certain distance as fast as possible or perform as much work as possible in a certain time (endurance performance). Studies using the above methods have demonstrated that heat stress significantly degrades aerobic performance (Galloway and Maughan, 1997; Tatterson et al., 2000). Galloway and Maughan (1997) conducted one of the first studies to examine the effects of temperature on endurance capacity. They used an ambient temperature range of 4–31 °C and demonstrated approximately 42 min shorter time to exhaustion (70% of VO_2max) in the warmest environment compared to the study optimum (11 °C). However, Ely et al. (2007) evaluated the impact of warm weather on endurance performance and found that marathon finishing times progressively worsened with increased wet-bulb globe temperature over a range of 5 °C to 25 °C. This finding seems true for both sexes, but data indicate that slower runners suffered a greater decrement in finishing time than faster runners.

The mechanisms responsible for the reduced endurance ability in the heat are not entirely clear and vary between current situations. Nevertheless, endurance ability could be impaired in the heat because of changes in energy metabolism, cardiovascular function and fluid balance, central nervous system (CNS) function and motor drive (Hargreaves, 2008). Cardiovascular mechanisms were for a long time assumed to be the primary factor impairing submaximal performance in the heat, but later findings (Nielsen et al., 1993; Nybo, 2008; 2012) confirmed the critical core body temperature (T_c) hypothesis according to which the primary mechanism impairing submaximal aerobic performance in

the heat is elevated core temperature that in turn causes hyperthermia. Hyperthermia occurring during exercise has been defined as an elevation of T_c above 38 °C (IUPS Thermal Commission, 1987). This hypothesis has been later marked as questionable and the new standpoint reflects that elevated skin temperature could be responsible for impaired endurance performance in the heat (Cheuvront et al., 2010; Sawka et al., 2012).

The water status of the organism influences skin and core temperature during exercise in the heat and thereby performance. When the ambient temperature is higher than skin temperature, the only mechanism by which heat can be lost from the body is evaporation of water from the skin and respiratory tract (Maughan, 2006). High fluid loss during exercise, which is not replaced, causes dehydration. This in turn reduces stroke volume, cardiac output, blood pressure, muscle blood supply and tolerance of hyperthermia (Nybo, 2008). Fatigue associated with dehydration may induce performance impairment and is most evident during endurance exercise in warm humid conditions where sweat rates are high (Maughan and Shirreffs, 2008). Already *ca* 2% of fluid loss from initial euhydrated body mass impairs endurance performance (Coyle, 2004) and in warm climate even lower dehydration level may impair performance (Bardis, 2013).

The combination of hyperthermia and dehydration is considered to be the main cause of fatigue and impaired performance in high-temperature environment (Hargreaves, 2008). Therefore, the primary goal should be preventing it. Dehydration can be avoided with sufficient drinking regimen and according to Hargreaves, (2008) hyperthermia can be attenuated by using strategies like acclimatization, pre-cooling and fluid ingestion. In addition to these factors, there are some dietary supplements that might help to improve endurance ability. CAF is the world's most widely consumed legal CNS stimulant and meta-analysis conducted by Doherty and Smith (2005) revealed that in temperate environmental conditions, CAF, compared with placebo (PLC), may increase exercise performance by approximately 11% and that an average 5.6% reduction in ratings of perceived exertion (RPE) obtained during exercise may account for approximately 29% of the ergogenic effect of this drug. A concious decision is necessary to start voluntary exercise and although it is not clear what forces the recruitment of motor units, the end of effort is again volitional and a concious decision precedes it (Kayser, 2003). Therefore, the perception of effort is an important factor that limits exercise. Reduction in RPE is considered one of the main CNS related actions of CAF leading to improved endurance in temperate environments (Tarnopolsky, 2008).

In elite cyclists competing in temperate environmental conditions modest BM loss of 1.3% has been observed (Ross et al., 2014), but in warm weather professional cyclists typically lose 2.1–4.5 kg of BM after a stage (Atkinson et al., 2003). Considering an average BM of 69 kg for a cyclist (Mujika and Padilla, 2001), this means dehydration in the range of 3.0–6.5% that may lead to a decrease in plasma volume (PV) up to 16% (Baker et al., 2009; van Rosendal et al., 2012). Decreased PV in turn impairs performance and the expansion of

PV has been suggested to improve thermoregulation and exercise performance in the heat (Sims et al., 2007b). Compared with temperate environmental conditions, muscle (Febbraio, 2001) and blood (Febbraio et al., 1994; McAnulty et al., 2005) lactate accumulation is augmented during exercise in the heat, suggesting more severe acidosis (Robergs et al., 2004). CIT or $NaHCO_3$ are used with intention of increasing extracellular fluid buffering capacity (Requena et al., 2005) that may improve performance in situations where marked disturbances in acid-base balance occur (Ibanez et al., 1995). Therefore, CIT supplementation may improve endurance ability in the heat by increasing both PV and extracellular buffering capacity.

2.3. Impact of caffeine on endurance ability

CAF (1,3,7-trimethylxanthine) is a widely used ergogenic supplement by athletes at different levels of fitness and different fields of sporting disciplines (Del Coso et al., 2011). CAF absorption from the gastrointestinal tract is very quick: elevated levels can appear in bloodstream within 15–45 min after ingestion and peak concentrations appear approximately 1 h post ingestion (Goldstein et al., 2010). Besides timing, CAF's ergogenic effect could be affected by the method of administration (capsule *vs* liquid), dose (3–6 $mg \cdot kg^{-1}$ is optimal), habituation and training status (Graham, 2001).

It is also relevant to note that the physiological and psychological responses to CAF may differ between the sexes (Temple and Ziegler, 2011). CAF-induced increases in subjective activation and decreases in somnolence are greater in men than in women (Adan et al., 2008); adolescent males compared with females feel caffeinated soda to be more reinforcing (Temple et al., 2009) and are more likely to perceive getting a rush, having more energy, or having an improved athletic performance from CAF (Temple et al., 2010). Increased activeness and alertness may provide a psychophysiological advantage in exercise performance (Hull et al., 2003). In addition, CAF has anxiety-related properties (Botella and Parra, 2003), and anxiety sensitivity has been found to moderate a negative psychological response to CAF (Keogh and Chaloner, 2002; Telch et al., 1996), especially in women (Keogh and Birkby, 1999). Goldstein and colleagues (2010) pointed out that research on the ergogenic effects of CAF in women is limited, exceptionally varied and, therefore, additional research is needed to determine if CAF is effective in enhancing performance in females.

CAF enhances performance in a wide range of physical activities, with the ergogenic effect occurring more consistently in endurance tasks than in high-intensity or strength and power exercises (Astorino and Roberson, 2010; Doherty and Smith, 2004; Goldstein et al., 2010). However, current knowledge of CAF's impact on endurance is based mainly on the findings of studies conducted in temperate environments, whereas data on the ergogenic effect of CAF in the heat are scarce and inconsistent.

2.3.1. Mechanism of action

The mechanisms that cause the ergogenic effects of CAF are not fully understood. Nevertheless, strong evidence from animal experiments (Davis et al., 2003) suggests that blockage of the adenosine receptors in the CNS may explain the fatigue-delaying and endurance-enhancing properties of CAF. According to Tarnopolsky, (2008) CAF is likely to improve endurance through CNS-related mechanisms in humans as well.

Adenosine is a neurotransmitter the molecule of which is similar in structure to CAF and has been shown to cause tiredness, enhanced pain perception and reduced arousal (Davis and Green, 2009). Adenosine receptors are found throughout the body and because of their similar chemical structure, CAF can bind adenosine receptors and work against the actions of adenosine (Burke et al., 2013). Adenosine receptors are classified into four: A_1, A_{2A}, A_{2B} and A_3 (George, 2003). The main targets for CAF are A_1 and A_{2A} receptors, which bind CAF already in small amounts. However, A_{2B} receptors respond only to high doses of CAF and A_3 receptors are not affected by CAF at all (Fredholm et al., 1999).

Adenosine inhibits the release of other neurotransmitters, whereas CAF causes elevated levels in them. CAF affects neurotransmitters like norepinephrine, dopamine, serotonin, acetylcholine, glutamate and gamma-aminobutyric acid (Fredholm et al., 1999). For example, the longer time the person is awake, the higher concentrations of adenosine in the brain are found, which in turn causes even greater fatigue (Fredholm et al., 1999). Inversely, CAF is known to have the opposite effect by stimulating alertness and reducing fatigue.

CAFcan pass through the blood-brain barrier and therefore it is very difficult to distinguish whether the performance enhancing effect results from the CNS or muscle level. One possible option is that CAF can affect substrate utilization during exercise and decreases reliance on glycogen (Goldstein et al., 2010). CAF ingestion leads to an increased lipolysis in adipose tissue and therefore increased level of free fatty acids. In turn, free fatty acids are alternative energy sources to spare the limited stores of glycogen in the muscle (Graham, 2001; Tarnopolsky, 1994). If CAF could increase fat utilization during exercise, it would allow glycogen stores to be used at a slower rate and be available at the end of longer exercise tasks (Burke et al., 2013) and thereby endurance ability could be improved.

2.3.2. Endurance performance

World Anti-Doping Agency (WADA) removed CAF from the prohibited list in 2004. Thereafter Del Coso et al. (2011) conducted a study to describe the use of CAF in sports. They found that 74% of elite athletes used CAF as an ergogenic aid during national and international competitions and endurance sports like triathlon, cycling and rowing showed the highest prevalence rate for CAF use.

According to Southward et al. (2018) acute CAF ingestion has a small but significant effect on endurance performance. In their meta-analysis, average TT time was 2.2% faster in CAF compared to PLC trials and mean power output followed the similar pattern. However, there are large inter-individual responses to CAF ingestion.

CAF ingestion shortens the time to finish a certain running (O'Rourke et al., 2008; Wiles et al., 1992) and cycling distances (Astorino et al., 2012; Skinner et al., 2013). Running times in 1500 m on a treadmill improved 4.2 s on average in CAF compared to PLC trial (Wiles et al., 1992). CAF ingestion also significantly improved 5-km running TT around a 400 m athletics track. In comparison to the PLC, the CAF trial resulted in 1.1% and 1.0% faster times for the well-trained and recreational runners, respectively (O'Rourke et al., 2008). Astorino et al. (2012) used 10-km cycling test on veloergometer to evaluate CAF impact on endurance performance on 2 consecutive test days. The results showed that with CAF ingestion the distance was covered 1.6% faster on the first day and 1.9% faster on the second day compared to PLC. Skinner et al. (2013) also found improved cycling performance when CAF was ingested 60 min prior to testing. Their participants cycled 40-km TT 70.5 s faster (2%) in CAF compared to PLC trial.

2.3.3. Endurance capacity

In previous well controlled laboratory studies, cycling TT protocols have been used exclusively (Southward et al., 2018). It is important to note that central fatigue (failure to sustain CNS drive to working muscle) is considered a more critical performance limiting factor in endurance running than in cycling (Millet et al., 2009). However, the vast majority of CAF studies conducted in the heat used cycle ergometer protocols (Cheuvront et al., 2009; Ganio et al., 2011; Pitchford et al., 2014; Roelands et al., 2011). Furthermore, a meta-analysis conducted by Doherty and Smith, (2004) revealed that time to exhaustion protocols are much more likely to elicit a positive effect of CAF on endurance ability than are TT-s or distance trials.

Data of CAF ingestion in the amount of 3–7 $mg \cdot kg^{-1}$ show *ca* 24% improved endurance capacity in time to exhaustion protocols in case of both treadmill and veloergometer trials (Goldstein et al., 2010). For example, CAF ingestion extended time to exhaustion 31% compared to PLC capsules and 22.8% compared to decaffeinated coffee in young fit adults who ran on a treadmill (Graham et al., 1998). However, Hogervorst et al. (2008) found 84% improvement in endurance capacity after CAF ingestion compared to PLC ingestion in cycling test until exhaustion.

2.3.4. Endurance ability in the heat

Although the ergogenic effect of CAF in thermoneutral environmental conditions has generally shown performance benefits, its efficacy in warm conditions is not so well known and results are controversial. Many researchers like Cohen et al. (1996), Cheuvront et al. (2009), and Roelands et al. (2011) observed no benefits of acute CAF ingestion on TT performance in the heat, but Ganio et al. (2011) found CAF to be equally ergogenic in both cool and warm environments. However, Pitchford et al. (2014) reported a worthwhile improvement in performance in the heat after CAF compared with PLC ingestion, although the difference between the two treatments did not reach statistical significance.

Cohen et al. (1996) conducted a field study with 7 endurance trained runners who performed 3 maximal-effort 21 km road races outdoors in warm (24–28 °C) and humid conditions. CAF ingestion 60 min before the start did not improve the running time. A possible explanation for this could be that the participants were *ca* 4% dehydrated by the end of the race. Dehydration, especially in excess of 2% of body mass and combined with a high skin temperature, markedly impairs endurance performance (Sawka et al., 2012). In Cohen et al. (1996) study the fatigue caused by dehydration assumably hindered the ergogenic effect of CAF. It is also important to note that the study conducted by Cohen and colleagues (1996) seems to be the only one where the potential ergogenic effect of CAF in warm environment has been examined in female participants.

An increase in T_c is considered to be one of the major causes of fatigue in warm climate (Hargreaves, 2008). It has been found that hyperthermia increases CNS fatigue, which in turn reduces performance (Nybo, 2008). Cheuvront et al. (2009) and Roelands et al. (2011) administered a single 6–9 $mg \cdot kg^{-1}$ dose of CAF to their participants and reported that CAF increased T_c during exercise in the heat. Furthermore, Roelands et al. (2011) suggested that this thermogenic effect might negate any potential ergogenic benefit of CAF in the heat. Pitchford et al. (2014) speculated on the basis of their own findings and those of Ganio et al. (2011) that relatively small doses of CAF, or a distribution of bigger doses, may prevent the thermogenic effect of CAF and increase the likelihood of an ergogenic effect of this drug in warm conditions.

The majority of studies have not found positive effect of CAF ingestion on endurance ability in the heat (Cheuvront et al., 2009; Cohen et al., 1996; Roelands et., 2011). The exception is Ganio et al. (2011) study, which compared the effect of CAF (6 $mg \cdot kg^{-1}$) on endurance performance in moderate (12 °C) and warm (33 °C) environment. In the latter study participants had to cycle 90 min with the intensity of 60–70% of their VO_2max and after that 15 min as fast as possible. High environmental temperature reduced endurance performance 19% regardless of the substance, while CAF improved endurance performance 5% compared to PLC in both environmental conditions. Therefore, it can be assumed that this time CAF shadowed hyperthermia.

2.4. Impact of sodium citrate on endurance ability

Several nutritional strategies have been studied over the decades to delay the onset of muscle fatigue, which is one of the main limiting factors affecting sports performance (Robergs et al., 2004). The problem is complex and scientists do not agree about the exact causes of fatigue. Muscle fatigue may originate in changes in the muscle itself (peripheral fatigue) or proceed from the CNS (central fatigue) (Fitts, 2004). Despite the lack of consensus, the accumulation of hydrogen ions (H^+) within the muscle cell has been emphasized as a major cause of fatigue during high-intensity, short duration exercise (Robergs et al., 2004). Therefore, many nutritional strategies aiming to increase intra- and extracellular buffering capacity, such as beta-alanine, $NaHCO_3$, CIT, have been investigated and applied to postpone muscle fatigue (Lancha Junior et al., 2015).

$NaHCO_3$ or CIT loading is not prohibited by the rules of sport and therefore, athletes have practiced "soda loading" for more than 80 years, with $NaHCO_3$ being ingested in the form of household product "bicarb soda" (Burke et al., 2006). Ingestion of alkalizing substances improves performance in high-intensity short duration exercise, but results from longer duration exercises that last more than 10 min are considered inconclusive (Carr et al., 2011; Requena et al., 2005). However, only three studies have addressed the potential ergogenic effects of these substances on endurance cycling. Two of these studies reported enhanced endurance performance owing to $NaHCO_3$ (McNaughton et al., 1999) or CIT (Potteiger et al., 1996) ingestion, whereas the third study (Schabort et al., 2000) found no impact of CIT on endurance performance. Studies where endurance ability was improved after CIT ingestion used 500 $mg \cdot kg^{-1}$ doses and the time interval from intake to the beginning of exercise was *ca* 120 min (Requena et al., 2005).

2.4.1. Mechanism of action

CIT does not exist in molecular form in body fluids and dissociates quickly into Na^+ and $citrate^-$ ions immediately after ingestion. The citrate anion is expelled from the plasma and electrical equilibrium becomes unbalanced (Kowalchuk et al., 1989). In order to restore this equilibrium, H^+ concentration in plasma decreases and HCO_3^- concentration increases, and thus the extracellular buffering capacity enhances (McNaughton and Cedaro, 1992). Ingestion of CIT increases HCO_3^- concentration in extracellular space, which in turn may facilitate a greater efflux of lactate and H^+ out of the working muscle (Lancha Junior et al., 2015). During high-intensity exercise accumulating H^+ binds with bicarbonate and forms carbonic acid, which reduces acidification of intracellular space and thereby may enhance performance (Requena et al., 2005).

The generation of lactic acid in the muscles during high-intensity exercise results in lowered pH of the muscle and blood due to the dissociation of lactic

acid into lactate and H^+ (Abbiss and Laursen, 2005). Increased lactate concentration together with concomitant rise in H^+ could induce fatigue by various mechanisms like inhibition of phosphofructokinase and thus the glycolytic rate, competitive inhibition of Ca^{2+} binding to troponin C reducing cross-bridge activation, and inhibition of sarcoplasmic reticulum ATPase (Fitts, 2004). However, it appears that the intracellular accumulation of lactate and H^+ *per se* are not the major factors in muscle fatigue (Allen et al., 2008). Despite those findings, high-intensity exercise still could be enhanced by increased extracellular (Carr et al., 2011) or intracellular (Quesnele et al., 2014) buffer capacity.

The majority of previous studies have focused on ergogenic effects of alkalizers based mainly on their property to increase extracellular buffer capacity (Bishop et al., 2004; McNaughton et al., 1999; Potteiger et al., 1996), whereas their impact on exercise-induced stress has received less attention. Stress is a complex and multifactorial phenomenon that affects performance, but physiological responses to stress generally comprise changes in neuroendocrine, hormonal and immune functions (Kozlov and Kozlova, 2014; Glaser and Kiecolt-Glaser, 2005). By increasing the body's buffer capacity (Potteiger et al., 1996; Timpmann et al., 2012) and PV (Timpmann et al., 2012; Ööpik et al., 2010), CIT may facilitate maintenance of homeostasis, i.e. reduce physiological stress during heavy exercise. Available data suggest that acute orally induced metabolic alkalosis alleviates exercise stress, as reflected in attenuated stress hormone responses to intense exercise (Bouissou et al., 1988; Gordon et al., 1994; Rojas Vega et al., 2006; Wahl et al., 2010; Ööpik et al., 2010). However, the findings of Marx et al. (2002) and Bracken et al. (2005) are not consistent with these data, as they did not observe any impact of $NaHCO_3$ or CIT, respectively, on blood catecholamine responses to high-intensity exercise.

2.4.2. Endurance performance

The ergogenic effects of $NaHCO_3$ and CIT on short-term high-intensity exercise have been well documented with numerous investigations (Burke et al., 2006; Carr et al., 2011; Requena et al., 2005). Results obtained after long-term exercise are much more limited, because investigations are scarce and the existing data are controversial (Requena et al., 2005).

Potteiger et al. (1996) investigated trained male cyclists who ingested 500 $mg \cdot kg^{-1}$ of CIT or PLC prior to 30-km TT. Significantly higher pH values were observed throughout the TT with CIT compared to PLC. The authors concluded that CIT ingestion created a favourable metabolic condition that probably contributed to 4% improvement during the time of covering 30-km TT. Improved performance was also found in McNaughton et al. (1999) study, where well-trained male cyclists ingested 300 $mg \cdot kg^{-1}$ of $NaHCO_3$ before 60 min maximal cycle ergometer test. The $NaHCO_3$ increased plasma bicarbonate concentration prior to the start of the test and the participants completed significantly more work in $NaHCO_3$ trial than in control or PLC trial. The

authors suggested that $NaHCO_3$ ingestion may be used to offset the fatigue process during high-intensity cycling.

Contrary to the two previous studies (McNaughton et al., 1999; Potteiger et al., 1996), Schabort et al. (2000) did not find any significant enhancement in 40-km TT time or in the average power output after administering 200–600 $mg \cdot kg^{-1}$ of CIT, although there emerged dose-dependent changes in pH, base excess and bicarbonate concentrations. The authors suggested that the failure of CIT to enhance performance was due to gastrointestinal discomfort and stomach cramps, which were experienced by 5 out of 8 trained cyclists.

Ööpik et al. (2003) studied well trained male college runners who performed a 5 km treadmill run with and without preceding CIT ingestion. The CIT dose was 500 $mg \cdot kg^{-1}$ dissolved in 1 litre of water two hours before the run. The authors reported significantly improved endurance performance in CIT compared to PLC trial. However, the subsequent study carried out on outdoor stadium where the participants (trained male runners) competed in pairs against each other, did not confirm the ergogenic effect of CIT (500 $mg \cdot kg^{-1}$ dissolved in water) on 5 km running performance (Ööpik et al., 2004). It remains unclear why this dietary manipulation enhances endurance performance on treadmill but not in field conditions. Ööpik et al. (2004) speculated that the ingestion time of CIT might have been too early before the run. The solution was consumed 180 min before exercise within 60 min period contrary to Ööpik et al. (2003) study, where CIT was consumed 120 min before the start within 10 min. Ööpik et al. (2004) also assumed that the positive effect of CIT might have been negated by the uncontrollable variables presented in the real competitive environment.

2.4.3. Endurance capacity

Data regarding the potential effects of CIT on endurance capacity are scarce. Ööpik et al. (2010) administered 500 $mg \cdot kg^{-1}$ of CIT to well trained runners who performed an incremental running test to volitional exhaustion on a treadmill. Solution containing CIT increased PV (*ca* 6.4%) more than PLC drink (*ca* 4.1%). According to Mora-Rodriquez and Hamouti (2013) expansion of PV in the range of 7–8% may improve endurance capacity. However, Ööpik et al. (2010) did not find differences between CIT and PLC treatments in performance time to exhaustion. Probably the increase in PV in CIT trial was not that much higher (*ca* 2.3%) compared to PLC trial. Another explanation why endurance capacity was not improved, could be that 12 participants out of 13 complained gastrointestinal distress symptoms after CIT ingestion.

2.4.4. Endurance ability in the heat

Compared with temperate environment, exercise in the heat places a higher demand on the body's thermoregulatory system and induces sweat losses that may reach 3–4 $L \cdot h^{-1}$ in trained athletes (Taylor and Machado-Moreira, 2013). Although lower sweating rates of 1.0–2.5 $L \cdot h^{-1}$ are more common (Sawka and Montain, 2000), athletes still do not drink enough to avoid dehydration, especially in the heat. It has been suggested that increased PV could improve thermoregulation and exercise performance in the heat (Sims et al., 2007b).

Acute increases in PV induced by pre-exercise ingestion of solutions containing CIT and NaCl were associated with improved endurance cycling (Sims et al., 2007a) and running (Sims et al., 2007b) capacity in the heat in endurance-trained women and men, respectively. However, Vaher et al. (2015) reported no impact of acute pre-exercise CIT ingestion that induced water retention and significant increase in PV, on 5 km running performance in warm environment in non-heat-acclimated endurance-trained males. The number of studies conducted in the heat is too small to draw any definite conclusions.

2.5. Summary of the review of literature

Major physiological determinants of endurance ability are VO_2max, lactate threshold and exercise economy (Joyner and Coyle, 2008; Midgley et al., 2007; Pate and Branch, 1992), which can all be significantly improved by systematic endurance training. Many nutritional factors like carbohydrates (Jeukendrup et al., 2014; Jeukendrup and Jentjens, 2000), water (Holland et al., 2017) and several dietary supplements (Burke, 2007; Maughan et al., 2018) may acutely influence endurance ability of trained as well as untrained individuals. Besides training and nutritional status, environmental factors like temperature and RH have impact on endurance ability (Cheuvront et al., 2010; Sawka et al., 2011).

As far as dietary supplements are concerned, the positive effect of CAF on endurance ability is well established (Burke, 2007; Burke et al., 2013; Doherty and Smith, 2004; Maughan et al., 2018). This ergogenic effect appears more clearly when CAF is administered to well trained persons in capsules containing optimal dose (3–6 $mg \cdot kg^{-1}$) of CAF (Graham, 2001), especially to men, who might be more responsive to CAF than women (Adan et al., 2008; Temple et al., 2009, 2010). Regarding CIT, there is some evidence suggesting that this substance may enhance endurance ability, but the number of relevant studies is small, and the findings are not consistent (Burke, 2007; Carr et al., 2011; Requena et al., 2005). In studies where performance has been improved, mostly 500 $mg \cdot kg^{-1}$ doses of CIT and short-term high-intensity exercise protocols have been applied (Carr et al., 2011; Requena et al., 2005).

In addition, the potential ergogenic properties of both CAF and CIT have been mainly evaluated under moderate environmental conditions, with only a few studies conducted in warm environments. Although it is widely recognized

that CAF improves endurance ability in temperate environmental conditions, its efficacy in the heat is not clear. Indeed, the majority of well-controlled studies (Cheuvront et al., 2009; Cohen et al., 1996; Roelands et al., 2011) have found no benefits of acute CAF ingestion on endurance ability in the heat, with one yielding equivocal results (Pitchford et al., 2014) and only one (Ganio et al., 2011) demonstrating ergogenic effect. Data on the potential impact of CIT on endurance ability in the heat are even more scarce. Two studies, where participants ingested CIT combined with NaCl, reported increased time to exhaustion in trained women in cycling (Sims et al., 2007a) and in trained men in running (Sims et al., 2007b), whereas one study did not find any impact, positive or negative, of CIT ingestion on 5 km running performance in trained men (Vaher et al., 2015).

The findings of the majority of relevant studies suggest that acute orally induced metabolic alkalosis alleviates exercise stress, as reflected in attenuated stress hormone responses to exercise (Bouissou et al., 1988; Gordon et al., 1994; Rojas Vega et al., 2006; Ööpik et al., 2010; Wahl et al., 2010). However, all these studies were carried out in temperate environmental conditions and investigated relatively short-duration high-intensity exercise bouts. On the other hand, prolonged exercise in the heat compared to the same exercise performed in a temperate environment induces greater increases in blood prolactin (PRL) (Burk et al., 2012; Sparks et al., 2005), growth hormone (GH) (Niess et al., 2003; Ööpik et al., 2014), cortisol (CORT) (Satarifard et al., 2012; Tamm et al., 2015) and aldosterone (ALDO) (Akerman et al., 2017) levels, but to the best of our knowledge, it is unknown whether CIT (or $NaHCO_3$) ingestion alleviates responses of stress hormones to prolonged endurance exercise in warm environments.

3. RESEARCH AIMS AND HYPOTHESIS

The main purpose of this study was to determine the effects of caffeine and sodium citrate ingestion on endurance ability in recreationally active female and male participants performing in the heat.

Specific aims and hypotheses of the study were:

1. To compare the impact of acute caffeine ingestion on psychological and physiological parameters in young female and male participants walking to exhaustion in the heat. We hypothesized that the endurance- and mood-enhancing effect of caffeine would appear in males only or would be greater in males than in females.
2. To assess the impact of sodium citrate ingestion during recovery from dehydrating execise on rehydration, plasma volume, ratings of perceived exertion and thermoregulation. We hypothesized that sodium citrate, stimulating rehydration and inducing expansion of plasma volume slow down increases in core body temperature and improve performance in warm environment in male non-heat-acclimated endurance athletes.
3. To test the hypothesis that sodium citrate ingestion during 16-h recovery period after dehydrating cycling exercise, in an amount that has been shown to induce metabolic alkalosis and expansion of plasma volume, would attenuate increases in the blood levels of cortisol, aldosterone, growth hormone and prolactin during subsequent 40-km time-trial in the heat.

4. METHODS

4.1. Participants

A total of forty three healthy, recreationally active male and female athletes (Table 1) volunteered to participate in 2 studies, the protocols of which were approved by the Research Ethics Committee of the University of Tartu, and these conformed to the standards set by the Declaration of Helsinki. Before the study, all participants were informed of the nature of the investigation, after which they gave written consent. All the volunteers were nonsmokers, they were involved in endurance-type recreational activities 3–4 times a week. They neither had history of heat illness nor had they travelled to a warm climate at least 2 months before the study. All female participants were users of contraceptive pills.

Table 1. Participants' anthropometric parameters

Study	Gender	Number of partici-pants	Age (years)	Body mass (kg)	Height (cm)	VO_2peak ($ml \cdot min^{-1} \cdot kg^{-1}$)
Caffeine:	Male	13	24.9 ± 4.1	78.8 ± 7.9	182.7 ± 5.5	51.7 ± 2.7
	Female	10	22.5 ± 2.0	61.0 ± 5.4	167.3 ± 5.2	45.6 ± 4.0
Sodium citrate	Male	20	30.8 ± 5.4	78.2 ± 8.4	182.5 ± 6.9	57.0 ± 5.9

Data are presented as mean ± SD.

4.2. Study designs

4.2.1. Caffeine study

The participants visited the laboratory on 4 occasions. During the first visit they were familiarized with the study protocol and the equipment to be affixed to their bodies. Anthropometric measurements were taken and peak oxygen uptake (VO_2peak) was determined in a temperate environment (air temperature, 21–22 °C; relative humidity (RH), 50–51%) using an online breath-by-breath metabolic system (MasterScreen CPX; Viasys Healthcare GmbH, Hoechberg, Germany) and a motorized treadmill (Viasys/Jaeger LE300 C; Viasys Healthcare GmbH), as described by Burk and colleagues (2012). During the second visit the participants completed a 20-min treadmill walk in warm-dry environmental conditions (42 °C; RH 20%) in a climatic chamber (Design Environmental Ltd.; Gwent, South Wales, UK). The purpose of this exercise session was to familiarize the participants with the walking exercise and psychological

testing procedures and to minimize any anxiety effects during the experimental trials.

During both the third and fourth visit to the laboratory, the endurance capacity of each participant was measured in the same environmental conditions (i.e., 42 °C; RH 20%). The participants walked on a treadmill at 60% of their thermoneutral VO_2peak until volitional exhaustion after CAF and PLC administration in a randomly assigned, double-blind, crossover manner, with 1 week between the 2 experimental trials.

The participants were instructed to refrain from alcohol, CAF, and strenuous exercise for 24 h before each visit to the laboratory. To ensure similar nutritional and hydration status and physical activity level before all the testing sessions, the participants were instructed to record their dietary intake and physical activity during the day before their first visit to the laboratory and to follow the same regimen before the consecutive visits.

4.2.2. Experimental trials in caffeine study

On each trial day (Figure 1), the participants reported to the laboratory 1 h after breakfast (i.e., at 8:00 am). They voided and donated a urine sample. Then, in the CAF trial, the participants were administered a total of 6 mg·kg^{-1} of CAF (Oriola, Finland) in gelatine capsules in 2 doses: 60 min (4 mg·kg^{-1}) and immediately (2 mg·kg^{-1}) before the endurance capacity test. In the PLC trial, the participants were administered wheat flour in the same amount and according to the same protocol. On both occasions, the participants were advised to ingest the capsules with a bolus of water.

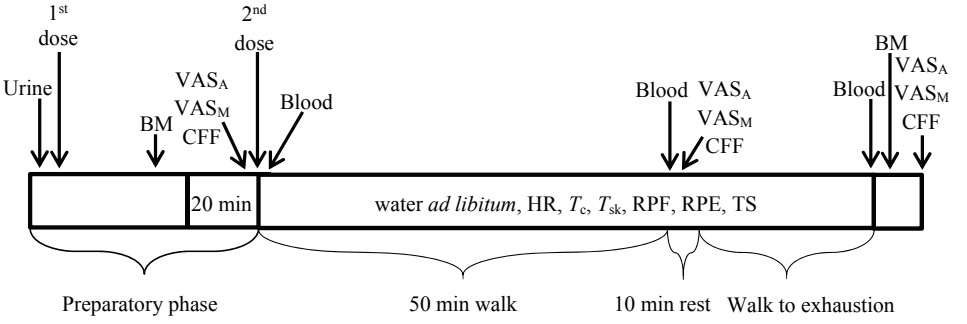

Figure 1. Testing day protocol. Arrows indicate time points at which outcome measures were collected or supplements administered. 20 min indicates the duration that participants spent in a standing position before blood sampling. BM, body mass; T_c, core body temperature; T_{sk}, skin temperature; HR, heart rate; 1st/2nd dose, caffeine or placebo administration; RPF, ratings of perceived fatigue; RPE, ratings of perceived exertion; TS, thermal sensation; VAS_A, visual analogue scale for perceived arousal; VAS_M, visual analogue scale for mood; CFF, critical flicker frequency.

Fifteen minutes before entering the climatic chamber, the nude BM of the participants was measured. Thereafter they positioned rectal temperature probe, heart rate (HR) transmitter strap and skin temperature probes were attached to their bodies.

After entering the climatic chamber, the participants stood still on the treadmill for 20 min before blood sampling and starting the endurance capacity test. During the test, the intensity of the exercise was adjusted individually to 60% of participant's personal thermoneutral VO_2peak by using a constant speed of 6 $km \cdot h^{-1}$ and regulating the grade of the treadmill. In both the CAF and the PLC trials, the endurance capacity test included 2 periods of walking separated by 10-min rest interval while participants stood on the treadmill. The first walking period lasted for 50 min, and the second until volitional exhaustion. One of the research group members was responsible for premature termination of the endurance capacity test if one of the following conditions was observed in the participant: (*i*) a rise of T_c to 40 °C for 5 min; (*ii*) a rise of HR to 95% of age-predicted maximal HR for 5 min; (*iii*) the occurrence of symptoms of exertional heat illness, such as nausea, dizziness, or headache; or (*iv*) a participant's request to stop.

During the endurance capacity test, the participants were allowed to drink water *ad libitum*, and the volume of water consumed was registered. Whole-body sweat production was calculated on the basis of changes in nude BM during exercise, taking into account the volume of water consumed. Capillary blood samples were taken from the fingertips of the participants immediately before the start of the endurance capacity test (Pre-Ex), immediately after the first 50-min walking period (Mid-Ex), and at the time of volitional exhaustion (Post-Ex).

Ratings of overall perception of exertion (RPE) and fatigue (RPF) (Borg, 1998) and thermal sensation (TS) (BSI, 2001) were recorded at 10-min intervals throughout each endurance capacity test. A visual analogue scale (VAS) for recording perceived arousal (VAS_A) and mood (VAS_M) and critical flicker frequency (CFF) for assessment of cortical arousal (Simonson and Brozek, 1952) were used before the start of the endurance capacity test, during the 10-min rest interval between the 2 walking periods, and at the cessation of the exercise test. Changes in cortical arousal and CNS activity have been registered reliably using the CFF threshold, in relation to CAF ingestion (Hindmarch et al. 1998) as well as to exercise (Davranche and Pichon 2005). The CFF threshold, defined as the frequency at which a flickering light is indistinguishable from a steady, nonflickering light, was used for further analysis.

After the endurance capacity test, the participants were asked to indicate whether they believed they had received CAF or PLC and how confident they were in the answer provided. These subjective ratings were converted into the confidence percentage of receiving caffeine (subjCAF).

4.2.3. Sodium citrate study

The study consisted of the preparatory phase and the main phase. The participants visited laboratory on 3 occasions in the preparatory phase and 4 times in the main phase. During the first visit to the laboratory, anthropometric measurements of the participants were collected, they were familiarized with the equipment to be affixed to their bodies as well as with all the experimental procedures. Their VO_2peak was determined in a temperate environment (21–22 °C; RH 50–51%) using the same online breath-by-breath metabolic system as in CAF study, but using different incremental workload test on a Cyclus 2 ergometer (RBM Elektronik-Automation GmbH, Leipzig, Germany). The protocol started with a warm-up period of 2.5 min at a workload of 100 W. Thereafter, the workload was increased by 50 W after every 2.5 min until exhaustion.

On the second and the third visits to the laboratory, the participants completed the 40-km familiarization cycling TTs in the heat (32 °C; RH 46%) to minimize any potential learning and anxiety effects.

On the first day of the main phase of the study participants exercised on cycle ergometer in the heat until they lost approximately 4% of BM. During 16-h recovery period participants drank bottled water *ad libitum*, ate prescribed diet and ingested gelatine capsules containing either CIT or PLC (sucrose) in randomly assigned double-blind crossover manner with 1 week between the 2 trials. In the morning of the next day, 2 h after having prescribed breakfast, participants performed 40-km TT in the heat (32 °C; RH 46%).

Participants were instructed to record their 24-h dietary intake before starting the main phase of the study with the PLC or the CIT trial. Before their second trial in the main phase, the records were returned to the participants with instructions to follow the same diet for 24 h prior to their visit to the laboratory. Participants were also instructed to refrain from exercise for 24 h before trials.

4.2.4. Experimental trials in sodium citrate study

In the afternoon of the first day of the main phase participants performed dehydrating cycling exercise (DE) followed by the 40-km TT in the next morning (Figure 2). Both trials took place in the climatic chamber where air temperature was set to 32 °C and RH to 46%.

Participants reported to the laboratory at 2:30 pm, voided, donated a urine sample, and then their nude BM was measured. Prior to entering climatic chamber, the participants positioned rectal temperature probe and they were fitted with a HR transmitter strap. After entering the climatic chamber, the participants sat on a chair for 20 min prior to blood sampling and start of DE. The intensity of work during DE was individually adjusted to 50%–60% of VO_2peak.

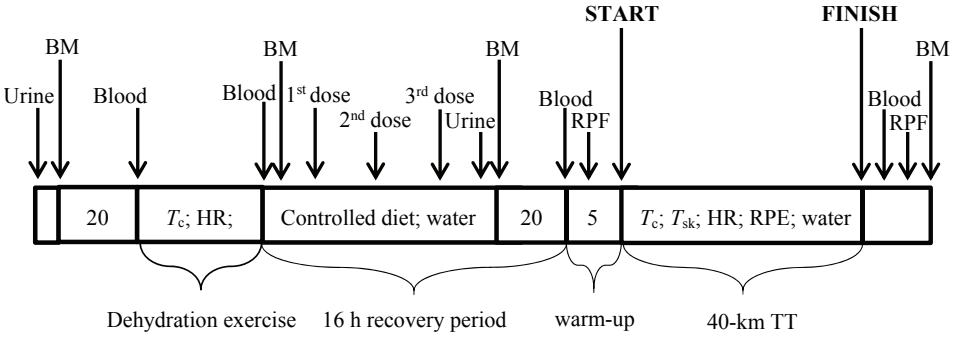

Figure 2. Main phase protocol. Arrows indicate time points at which outcome measures were collected, supplements administered, or TT started/finished. 20 min indicate the duration that participants spent in a sitting position before blood sampling and 5 min indicate the duration of standardized warm-up. BM, body mass; T_c, core body temperature; T_{sk}, skin temperature; HR, heart rate; $1^{st}/2^{nd}/3^{rd}$ dose, sodium citrate or placebo administration; RPF, ratings of perceived fatigue; RPE, ratings of perceived exertion.

Initially all participants performed 3 sets of 20-min cycling followed by a 10-min rest without drinking. During the third 10-min rest, participants towel-dried themselves and they were weighed wearing only cycling shorts and socks. On the basis of individual BM loss it was calculated how many 20-min cycling sets each participant had to complete to achieve 4% dehydration level. Immediately after DE participants removed all the affixed equipment, towel-dried themselves, and their nude BM was measured for calculating the actual dehydration level achieved.

During 16-h recovery period participants were allowed to drink bottled water *ad libitum*, and the volume consumed was precisely recorded. After DE, at approximately 40 min prior to leaving the laboratory, all participants ate a meal that provided 20 kcal·kg^{-1} initial BM (69% carbohydrates, 11% proteins, 20% fats). Right before this meal, participants were administered the first dose (200 mg·kg^{-1} initial BM) of CIT or PLC. When leaving the laboratory, each participant received a small plastic bag with gelatine capsules containing the second 200 mg·kg^{-1} dose of CIT or PLC. Participants were instructed to ingest this dose of supplement with a bolus of water at approximately 1 h before bedtime at home.

Next morning the participants reported to the laboratory at 8:00 am and ate breakfast, which, together with the third 200 mg·kg^{-1} dose of CIT or PLC, provided 12 kcal·kg^{-1} BM (80% carbohydrates, 9% proteins, 11% fats). About an hour after the breakfast participants voided and donated a urine sample. Their nude BM was measured, they positioned rectal temperature probe and HR transmitter strap, and skin temperature probes were attached to their bodies. After entering the climatic chamber, participants sat still on a chair for 20 min. Then venous and capillary blood samples were collected, RPE and RPF were recorded (Borg, 1998).

Cycling started at 2 h after breakfast. Initially participants performed a 5-min warm-up and after 2-min rest they started a flat 40-km TT, the profile of which was created using the Cyclus 2 software (Skorski et al. 2015). Participants were instructed to complete the TT as fast as possible and they were given verbal encouragement by one of the research group members. The same range of electronic gear ratios was used for each trial, and participants started both trials with the same gear ratio. RPE was recorded at 5-km intervals throughout TT and RPF right before and after the 40-km TT. During TT, the participants were allowed to drink water *ad libitum*. After TT participants were asked to indicate whether they believe they had ingested CIT or PLC.

4.3. Blood sampling and biochemical analyses

In both of the studies (CAF and CIT) lactate concentration in the capillary blood samples was measured enzymatically (Dr Lange Cuvette Test LKM 140; Dr Lange, Berlin, Germany) using the miniphotometer LP 20 Plus (Dr Lange, Berlin, Germany). Hemoglobin concentration and hematocrit were measured from capillary blood samples in CAF study, and from venous blood samples in CIT study, using blood analyzer Celltac α MEK-6108K (Nihon Kohden, Tokyo, Japan). The values obtained were used for calculation of relative changes in PV (Dill and Costill, 1974).

In CIT study venous blood samples were collected from antecubital vein before and after DE and TT into sterile serum Vacutainer tubes as well as into Vacutainer tubes containing EDTA. The collected blood was allowed to clot for 10 min at room temperature and was then centrifuged (Eppendorf 5804R; Eppendoff AG, Hamburg, Germany) for 10 min at 3000 rpm at 4 °C. The separated serum was used for assessment of hydration status (for details see below). Serum and plasma specimens were maintained at –25 °C until assayed for hormones and metabolites.

Glucose concentration in serum was measured using the hexokinase enzymatic method on Roche/Hitachi Cobas 6000 c501 analyser (Roche Diagnostics GmbH, Mannheim, Germany).

ALDO and GH concentrations were measured in plasma and serum, respectively, with chemiluminescence immunoassay method and using IDS-iSYS Multi-Discipline Automated Analyser (Immunodiagnostic Systems Limited, Boldon Colliery, UK). Concentrations of PRL and CORT were measured in serum using electrochemiluminescence immunoassay "ECLIA" on Cobas 6000 e601 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

4.4. Thermoregulatory measurements

Core body temperature (T_c) was measured using a rectal temperature probe (REC-UU-VL5-0; Grant Instruments Ltd, Cambridge, UK), which participants positioned at 10 cm beyond the anal sphincter. T_c was recorded every 1 min using an electronic data logger (SQ2020-1F8; Grant Instruments Ltd, Cambridge, UK).

Skin temperature was registered in 4 sites (arm, chest, thigh, leg) by means of 4 miniature temperature probes DS1922L (Maxim Integrated Products Inc., San Jose, CA, USA), which were attached on the same side of the body using a sticking plaster. Skin temperature was recorded every 1 min and the data were transferred to a computer using the appropriate device (DS1401-4+; Maxim Integrated Products Inc., San Jose, CA, USA).

Weighted mean skin temperature (T_{sk}) was calculated according to Ramanathan (1964). T_c and T_{sk} were used for calculation of temperature gradient (T_{gr}): $T_{gr} = T_c – T_{sk}$ in CIT study, and for calculation of mean body temperature (T_b) according to Colin et al. (1971): $T_b = 0.2\ T_{sk} + 0.8\ T_c$, in CAF study.

4.5. Assessment of hydration status

Hydration status in CAF study was assessed on the basis of urine specific gravity (USG), urine osmolality (UOsm), BM, sweat production, water intake, and changes in PV. Hydration status during the main phase of the CIT study was assessed on the basis of USG, serum osmolality (SOsm), BM, water retention, and changes in PV. USG was measured by means of a digital clinical refractometer (PDX-CL; VeeGee Scientific Inc., Kirkland, WA, USA). UOsm and SOsm were measured using freezing point depression osmometer Model 3250 (Advanced Instruments Inc., Norwood, Mass., USA). BM changes were calculated on the basis of values measured before and after trial using electronic scale (CH3G–150I Combics; Sartorius AG, Goettingen, Germany). Whole-body sweat production was calculated on the basis of changes in nude BM, taking into account the volume of water consumed during the trial, which was precisely registered by one of the research group members. Water retention during the 16-h recovery period in CIT study was calculated as difference between the volumes of water consumed and urine passed.

4.6. Heart rate monitoring

In CAF study the participants were fitted with a HR transmitter strap (Suunto Dual Belt; Suunto OY, Vantaa, Finland) and their HR was recorded by means of a telemetry system (Suunto PC POD; Suunto OY, Vantaa, Finland).

In CIT study the participants were also fitted with a HR transmitter strap, but HR was recorded continuously with heart rate monitor Polar RS800CX and analyzed with Polar Protrainer 5 software (Polar Electro Oy, Kempele, Finland).

4.7. Gastrointestinal distress monitoring

Gastrointestinal symptoms monitoring was done only in CIT study. Gastrointestinal symptoms experienced by the participants during the 16-h recovery period and TT were recorded as described by Cameron et al. (2010). Questionnaires consisted of eight 100-mm VAS with no symptoms on the left-hand side and severe symptoms on the right-hand side. Participants were asked to rate their symptoms. If they had no symptoms, they made no mark on the VAS. If they were experiencing some symptoms, they indicated their overall rating by placing a vertical mark on the appropriate scale. The VAS was used to measure symptoms of nausea, flatulence, stomach cramping, stomachache, belching, vomiting, bowel urgency, and diarrhea.

4.8. Statistical analysis

In both studies the Statistica software was used for performing statistical analysis. Data are presented as mean ± SD. All data were checked for normal distribution using Kolmogorov-Smirnov test. In CAF study three-factor mixed-model analysis of variance (ANOVA) was used to examine the effects of gender (between factor) and trial and time (within factors) for T_c, T_{sk}, T_b, HR, BM, blood lactate concentration and perceptual ratings. Two-factor mixed-model ANOVA was employed for analysis of the effect of gender (between factor) and trial (within factor) on USG, UOsm, water intake, sweat production and PV data. In CIT study two-factor (trial x time) repeated-measures ANOVA was used to evaluate the differences within and between the trials for BM, SOsm, PV, T_c, T_{sk}, T_{gr}, RPE, RPF, HR, blood biochemical parameters, split times and power output. The number of time points assessed varied with measurements, depending on the specific outcome measure. In case a significant main effect was observed, Tukey's honestly significant difference post hoc analysis was used to locate differences between the means. Differences between PLC and CIT trials in variables measured at a single time point (energy intake, water intake, sweat loss, urine volume, water retention, USG, TT time and work done) were analyzed using paired-samples Student's *t*-test. The χ^2 analysis was used to compare the prevalence of gastrointestinal distress

symptoms in the PLC and CIT trials. Significant deviations from sphericity were tested with Mauchly's sphericity test. When a violation of sphericity was observed, the Greenhouse-Geisser correction was employed and corrected p values are reported in the CAF study. In both studies relationships between variables were determined on the basis of Pearson correlation coefficient (r). Significance was set at $p < 0.05$ level while the p values < 0.1 are reported to indicate trends. The hormone and glucose concentrations measured after the 40-km TT in the CIT study are presented as corrected for the individual changes in PV.

5. RESULTS

5.1. Caffeine study

Endurance capacity

There was no main effect of CAF ingestion (F = 2.22, p = 0.151) or gender (F = 0.40, p = 0.534) on endurance capacity: volitional exhaustion occurred after 82.1 ± 15.1 min and 75.6 ± 11.2 min of exercise in females and after 83.1 ± 16.6 min and 81.6 ± 14.4 min in males in the PLC and CAF trials, respectively.

Blood lactate concentration

Significant main effects of time (F = 40.99, p < 0.001) and trial (F = 43.06, p < 0.001), with no between-gender difference (F = 0.045, p = 0.834) were observed for blood lactate concentration. Overall, blood lactate concentration was higher in the CAF (3.11 ± 1.76 mmol·L^{-1}) than in the PLC (2.12 ± 0.87 mmol·L^{-1}) trial (p < 0.001). Analyses of the pooled data of males and females revealed similar Pre-Ex blood lactate concentrations in the 2 trials, but Mid-Ex and Post-Ex blood lactate levels were significantly higher in the CAF compared with the PLC trial (Figure 3).

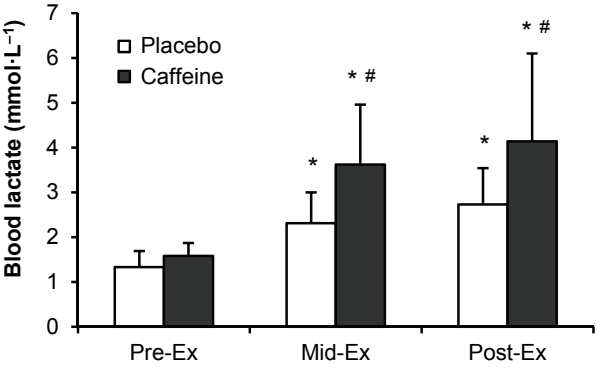

Figure 3. Blood lactate concentration during endurance capacity test. Data are presented as mean ± SD. Significantly different (p < 0.05): * from Pre-Ex; # from placebo.

Thermoregulatory responses

Significant main effects of gender ($F = 5.17$, $p = 0.034$) and time ($F = 361.14$, $p < 0.001$), with no between-trial difference ($F = 0.17$, $p = 0.688$), were evident for T_c that increased with the duration of exercise in both females and males. Overall, T_c was lower in males (38.2 ± 0.8 °C) compared with females (38.4 ± 0.8 °C; $p = 0.034$), but post hoc analysis revealed no significant difference between males and females at any time point (Figure 4A).

There was a significant main effect of time ($F = 108.29$, $p < 0.001$), but not of trial ($F = 0.35$, $p = 0.562$) or gender ($F = 3.09$, $p = 0.093$) on changes in T_{sk}. T_{sk} increased with the duration of exercise in both trials, with no difference between males and females (Figure 4B).

Significant main effects of gender ($F = 5.12$, $p = 0.034$) and time ($F = 344.99$, $p < 0.001$), with no between-trial difference ($F = 0.25$, $p = 0.623$) were evident for T_b that increased with the duration of exercise in both females and males. Overall, T_b was lower in males (38.0 ± 0.8 °C) than in females (38.2 ± 0.7 °C; $p = 0.034$), but post hoc analysis revealed no significant difference between males and females at any time point.

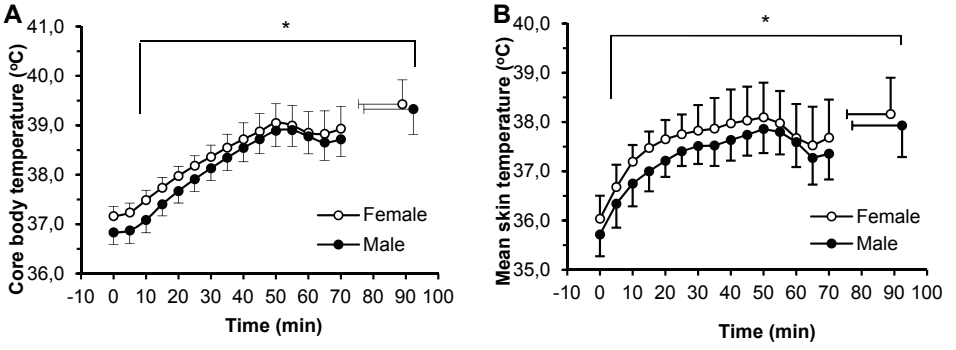

Figure 4. Core body temperature (**A**) and mean skin temperature (**B**) during endurance capacity test. Data are presented as mean ± SD. *Significantly different ($p < 0.05$) from time point 0.

Body mass and hydration status

In both females and males USG and UOsm measured in the morning (on arrival of the participants in the laboratory) and BM measured before the endurance capacity test were similar in the CAF and PLC trials (Table 2). Males were significantly heavier compared with females (main effect of gender: $F = 38.06$, $p < 0.001$), but there was no between-gender difference ($F = 0.82$, $p = 0.376$) in the magnitude of BM loss during the endurance capacity test (Table 2). There were no between-trial differences in total water intake ($F = 0.40$, $p = 0.534$) and whole-body sweat production ($F = 0.002$, $p = 0.966$) or rates of water intake ($F = 0.11$, $p = 0.740$) and whole-body sweat production ($F = 3.00$, $p = 0.098$)

during the endurance capacity test. However, all these parameters were significantly lower in females compared with males (Table 2). Relative change in PV during the endurance capacity test was similar in the 2 trials (F = 1.84, p = 0.189), with no difference between males and females (F = 0.00006, p = 0.994) (Table 2).

Table 2. Body mass and hydration status

Parameter	Female (n = 10)		Male (n = 13)	
	PLC	CAF	PLC	CAF
Body mass, kg				
Pre-Ex	60.96±5.55	61.03±5.25	78.61±7.82$^{\$}$	79.10±7.84$^{\$}$
Post-Ex	60.31±5.53*	60.29±5.18*	77.75±7.84*$^{\$}$	78.21±7.70*$^{\$}$
Change	–0.65±0.49	–0.74±0.60	–0.85±0.49	–0.89±0.56
USG	1.0126±0.0094	1.0138±0.0100	1.0198±0.0088	1.0188±0.0059
UOsm, mOsmol·kg^{-1}	456±303	521±359	791±349$^{\$}$	774±228
Water intake:				
mL	933±457	829±399	1442±690$^{\$}$	1426±739$^{\$}$
mL·min^{-1}	10.2±4.8	9.8±4.8	15.4±6.4$^{\$}$	15.2±7.2$^{\$}$
Sweat production				
mL	1581±440	1566±459	2294±505$^{\$}$	2316±515$^{\$}$
mL·min^{-1}	16.9±3.1	18.1±4.2	24.6±3.2$^{\$}$	25.3±3.7$^{\$}$
Change in PV (%)	–5.1±6.1	–4.4±5.3	–5.3±5.1	–4.2±5.0

Data are presented as mean ± SD. Significantly different (p < 0.05): * from Pre-Ex; $^{\$}$ from female participants. Pre-Ex, before test exercise; Post-Ex, after test exercise; USG, urine specific gravity; UOsm urine osmolality; PV, plasma volume.

Heart rate

Significant main effects of time (F = 564.22, p < 0.001), trial (F = 7.84, p = 0.011), and time by trial interaction (F = 4.00, p = 0.002) were evident for HR, with no between-gender difference (F = 4.16, p = 0.054). Overall, HR was higher in the CAF (158.0 ± 27.4 beats·min^{-1}) than in the PLC (154.9 ± 26.8 beats·min^{-1}) trial (p = 0.009). Analysis of the pooled data of females and males revealed a significantly lower HR in the PLC compared with the CAF trial after 40 min of exercise and during the 10-min rest interval between the 2 exercise periods (Figure 5).

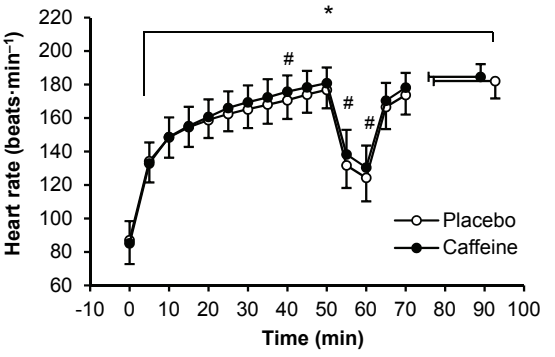

Figure 5. Heart rate during endurance capacity test. Data are presented as mean ± SD. Significantly different ($p < 0.05$): * from time point 0; # from placebo.

Ratings of perceived exertion, fatigue and thermal sensation

There was a significant main effect of time ($F = 135.15$, $p < 0.001$), but not of trial ($F = 2.41$, $p = 0.136$) or gender ($F = 0.004$, $p = 0.951$) for the changes in RPE. However, significant trial by gender interaction ($F = 6.33$, $p = 0.020$) revealed an overall between-trial difference in males (13.8 ± 4.3 and 12.7 ± 3.9 in the PLC and CAF trials, respectively; $p = 0.022$) but not in females (13.2 ± 3.3 and 13.4 ± 3.8, respectively; $p > 0.05$). Post hoc analysis revealed significantly lower RPE in the CAF than in the PLC trial in males after 50 min of exercise (Figure 6A), but there was no between-trial difference in RPE in females at any time point (Figure 6B).

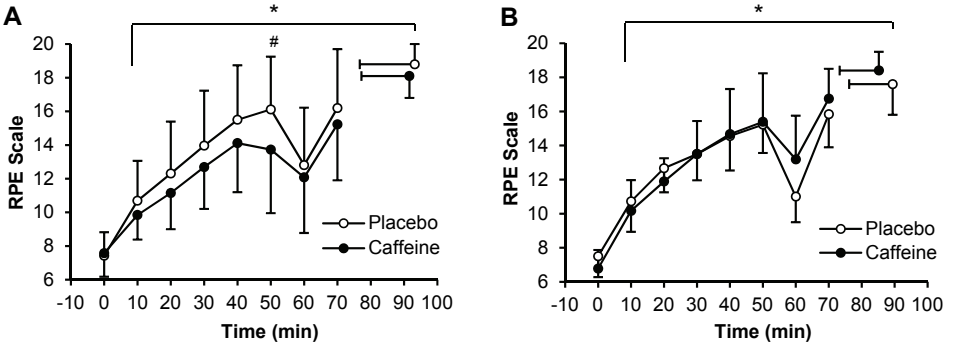

Figure 6. Rating of perceived exertion during endurance capacity test in males (**A**) and females (**B**). Data are presented as mean ± SD. Significantly different ($p < 0.05$): *from time point 0; # from caffeine.

Significant main effects of time ($F = 90.06$, $p < 0.001$) but not of trial ($F = 0.25$, $p = 0.624$) or gender ($F = 0.23$, $p = 0.635$) were observed for the changes in RPF. Significant trial by gender interaction ($F = 11.03$, $p = 0.003$) revealed an overall between-trial difference in males (5.1 ± 3.5 and 4.3 ± 3.1 in the PLC and

CAF trials, respectively; $p = 0.034$) but not in females (4.1 ± 2.2 and 4.7 ± 2.7, respectively; $p = 0.286$). Post hoc analysis revealed significantly lower RPF in the CAF than in the PLC trial in males after 40 min of exercise (Figure 7A), but there was no between-trial difference in RPF in females at any time point (Figure 7B).

There was a significant main effect of time ($F = 10.11$, $p < 0.001$), but not of trial ($F = 1.00$, $p = 0.330$) or gender ($F = 0.005$, $p = 0.944$) for the changes in TS. TS increased with the duration of exercise in both trials in both males and females.

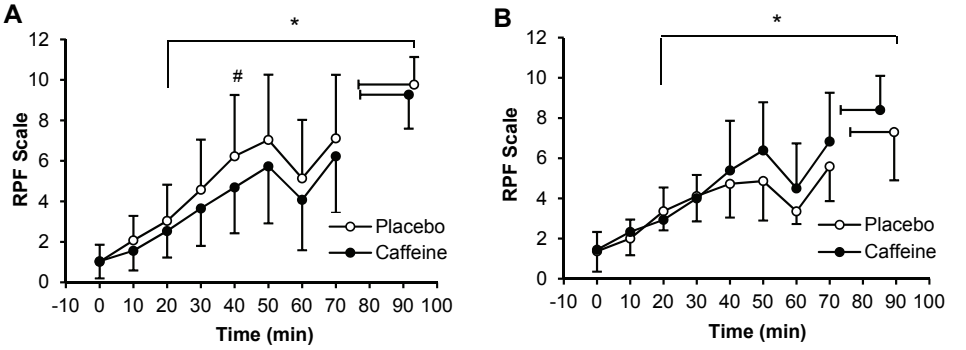

Figure 7. Rating of perceived fatigue during endurance capacity test in males (**A**) and females (**B**). Data are presented as mean ± SD. Significantly different ($p < 0.05$): *from time point 0; # from caffeine.

Mood and arousal

Significant main effect of time ($F = 13.15$, $p < 0.001$), but not of trial ($F = 1.23$, $p = 0.20$) or gender ($F = 0.15$, $p = 0.705$) was observed for VAS_M. VAS_M was rated lower during the 10-min rest interval between the 2 walking periods (6.0 ± 2.2 and 5.5 ± 2.1 in the PLC and CAF trials, respectively) and after cessation of the endurance capacity test (6.4 ± 2.1 and 6.5 ± 2.3 in the PLC and CAF trials, respectively) compared with the pre-test measure in both trials (8.0 ± 1.2 and 7.4 ± 1.6 in the PLC and CAF trials, respectively).

Significant main effect of time ($F = 41.41$, $p < 0.001$) also occurred for VAS_A, with lower ratings during the 10-min rest interval between the 2 walking periods (3.5 ± 2.0 and 3.9 ± 2.0 in the PLC and CAF trials, respectively) and after cessation of the endurance capacity test (3.2 ± 1.7 and 3.3 ± 2.2 in the PLC and CAF trials, respectively) in comparison with the pre-test ratings (6.7 ± 1.6 and 7.2 ± 1.4 in the PLC and CAF trial, respectively). No significant effects of trial ($F = 0.56$, $p = 0.462$) or gender ($F = 0.72$, $p = 0.407$) were observed for VAS_A. Although significant interaction was observed between time and gender ($F = 3.63$, $p < 0.05$), post hoc analysis did not show any significant differences between single time point measures.

For CFF, the main effect of time was significant (F = 5.40, p < 0.01), indicating a higher threshold measure during the 10-min rest interval between the 2 walking periods (32.3 ± 4.2 and 32.4 ± 3.9 Hz in the PLC and CAF trials, respectively) compared with the pre-test values (31.2 ± 3.5 and 31.2 ± 3.7 Hz in the PLC and CAF trials, respectively) and the measures taken after cessation of the endurance capacity test (31.1 ± 3.8 and 31.4 ± 3.7 Hz for the PLC and CAF trials, respectively). No significant effects of trial (F = 0.95, p = 0.343) or gender (F = 1.69, p = 0.210) were observed for CFF.

Correlation analysis

In females, endurance capacity was significantly related to the confidence ratings on perception of receiving caffeine (subjCAF; r = –0.71, p < 0.05) in the PLC trial, whereas in the CAF trial, endurance capacity was related to RPE (r = –0.79, p < 0.05) and RPF (r = –0.70, p < 0.05) recorded at the end of the first walking period (at 50 min). In males, endurance capacity was associated with RPE and RPF recorded at the end of the first walking period in both the PLC trial (RPE, r = –0.78, p < 0.01; RPF, r = –0.87, p < 0.001) and the CAF trial (RPE, r = –0.68, p < 0.05; RPF, r = –0.79, p < 0.01).

5.2. Sodium citrate study

Dehydrating exercise

There was main effect of time (F = 193.46, p < 0.001) and significant trial by time interaction (F = 63.18, p < 0.001) but no main effect of trial (p > 0.05) for BM (Table 3). Likewise, main effect of time (F = 45.80, p < 0.001) and significant trial by time interaction (F = 9.11, p < 0.001) but no main effect of trial (p > 0.05) were observed for SOsm. Regarding changes in PV, main effects of trial (F = 22.51, p < 0.001) and time (F = 131.57, p < 0.001) and significant trial by time interaction (F = 27.49, p < 0.001) occurred (Figure 8).

Prior to DE, BM, SOsm, and USG did not differ in the PLC and CIT trials (Table 3). During DE, similar sweat losses and changes in BM, SOsm and PV (Figure 8) occurred in the 2 trials. Total time spent in climatic chamber to achieve the target dehydration level was 146.8 ± 10.8 min in the PLC and 147.2 ± 11.1 min in the CIT trial (p > 0.05).

Table 3. Body mass and hydration status.

Parameter	Placebo		Citrate	
	DE	40-km TT	DE	40-km TT
Body mass, kg				
Pre-Ex	79.83±9.00	79.70±9.03	79.63±8.71	80.29±8.99 #$
Post-Ex	76.90 ± 8.88*	78.35±9.03*	76.71±8.59*	79.11±7.70*
Body mass change				
kg	–2.93±0.42	–1.36±0.60	–2.92±0.43	–1.18±0.69 $
%	–3.69±0.56	–1.72±0.79	–3.69±0.56	–1.49±0.89 $
Pre-Ex USG	1.0113±0.0086	1.0119±0.0093	1.0119±0.0061	1.0113±0.0074
SOsm, mOsmol·kg^{-1}				
Pre-Ex	292.0±3.8	292.2±2.9	290.8±3.8	295.5±3.3 #$
Post-Ex	302.1±5.1*	296.4±6.3*	302.3±3.8*	298.2±6.7*
Water intake, mL	–	1285±420	–	1529±611 $
Sweat production				
mL	2926±416	2642±393	2922±430	2711±381
L·h^{-1}	–	2.33±0.35	–	2.39±0.36

Data are presented as means ± SD, n = 20. Significantly different (p < 0.05): * from Pre-Ex; # from DE; $ from placebo trial. DE, dehydrating cycling exercise; Pre-Ex, before test exercise; Post-Ex, after test exercise; SOsm, serum osmolality; TT, time-trial; USG, urine specific gravity.

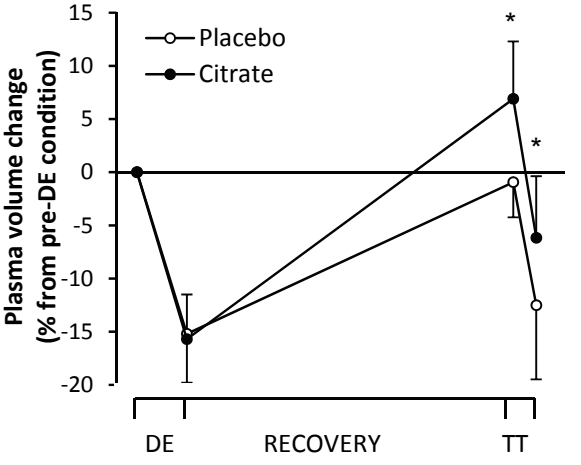

Figure 8. Changes in plasma volume during dehydrating cycling exercise (DE), 16-h recovery period (RECOVERY), and subsequent 40-km time-trial (TT). Data are presented as means ± SD, n = 20. *Significantly different (p < 0.05) from placebo.

Recovery after dehydrating exercise

Actual energy intake within the 16-h recovery period after DE was similar in the 2 trials. During evening meal participants consumed 1585 ± 226 kcal (20.1 ± 3.3 kcal·kg^{-1}) in the PLC and 1589 ± 204 kcal (20.1 ± 3.0 kcal·kg^{-1}) in the CIT trial ($p > 0.05$). During breakfast their energy intake was 851 ± 182 kcal (10.8 ± 2.6 kcal·kg^{-1}) in the PLC and 825 ± 186 kcal (10.4 ± 2.6 kcal·kg^{-1}) in the CIT trial ($p > 0.05$).

Water content of the food consumed during recovery period was similar in the PLC and CIT trials (2106 ± 310 mL and 2131 ± 300 mL, respectively; $p > 0.05$). However, drinking water consumption (2855 ± 684 mL in PLC and 3364 ± 612 mL in CIT; $p < 0.001$) and total water intake (4962 ± 739 mL; 62.6 ± 10.4 mL·kg^{-1} in PLC and 5495 ±721 mL; 69.4 ± 9.7 mL·kg^{-1} in CIT; $p < 0.001$) were greater in the CIT trial. The volume of urine passed during recovery period did not differ in the 2 trials (1500 ± 653 mL in PLC and 1369 ± 394 mL in CIT; $p > 0.05$), but water retention was significantly ($p < 0.001$) higher in the CIT trial (4126 ± 592 mL) than in the PLC (3462 ± 486 mL) trial. Greater increase in PV occurred in the CIT trial compared with the PLC trial ($p < 0.001$; Figure 8).

By the end of recovery period, i.e., immediately prior to 40-km TT, SOsm had returned to the level observed prior to DE in the PLC trial, but still remained elevated in the CIT trial (Table 3). Moreover, immediately prior to the 40-km TT, SOsm was higher in the CIT trial compared with the PLC trial. BM gain in the CIT trial (4.68% ± 0.71%) significantly ($p < 0.001$) exceeded that observed in the PLC trial (3.67% ± 0.75%). In both the PLC and CIT trials, changes in BM strongly correlated with water retention: $r = 0.854$ and $r = 0.932$, respectively, in both occasions $p < 0.001$. At the end of the 16-h recovery period, i.e., immediately prior to 40-km TT, BM of the participants was greater in the CIT than in the PLC trial (Table 3).

Time-trial performance

Cycling 40-km TT time in the PLC and CIT trials (68.1 ± 2.9 min and 68.1 ± 3.3 min, respectively) did not differ ($p > 0.05$). There was no main effect of trial on split times (Figure 9A) or power output (Figure 9B, $p > 0.05$ in both cases), but significant main effect of time was evident on both split times ($F = 16.40$, $p < 0.001$) and power output ($F = 22.52$, $p < 0.001$). Significant trial by time interaction on split times ($F = 2.146$, $p = 0.044$) and power ($F = 3.599$, $p < 0.01$) was observed, but post hoc analysis did not reveal any significant between-trial difference in split times or power output during any single 5-km segment. Nevertheless, a trend ($p = 0.079$) towards higher power output occurred in the CIT trial (238.0 ± 32.8 W) compared with the PLC trial (226.9 ± 28.8 W) during the final 5-km segment of the 40-km TT.

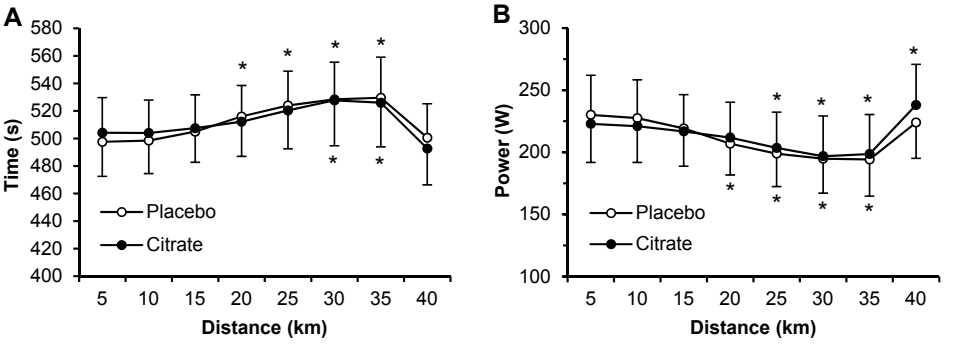

Figure 9. Average 5-km split times (A) and average power output on 5-km segments (B) of the 40-km time-trial. Data are presented as means ± SD, n = 18. *Significantly different ($p < 0.05$) from the first 5-km segment.

Blood Biochemical Parameters

Significant main effects of time ($F = 96.83$, $p < 0.001$), trial ($F = 32.18$, $p < 0.001$) and trial by time interaction ($F = 23.11$, $p < 0.001$) were evident for plasma ALDO concentration. ALDO levels were lower in the CIT trial compared with the PLC trial before ($p < 0.001$) and after ($p < 0.001$) TT (Figure 10A). During 40-km TT ALDO levels increased in both trials, but the magnitude of the increase was smaller in the CIT trial than in the PLC trial ($p < 0.001$).

Regarding serum CORT levels, time ($F = 34.23$, $p < 0.001$) and trial ($F = 21.41$, $p < 0.001$) significant main effects, but no trial by time interaction ($F = 1.36$, $p = 0.257$) were found. CORT levels were lower in the CIT trial compared with the PLC trial before ($p = 0.039$) and after ($p = 0.001$) 40-km TT (Figure 10B). However, the magnitude of increases in CORT levels during 40-km TT was similar in the 2 trials.

Serum PRL and GH concentrations showed significant main effects of time ($F = 84.91$, $p < 0.001$ and $F = 99.02$, $p < 0.001$, respectively), but no main effects of trial ($F = 3.00$, $p = 0.099$ and $F = 1.39$, $p = 0.254$, respectively) or time by trial interactions ($F = 3.87$, $p = 0.064$ and $F = 0.57$, $p = 0.460$, respectively). Significant increase in concentrations of PRL ($p < 0.001$) and GH ($p < 0.001$) occurred during 40-km TT, but the magnitude of these increases was similar in the 2 trials (Figure 10C and 10D).

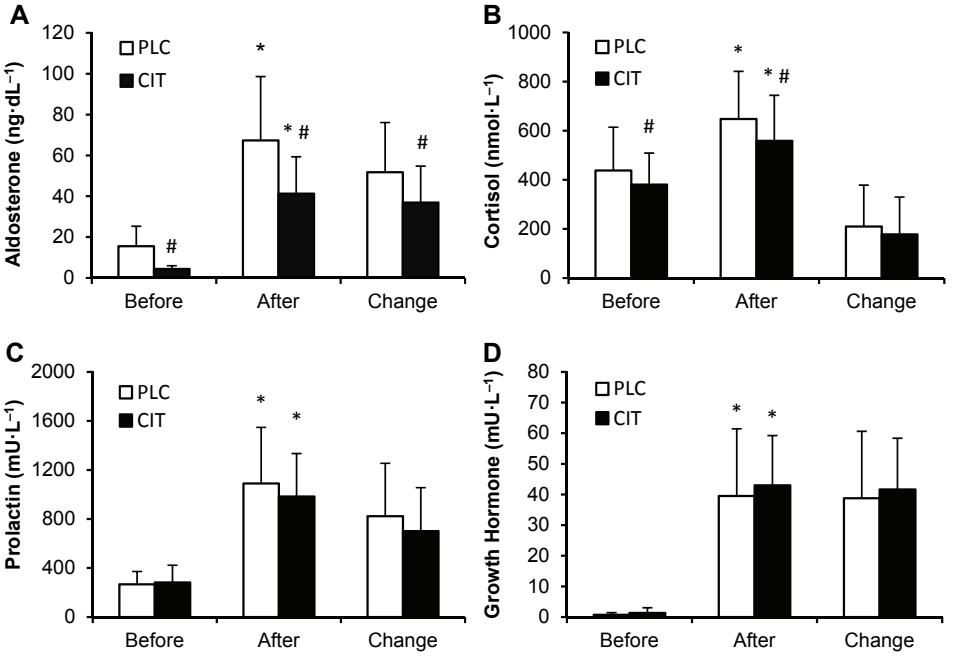

Figure 10. Plasma aldosterone (A), serum cortisol (B), prolactin (C), and growth hormone (D) concentration in placebo (PLC) and sodium citrate (CIT) trials before and after 40-km time-trial. "Change" was calculated as hormone concentration "After" minus hormone concentration "Before". Data are presented as means ± SD, n = 20. Significantly different ($p < 0.05$): * from Before; $^{\#}$ from PLC.

Significant main effects of trial ($F = 23.42$, $p < 0.001$) and time ($F = 132.13$, $p < 0.001$) and trial by time interaction ($F = 17.71$, $p < 0.001$) for blood lactate concentration were observed. Prior to 40-km TT blood lactate levels were similar in the PLC and CIT trials (Figure 11A). However, during 40-km TT, the extent of an increase in blood lactate concentration was greater ($p < 0.001$) in the CIT trial than in the PLC trial. Therefore, after finishing the 40-km TT higher blood lactate level was observed in the CIT trial compared with the PLC trial ($p < 0.001$).

Regarding serum glucose concentration, a significant main effect of time ($F = 5.40$, $p = 0.031$) and time by trial interaction ($F = 6.43$, $p = 0.020$), but no trial main effect ($F = 1.01$, $p = 0.327$) were found. Prior to 40-km TT, serum glucose concentration did not differ in the 2 trials ($p = 0.844$), but after exercise glucose level tended to be lower in the CIT trial compared with the PLC trial ($p = 0.055$) (Figure 11B). However, during 40-km TT, the extent of a decrease in serum glucose concentration was greater in the CIT trial compared with the PLC trial ($p = 0.020$). Therefore, after finishing the 40-km TT, serum glucose concentration had fallen below the before exercise level in the CIT ($p < 0.001$) but not in the PLC trial ($p = 0.643$).

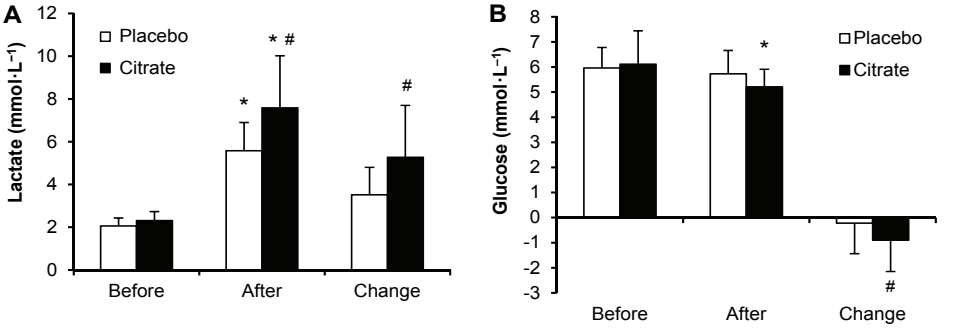

Figure 11. Blood lactate (A) and serum glycose (B) concentration before and after 40-km TT. "Change" was calculated as lactate/glucose concentration "After" minus lactate/glucose concentration "Before". Data are presented as means ± SD, n = 20. Significantly different ($p < 0.05$): * from before; # from placebo.

Thermoregulatory responses during time-trial

Significant main effect of time ($F = 243.53$, $p < 0.001$) and time by trial interaction ($F = 3.10$, $p < 0.001$) were evident for changes in T_c. Post hoc analysis revealed significantly higher T_c ($p = 0.033$) in the PLC trial (39.65 ± 0.52 °C) than in the CIT trial (39.54 ± 0.50 °C) at the finish of the 40-km TT (Figure 12A).

There was a significant main effect of time ($F = 60.77$, $p < 0.001$) but not of trial ($p > 0.05$) for mean T_{sk} (Figure 12B). No time by trial interaction effect ($p > 0.05$) was observed for T_{sk}.

Significant main effect of time ($F = 119.79$, $p < 0.001$) was evident for T_{gr}; however, no main effects of trial or trial by time interaction ($p > 0.05$ in both cases) for T_{gr} were observed. T_{gr} increased from 2.40 to 3.34 °C and from 2.39 to 3.31 °C in the PLC and CIT trials, respectively (in both occasions $p < 0.001$).

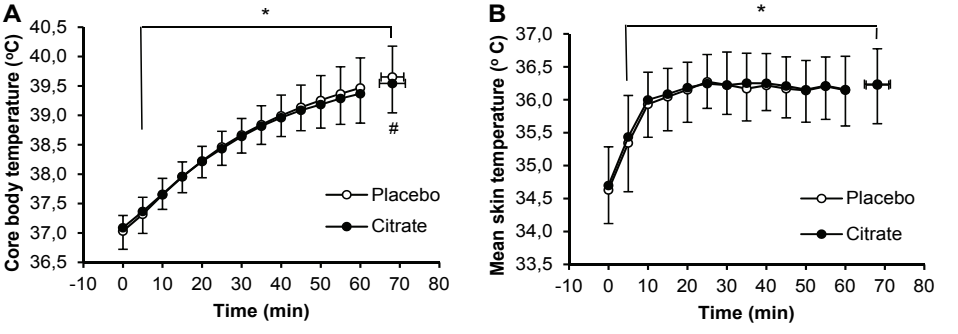

Figure 12. Core body temperature (A) and mean skin temperature (B) during 40-km time-trial. Data are presented as means ± SD, n = 20. Significantly different ($p < 0.05$): * from time point 0; # from placebo.

Hydration status during time-trial

During 40-km TT, reduction in BM was greater in the PLC trial compared with the CIT trial (Table 3). Water intake in the CIT trial significantly (19%, $p = 0.002$) exceeded that observed in the PLC trial, but sweat losses were similar in the 2 trials. Likewise, the extent of an increase in SOsm and that of a decrease in PV (Figure 8) did not differ in the 2 trials during 40-km TT. However, at the end of the performance test compared with the pre-DE time point, PV had decreased to a lower level in the PLC trial than in the CIT trial ($p < 0.001$; Figure 8).

Heart rate during time-trial

A significant main effect of time ($F = 297.94$, $p < 0.001$) and trial by time interaction ($F = 3.70$, $p < 0.001$) were evident for HR, but there was no main effect of trial ($p > 0.05$). HR registered immediately before the start of the 40-km TT was higher ($p < 0.001$) in the CIT trial (95.6 ± 12.9 beats·min^{-1}) compared with the PLC trial (89.6 ± 11.5 beats·min^{-1}) (Figure 13).

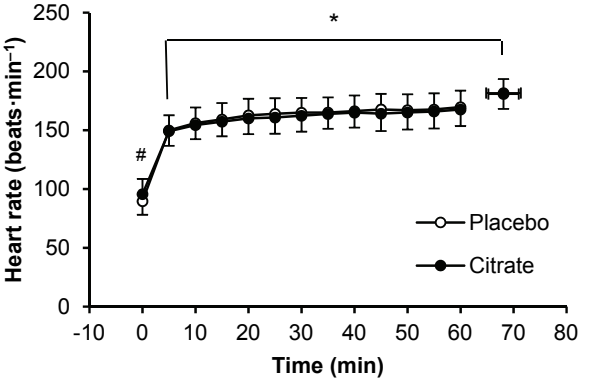

Figure 13. Heart rate during 40-km time-trial. Data are presented as means ± SD, $n = 19$. Significantly different ($p < 0.05$): * from time point 0; # from placebo.

Ratings of perceived exertion and fatigue during time-trial

No main effect of trial was observed for RPE or RPF ($p > 0.05$ in both cases), but main effect of time was evident for both RPE ($F = 439.83$, $p < 0.001$) and RPF ($F = 544.42$, $p < 0.001$). Significantly higher ($p < 0.05$) RPE levels were evident already after 5-km of cycling compared to time point 0. There was no trial by time interactions for RPE or RPF ($p > 0.05$ in both cases). At the beginning (CIT, 6.1 ± 0.3; PLC, 6.5 ± 1.0; $p > 0.05$) and end (CIT, 19.3 ± 0.9; PLC, 19.5 ± 0.8; $p > 0.05$) of the 40-km TT similar levels of RPE were recorded in the 2 trials. Also similar levels of RPF in both trials were recorded at the

beginning (CIT, 1.1 ± 0.9; PLC, 1.2 ± 1.1; $p > 0.05$) and end (CIT, 9.0 ± 1.6; PLC, 9.4 ± 1.2; $p > 0.05$) of the 40-km TT.

Gastrointestinal distress

Prevalence of GI symptoms was similar in the PLC (6 reports) and CIT (10 reports) trials ($\chi^2 = 1.67$, $p > 0.05$). Four participants reported moderate flatulence and one felt bowel urgency in the evening preceding the 40-km TT in the PLC trial. Two of these four participants mentioned very mild stomach ache in the evening preceding the 40-km TT in the CIT trial. Three participants reported mild diarrhea before 40-km TT only in the CIT trial. One participant vomited during 40-km TT in both trials. At the end of the experimental part of the study, exactly half of the participants correctly figured out the substance that they had administered in the 2 trials.

Correlation analysis

During TT, the only hormone the increases of which significantly correlated with increases in blood lactate concentration was CORT and the relationship occurred only in the PLC trial: $r = 0.566$, $p = 0.009$. The only hormone the increases of which significantly correlated with increases in T_c was PRL: $r = 0.609$ ($p = 0.004$) in the PLC trial and $r = 0.572$ ($p = 0.008$) in the CIT trial. Based on pooled data from PLC and CIT trials, plasma ALDO levels before TT correlated with changes in PV during time interval between the beginning of DE and end of the recovery period ($r = –0.520$, $p = 0.001$). Moreover, plasma ALDO levels after TT correlated with changes in PV during time interval between the beginning of DE and end of TT ($r = –0.338$, $p = 0.033$). Finally, based on pooled data from PLC and CIT trials, serum CORT levels before TT correlated with serum glucose concentrations after exercise ($r = 0.429$, $p = 0.006$).

6. DISCUSSION

6.1. Caffeine study

This study was unique because its design allowed direct comparison of the effects of CAF in female and male participants exercising in the heat. We hypothesized that the endurance-enhancing effect of CAF would appear in males only or would be greater in males than in females. The results demonstrate that CAF did not reveal any ergogenic effect in female participants, but, contrary to our hypothesis, it also had no impact on endurance capacity in males. Nevertheless, CAF decreased RPE and RPF in males, but not in females.

Our participants became hyperthermic (T_c above 38 °C) within the first 20–30 min of moderate-intensity exercise. Hyperthermia induced central fatigue appears to become most relevant during prolonged moderate-intensity exercise, whereas reduced oxygen delivery to active muscles may limit performance during high intensity exercise only (Nybo, 2008). Martin and Rattey (2007) and Russ and Kent-Braun (2003) have shown that central fatigue is a more important performance-limiting factor in men than in women. Recently, Adan and colleagues (2008) demonstrated greater effects (less somnolence and greater activation) of CAF in men than in women, and this observation is in line with that of Botella and Parra (2003). On the other hand, there is strong evidence for a CNS-mediated endurance-enhancing effect of CAF from animal experiments (Davis et al., 2003), and a central mechanism of action of CAF is considered to be involved in improving endurance in humans as well (Tarnopolsky, 2008). Taken together, these data (Adan et al., 2008; Botella and Parra, 2003; Davis et al., 2003; Martin and Rattey, 2007; Russ and Kent-Braun, 2003; Tarnopolsky, 2008) suggest that, regarding endurance capacity in the heat, females may benefit less than do males, or even not at all, from acute pre-exercise CAF ingestion. The findings of the current study support this suggestion, because CAF had no ergogenic effect at all in our female participants.

Another unique aspect of this study was the use of a test that consisted of a treadmill walk to exhaustion. We used a time to exhaustion treadmill protocol with the intention of creating the most favorable conditions for detecting a putative endurance-enhancing effect of CAF in a warm environment. Considering that even the potentially less appropriate cycling TT-s have yielded some evidence for the presence of an ergogenic effect of CAF in the heat in non-heat-acclimated males (Ganio et al., 2011; Pitchford et al., 2014), we expected to corroborate these data. However, contrary to our expectation, but similar to the findings of Cheuvront and colleagues (2009) and Roelands and colleagues (2011), CAF had no impact on endurance ability in our male participants.

Cheuvront and colleagues (2009) and Roelands and colleagues (2011) administered a single 6–9 $mg \cdot kg^{-1}$ dose of CAF to their participants and reported that CAF increased T_c during exercise in the heat. Furthermore,

Roelands and colleagues (2011) suggested that such thermogenic effect might negate any potential ergogenic benefit of CAF in the heat. Pitchford and colleagues (2014), on the basis of their own findings and those of Ganio and colleagues (2011), speculated that relatively small doses of CAF, or a distribution of bigger doses, may prevent the thermogenic effect of CAF and increase the likelihood of an ergogenic effect of this drug in warm conditions. Our findings partially support this view, because we administered CAF in 2 small doses and observed similar T_c, T_{sk}, and T_b in the CAF and PLC trials. However, prevention of CAF's thermogenic effect did not improve endurance capacity in our participants. Consequently, the thermogenic potential of CAF is not a satisfactory explanation for the absence of an ergogenic effect of this drug in warm environments.

Our observation of an increased blood lactate response to exercise in the CAF compared with the PLC trial is in accordance with the findings of Cheuvront and colleagues (2009), but most other relevant studies (Cohen et al., 1996; Ganio et al., 2011; Roelands et al., 2011) have reported no impact of CAF on blood lactate levels during exercise in the heat. Exercise HR in the heat has been reported to be not influenced by CAF (Cheuvront et al., 2009; Ganio et al., 2011; Pitchford et al., 2014; Roelands et al., 2011; Zhang et al., 2014), but our participants' HRs were greater in the CAF than in the PLC trial, although the difference was small (approximately 3 beats·min^{-1}). As a novel finding, we observed no between-gender differences in the influence of CAF on blood lactate levels or HR during exercise in the heat.

Dehydration, especially in excess of 2% of BM and combined with a high T_{sk}, markedly impairs endurance performance (Sawka et al., 2012). Cohen and colleagues (1996) suggested that dehydration in the magnitude of approximately 4% of BM, with a concomitant > 10% average reduction in PV, abolished any possible ergogenic effect of CAF in their runners. Our participants were hydrated equally at the beginning of the PLC and CAF trials, as evidenced by similar morning USG and UOsm and pre-exercise BM. Approximately 59%–63% of sweat loss that occurred during exercise was compensated for by simultaneous water intake, resulting in merely a 1.1%–1.2% BM loss and a 4.2%–5.3% reduction in PV, both of which could be considered modest changes. Similar BM and PV decreases in the PLC and CAF trials suggest that CAF had no impact on hydration status during exercise. Average T_{sk}, however, reached 38 °C during exercise. This value is much higher than that (approximately 33 °C) observed in participants who improved endurance performance in the heat with CAF ingestion (Ganio et al., 2011). Hot skin is considered a primary factor impairing aerobic exercise performance, independent of hydration status and T_c (Sawka et al., 2012). Thus, a high T_{sk} may be one factor that abolished the endurance-enhancing effect of CAF in our participants.

Meta-analysis revealed that in temperate environmental conditions, CAF, compared with PLC, may increase exercise performance by approximately 11% and that an average 5.6% reduction in RPE obtained during exercise may account for approximately 29% of the ergogenic effect of this drug (Doherty

and Smith, 2005). The same meta-analysis concluded that effort sense and fatigue experienced at the conclusion of exhausting exercise are essentially the same, regardless of treatment. There is a paucity of data concerning the impact of CAF on RPE during exercise in the heat. Some authors have reported similar RPE values in CAF and PLC trials in males, but the measures were taken in the final minute only (Cheuvront et al., 2009) or at the end of the performance test (Ganio et al., 2011; Roelands et al., 2011). Pitchford and colleagues (2014) and Zhang and colleagues (2014) obtained RPE values from males, and Cohen and colleagues (1996) from a mixed group of males and females, at several time points during exercise and also found that the average RPE in the CAF-supplemented trial did not differ from that in the PLC trial. In our male participants, the average RPE was significantly (7.5%) lower during exercise in the CAF than in the PLC trial, but contrary to the males, there was no impact of CAF on RPE in females. Reduction in RPE is considered one of the main CNS related actions of CAF leading to improved endurance in temperate environments (Doherty and Smith, 2005; Tarnopolsky, 2008). Thus, the data regarding our male participants suggest that some factor, possibly a fed state and/or a high T_{sk}, may abolish the beneficial effect of lowered RPE on endurance ability in the heat.

Similar to RPE scores, RPF scores obtained from our male participants during exercise were significantly (on an average, 15.5%) lower in the CAF compared with the PLC trial, with no between trial difference in females. Generally, our RPE and RPF data are in accordance with previous observations (Adan et al., 2008; Botella and Parra, 2003; Temple et al., 2009, 2010) about a greater sensitivity of men than women to acute CAF ingestion. In line with the reports of others (Cheuvront et al., 2009; Roelands et al., 2011), our study showed that CAF ingestion did not change TS in our participants.

Similar to Zhang and colleagues (2014), we observed deterioration in mood and perceived arousal during exercise in the heat, but no effect of CAF on these estimates. At the same time, the CFF threshold indicated an increase in cortical arousal. Considering that CAF's stimulating impact on mood is well documented in temperate environmental conditions (Lorist and Tops, 2003; Smith et al., 1999), our data and that of Zhang and colleagues (2014) suggest that exposure to combined exercise and heat stress may mask the effects of CAF on the psychological state.

The PLC effect, defined as a change attributable only to an individual's belief in the efficacy of treatment, has been demonstrated to improve physical performance in well-trained male cyclists (Beedie et al., 2006) and in untrained healthy males (Pollo et al., 2008) who believed they had ingested CAF but were actually administered PLC. Surprisingly, in our female participants, but not in our male participants, a stronger belief that they had been administered CAF was associated with a lower endurance capacity. To the best of our knowledge, only Adan and colleagues (2008) have previously reported gender differences in the occurrence of a PLC effect: they found that decaffeinated coffee produced a greater decrease in somnolence and a more pronounced increase in activation in

women than in men. In addition, anxiety sensitivity has been found to moderate a negative psychological response to CAF (Keogh and Chaloner, 2002), especially in women (Keogh and Birkby, 1999). The underlying mechanism of these effects is presumed to be the negative interpretive bias: individuals high in anxiety sensitivity might misinterpret bodily sensations associated with CAF ingestion and respond in a more negative manner (Telch et al., 1996). These moderating effects of anxiety sensitivity might explain the observed negative association between the belief of ingesting CAF and endurance capacity in the heat in females. However, because we did not measure anxiety sensitivity in our participants, further studies are needed to explore in more detail the occurrence of gender differences in psychological response to endurance exercise in the heat after CAF ingestion.

Our CAF study has limitations. First, the type of oral contraceptives used by the female participants and the participants' stage in their contraceptive cycle was not controlled. Oral contraceptives prolong the elimination half-life of CAF (Patwardhan et al., 1980) and may have led to different CAF levels between the sexes, even though the women and men ingested the same relative dose. Oral contraceptives may also influence HR and T_c responses during exercise in the heat (Martin and Buono, 1997). Considering that our female participants used contraceptives in both trials, the specific impact of contraceptives on HR and T_c could be expected to be more or less equal under the CAF and PLC treatments.

Second, the nutrient content of the breakfast on the trial days was not analyzed quantitatively. However, the dietary intake of the participants was well controlled qualitatively to ensure similar nutritional and hydration status before all the testing sessions. Nevertheless, the findings of Desbrow and colleagues (2009) and Acker-Hewitt and colleagues (2012) suggest that a fed state may eliminate the ergogenic effect of CAF on endurance performance that has often been observed in fasted participants. Because our participants were tested 2 h after breakfast, their fed state may be one factor that hindered the occurrence of the endurance-enhancing properties of CAF.

6.2. Sodium citrate study

Our study is the first to investigate whether CIT ingestion during recovery from DE improves cycling performance during subsequent 40-km TT in a warm environment. Also this study investigates whether CIT ingestion in the amount that has been shown to induce metabolic alkalosis and an acute increase in PV (Timpmann et al., 2012) influences the responses of blood stress hormones to prolonged self-paced cycling exercise in a warm environment. The main findings of the study are that CIT compared with PLC enhanced rehydration and increased PV, but had minor impact on thermoregulation and did not influence cycling performance in the heat in recreationally active male non-heat-acclimated endurance athletes. The novel aspect demonstrates the capacity of CIT to reduce blood ALDO levels during exercise-heat stress that is strong

stimulus for ALDO secretion (Akerman et al., 2017). Similarly to ALDO, after CIT ingestion, significantly lower blood CORT levels occurred prior to and immediately after completing the TT. However, there was no between-trial difference in the magnitude of the increases in CORT levels that occurred during exercise. CIT ingestion did not influence GH and PRL levels in our participants.

Assuming that our participants were in a euvolemic status before DE, prior to 40-km TT their PV had expanded by 7.8% in the CIT trial compared with 0.9% shrinkage observed in the PLC trial. Acute PV expansion in the range of 7%–8% may improve endurance ability by 20%–25% (Mora-Rodriguez and Hamouti, 2013), but our participants' TT time was similar after CIT and PLC ingestion. We are aware of 4 published studies addressing the potential impact of acute PV expansion or pre-exercise hyperhydration on endurance cycling ability in a warm environment. Improved TT performance (Hamouti et al., 2014) and prolonged time to exhaustion (Sims et al., 2007a) were observed in association with 4%–5% increases in PV, and improved TT performance was also reported owing to increased pre-exercise water retention in the range of 577–673 mL (Morris et al., 2015). In contrast to these results, Watt et al. (2000) found no influence of 13.1% acute PV expansion on TT performance.

Several factors may explain discrepancy in the results of different studies regarding the impact of acute pre-exercise PV expansion or hyperhydration on endurance cycling performance in the heat. First, TT performance may be influenced by the ability to maintain increased PV throughout exercise bout. Indeed, participants studied by Hamouti et al. (2014) maintained hypervolemic state throughout TT and achieved higher mean power output compared with euvolemic condition, whereas participants in the study conducted by Watt et al. (2000) experienced rapid loss of PV expansion at an early stage of exercise and did not improve performance. Nevertheless, in our participants persistent hypervolemic and hyperhydrated state in the CIT trial compared with the PLC trial did not result in improved performance.

Second, endurance cycling ability after acute pre-exercise PV expansion or hyperhydration may depend on the availability of drinking water during exercise in the heat. Notably, in all previous studies that reported improved TT performance (Hamouti et al., 2014; Morris et al., 2015) or prolonged time to exhaustion (Sims et al., 2007a), no fluids were consumed during exercise, whereas our participants drank water *ad libitum*. Arnaoutis et al. (2012) demonstrated that, possibly through activation of pharyngeal receptors, even a small volume of water ingested during endurance exercise increased time to exhaustion in the heat in dehydrated trained cyclists. Thus, it seems possible that freely available drinking water during TT masked the potential ergogenic effect of acute pre-exercise PV expansion in our participants.

Third, the impact of acute pre-exercise PV expansion or hyperhydration on cycling performance may be influenced by the duration of TT in the heat. In previous studies (Hamouti et al., 2014; Morris et al., 2015) performance tests began with 120 and 60 min of cycling at constant work rate, but the TT segment of the tests lasted merely 10.0–10.4 min and 12.2–14.4 min, respectively. As we

intended to mimic a cycling competition, our participants completed a 40-km TT, the duration of which was much longer (~68 min). Hamouti et al. (2014) and Morris et al. (2015) reported 7.4% higher mean power output and 9.2%–11.4% shorter TT completion time after inducing pre-exercise hypervolemia or hyperhydration, respectively. We only observed a tendency towards greater power output during the final 5-km segment of the 40-km TT in the CIT trial compared with the PLC trial. Thus, it appears that a chance for improving performance owing to acute pre-exercise hypervolemia or hyperhydration may be greater during relatively short (up to approximately 15 min) than in longer (over 1 h) cycling TTs in the heat.

Sims et al. (2007a, 2007b) suggested that increased PV provides more fluid for redistribution, a benefit of which is slower rise in T_c that may contribute to increased time to exhaustion during constant-pace cycling or running in the heat. This suggestion is supported by observation of enhanced sweating responsiveness during exercise in the heat in hypervolemic state (Fortney et al., 1981). However, in our participants during the CIT trial, persistent hypervolemia was observed, but the time course of rise in T_c and sweat rate were similar after CIT and PLC supplementation. Marginally (by 0.1 °C) lower T_c in the CIT trial compared with the PLC trial only occurred at the end of 40-km TT. Similarly, Hamouti et al. (2014) and Morris et al. (2015) observed minor impact of acute PV expansion or hyperhydration on T_c and sweat rate during subsequent cycling TT in the heat. Nevertheless, contrary to us, both groups (Morris et al., 2015; Hamouti et al., 2014) reported improved TT performance in conditions of hypervolemia or hyperhydration. Altogether the literature and the data of the current study suggest that better control of T_c achieved through acute PV expansion may partially explain improved endurance capacity in the heat, but the influence of hypervolemia on cycling TT performance appears to depend on other mechanisms.

Hamouti et al. (2014) concluded that pre-exercise PV expansion improves cycling TT performance in the heat through better maintenance of stroke volume and cardiac output during exercise. However, actually they measured these parameters during cycling at constant work rate, but not during subsequent TT. We only recorded HR that was not influenced by PV expansion during 40-km TT. This finding is consistent with the observations of others (Hamouti et al., 2014; Morris et al., 2015; Watt et al., 2000).

There is evidence that hot skin (defined as $T_{sk} > 35$ °C), not high T_c, may be the primary factor limiting aerobic exercise performance in the heat (Cheuvront et al., 2010; Sawka et al., 2012). Hot skin narrows the T_{gr}, which increases skin blood flow and may limit muscle as well as cerebral blood flow (Sawka et al., 2012). In our participants in both the PLC and CIT trials, narrow T_{gr} of 2.4–3.4 °C was observed and T_{sk} exceeded the level of 35 °C already 5 min after the start of 40-km TT and remained persistently above 36 °C since the fifteenth minute of exercise. Hamouti et al. (2014) did not report T_{sk} during TT, but during preceding cycling at constant work rate it remained below 35 °C independently of PV status. Thus, higher T_{sk} during TT in our participants

compared with the subjects studied by Hamouti et al. (2014) may be one of the factors that explain the absence of ergogenic effect of acute hypervolemia in our participants.

Serum hyperosmolality inhibits thermoregulatory responses to heat stress (Sawka et al., 2000; Takamata et al., 2001) and acute ingestion of CIT is known to result in elevated blood Na^+ concentration (Ööpik et al., 2010; Vaher et al., 2015) that may increase SOsm. In our participants, higher SOsm occurred in the CIT trial compared with the PLC trial immediately prior to 40-km TT. However, in absolute terms the between-trial difference was small and in either trial SOsm did not reach the levels that have been shown to inhibit thermoregulatory responses (Takamata et al., 2001). Furthermore, there was no between-trial difference in SOsm at the end of 40-km TT. Therefore, we consider unlikely that the absence of meaningful thermoregulatory effects was caused by increased SOsm in the CIT trial.

Heat stress increases RPE while anything that alters RPE may affect motivation-driven motor-neural firing and influence physical work ability (Cheuvront et al., 2010). Pre-exercise acute hypervolemia was associated with decreased RPE and increased time to exhaustion during constant-load exercise in the heat (Sims et al., 2007a, 2007b). Hamouti et al. (2014) and Coles and Luetkemeier (2005) reported that in acute hypervolemic state RPE tended to be lower at the end of constant-load cycling exercise in the heat and temperate environment, respectively, but they did not register RPE during TT that followed. Our results suggest that in the presence of hot skin, approximately 8% expansion of PV has no impact on RPE or 40-km cycling performance in the heat.

CIT ingestion may improve physical performance in high-intensity exercise through inducing metabolic alkalosis and increasing blood buffering capacity (Burke et al., 2006; Carr et al., 2011; Requena et al., 2005). In the current study we did not measure blood pH, HCO_3^- concentration, or base excess. However, Timpmann et al. (2012), using the same CIT dose and administration protocol, observed significant increases in all these 3 parameters. Therefore, we assume that CIT provoked metabolic alkalosis in our participants. Considering that elevated post-exercise blood lactate level in alkalotic state is a typical finding in previous studies (Requena et al., 2005), higher blood lactate concentration in the CIT trial than in the PLC trial observed after the 40-km TT could be interpreted as indirect evidence of increased blood-buffering capacity. Nevertheless, some well-controlled studies have revealed no impact of alkalosis induced by CIT (Schabort et al., 2000) or $NaHCO_3$ (Northgraves et al., 2014; Peart et al., 2013; Price et al., 2003; Raymer et al., 2004) ingestion on blood lactate response to exercise. Therefore, lack of definite data characterizing blood-buffering capacity in our participants should be considered one of the limitations of our study.

Potteiger et al. (1996) and Schabort et al. (2000) assessed the potential impact of CIT on cycling performance in temperate environment. Potteiger et al. (1996) concluded that alkalosis induced by 500 $mg \cdot kg^{-1}$ of CIT ingestion

improved 30-km TT time, whereas Schabort et al. (2000) reported dose-dependent increases in blood buffering capacity without any change in 40-km TT time after administering 200–600 $mg \cdot kg^{-1}$ of CIT. Our findings add to these data, showing that ingestion of 600 $mg \cdot kg^{-1}$ of CIT during recovery after DE has no impact on subsequent 40-km cycling performance in the heat.

In goats, metabolic alkalosis suppressed blood ALDO concentration in the absence of detectable changes in plasma K^+ concentration or renin activity (Augustinsson et al., 1989), which are considered primary regulators of ALDO secretion (Bollag, 2014; El Ghorayeb et al., 2016). However, in sedentary rats, alkalosis did not decrease, but rather increased plasma ALDO concentration (Lieu et al., 2013). In young men, ingestion of either $NaHCO_3$ or $KHCO_3$ induced metabolic alkalosis, but neither of these substances suppressed plasma ALDO levels (Lindinger et al., 2000). Considering these data, it seems unlikely that reduced ALDO levels were induced by metabolic alkalosis in our participants in CIT trial.

In temperate environmental conditions, CIT compared to PLC ingestion induced higher serum Na^+ and lower K^+ concentrations (Ööpik et al., 2010) and exactly the same pattern of changes in blood ALDO levels as observed in the current study. Therefore, it is plausible that a similar impact of CIT on blood electrolyte levels occurred in our participants. Increase in K^+ is considered a strong, and decrease in Na^+ a relatively weak, stimulus for ALDO secretion (Bollag, 2014; El Ghorayeb et al., 2016). In light of these data, it is likely that CIT-induced changes in blood K^+ and Na^+ concentrations were among the factors that reduced ALDO levels in our participants in resting state and during exercise in the heat. Unfortunately, the lack of definite data regarding the electrolyte levels may be considered as a limitation of this study.

In temperate environmental conditions, expanded PV and decreased blood ALDO levels after CIT ingestion were observed (Ööpik et al., 2010). Others (Grant et al., 1996; Roy et al., 2001) induced an acute pre-exercise PV expansion by infusion of dextran solution and reported blunted blood ALDO responses to prolonged constant-load cycling exercise. As with Grant et al. (1996), we observed an inverse relationship between the PV changes and blood ALDO levels. Nevertheless, in the study by Grant et al. (1996) the effect of PV expansion was manifested only during exercise, whereas in our participants decreased blood ALDO level occurred already at resting state prior to the TT. This discrepancy could be explained by the specifics of the methods used for inducing PV expansion (dextran infusion vs CIT ingestion) and/or by different time interval during which pre-exercise PV expansion was achieved (2 h vs 16 h). Thus, in light of previous data (Grant et al., 1996; Roy et al., 2001; Ööpik et al., 2010) our findings suggest that expansion of PV was one of the factors that decreased blood ALDO levels at resting state and during exercise in the heat.

Cortisol is among the most widely studied markers of stress (Hackney, 2006; Staufenbiel et al., 2013). In a temperate environment, $NaHCO_3$ compared to PLC ingestion attenuated serum CORT responses to four consecutive 30 s bouts

of maximal cycling exercise (Wahl et al., 2010). These authors concluded that exercise-induced acidosis stimulated CORT secretion and that $NaHCO_3$ induced metabolic alkalosis was partly responsible for blunted CORT response. Without ingestion of alkalizing substances, parallel increases in blood lactate level and acidity occur during exercise (Robergs et al., 2004). Therefore, observation that during TT in PLC trial increases in serum CORT levels correlated positively with changes in lactate concentrations suggests that exercise-induced acidosis influenced CORT secretion in our participants. The absence of such correlation in CIT trial may be explained by the fact that induced metabolic alkalosis facilitates efflux of both lactate and H^+ from contracting muscle cells and at the same time more efficiently buffers H^+, leading to greater elevations in blood lactate levels, but attenuating increases in blood acidity (Bishop et al., 2004; McNaughton et al., 1999; Potteiger et al., 1996) and CORT levels.

Cooper et al. (2010) and Rhind et al. (2004) reported a significant relationship between exercise-induced increases in T_c and plasma CORT levels. In our participants, CIT ingestion induced acute PV expansion that may slow down increases in T_c during exercise (Mora-Rodriguez and Hamouti, 2013). However, lower serum CORT level in CIT trial occurred already before the start of the TT when T_c did not differ. Immediately after exercise, T_c was 0.1 °C lower in CIT trial, but the between-trial difference in serum CORT level was similar to that observed prior to exercise. Furthermore, increases in T_c did not correlate with increases in serum CORT levels during exercise in either trial. Thus, it is unlikely that T_c induced between-trial differences in serum CORT level in our participants.

Nutritional status, nutrient timing, intensity and duration of exercise and total amount of work completed are important factors influencing endocrine responses to exercise (Hackney and Smith-Ryan, 2013; McMurray and Hackney, 2000). Our participants' energy and nutrient intake and timing was carefully controlled and kept identical during the 16-h recovery period. Time-trial time and total amount of work completed during exercise did not differ in the two trials. Thus, these factors could not induce the between-trial differences observed in ALDO and CORT levels. Similarly, these factors do not explain greater decrease in serum glucose concentration in CIT compared to PLC trial during exercise. On the other hand, CORT possesses a crucial role in maintaining blood glucose level (McMurray and Hackney, 2000), and it has been shown that during prolonged exercise, lower CORT levels are associated with greater decreases in plasma glucose concentrations (Scheen et al., 1998). Considering positive correlation observed between pre-exercise CORT and post-exercise glucose concentrations in our participants, it is plausible that during TT in CIT trial, the greater decrease in serum glucose concentration was at least partly due to lower CORT level.

Several-fold increases in serum PRL concentrations occurred during TT, but the magnitude of this response was similar in the two trials. Exercise stimulates PRL secretion (Rojas Vega et al., 2012), whereas the magnitude of rise in blood PRL level in response to similar exercise demands is markedly greater in the

heat compared to temperate (Burk et al., 2012; Mündel et al., 2010) or cold (Brisson et al., 1986; Pitsiladis et al., 2002) environments. De Meirleir et al. (1985) suggested that the trigger mechanism of PRL release is exercise-induced metabolic acidosis, and Rojas Vega et al. (2006) found that metabolic alkalosis reduced PRL response to short-duration exhausting exercise in a temperate environment. Considering the proven alkalotic effect of the dose used and protocol of administration of CIT (Timpmann et al., 2012), our findings suggest that orally induced metabolic alkalosis did not affect serum PRL response to TT in the heat.

A significant relationship occurs between T_c and PRL response to exercise (Bridge et al., 2003; Brisson et al., 1991; Burk et al., 2012; Strachan et al., 2004). Our data are consistent with this observation as exercise-induced increases in serum PRL concentrations correlated with increases in T_c. Taken together, our data suggest that increases in T_c stimulate PRL secretion during prolonged cycling exercise in the heat and that CIT ingestion does not modulate serum PRL response in this situation.

Secretory stimuli are similar for PRL and GH (Rojas Vega et al., 2006). Similar general patterns of GH and PRL serum levels in our participants, including the absence of any effect of CIT ingestion, are in accordance with this notion. However, Gordon et al. (1994) and Wahl et al. (2010) reported that metabolic alkalosis attenuated blood GH response to short-term high-intensity exercise in temperate environmental conditions. Assuming that our participants were in an alkalotic state in CIT trial, our data suggest that alkalosis did not modulate serum GH responses to prolonged exercise in the heat.

Literature shows, with a few exceptions (Bridge et al., 2003; Laing et al., 2008), that GH secretion is sensitive to increases in T_c (Christensen et al., 1984; Niess et al., 2003; Pitsiladis et al., 2002), and that positive relationships occur between T_c and GH levels during prolonged exercise in the cold (Wheldon et al., 2006) and in the heat (Ftaiti et al., 2008; Rhind et al., 2004; Ööpik et al., 2014). However, all previous studies which revealed direct relationship between exercise-induced changes in T_c and blood GH levels employed constant-load exercise of fixed duration (Ftaiti et al., 2008; Rhind et al., 2004; Wheldon et al., 2006) or to volitional exhaustion (Ööpik et al., 2014), whereas in the current study, the participants performed self-paced TT. Thus, the mode of self-pacing protocol might be a factor confounding the relationship between changes in T_c and serum GH in our participants. Nevertheless, our data reveal that in the heat, prolonged self-paced cycling exercise induces immense increases in serum GH levels, and that CIT ingestion does not modulate GH response in these conditions.

Gastrointestinal distress concomitant with ingestion of alkalizing substances is considered an important factor reducing potential performance-enhancing effect and limiting the use of these substances in sports (Burke and Pyne, 2007). Schabort et al. (2000) considered it possible that failure of CIT to produce improvements in cycling performance could have been due to gastrointestinal distress experienced by their participants. In the current study, 7 participants

reported mild gastrointestinal symptoms, but the prevalence and severity of these symptoms did not differ in the 2 experimental trials. Thus, gastrointestinal distress was apparently not the reason for absence of ergogenic effect of CIT in our participants.

To summarize, the main likely reasons why CIT supplementation, despite exerting positive impact on rehydration and PV, did not influence endurance cycling performance were availability of drinking water and occurrence of high T_{sk} during TT. Our findings of reduced ALDO and CORT levels before and after TT and blunted acute response of ALDO to TT suggest that CIT ingestion after DE alleviates stress during subsequent heavy endurance cycling bout in a warm environment. As suggested by Peart et al. (2013), reduced stress during exercise may lead to improved recovery. This possibility deserves further research, because in multi-stage road cycling races, improved recovery between stages may strongly influence the final rankings of the competitors. Therefore, investigating only one exercise-recovery-exercise cycle may be considered an important limitation of this study.

7. CONCLUSIONS

1. Caffeine induced greater increases in heart rate and blood lactate concentrations during exercise in the heat in both non-heat-acclimated males and females, but had no impact on changes in body temperature, cortical arousal, perceived arousal, or mood in participants of either gender. Caffeine decreased ratings of perceived exertion and ratings of perceived fatigue in males, but not in females. Nevertheless, caffeine did not influence endurance capacity in the heat in participants of either gender.

2. Sodium citrate ingestion during the 16-h recovery period after exercise-induced dehydration promotes water retention, enhances rehydration, increases plasma volume, and facilitates body mass regain, but has no impact on thermoregulation, ratings of perceived exertion, or 40-km cycling time-trial time in warm environment in male non-heat-acclimated endurance athletes.

3. Sodium citrate compared to placebo ingestion during 16-h recovery period after dehydrating cycling exercise results in decreased blood aldosterone and cortisol levels prior to and after cycling time-trial in warm environment in male non-heat-acclimated endurance athletes. Sodium citrate ingestion also reduces the magnitude of acute increases in blood aldosterone, but not cortisol level, during time-trial. Sodium citrate ingestion has no influence on blood levels of prolactin or growth hormone in these experimental conditions.

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SUMMARY IN ESTONIAN

Toidulisandite mõju hindamine vastupidavuslikule töövõimele kõrge temperatuuriga keskkonnas

Sissejuhatus

Vastupidavuslikul töövõimel on spordis ja militaarses kontekstis sageli selgesti erinev rakenduslik tähtsus. Spordivõistlustel on praktiliselt alati esmatähtis võime läbida kindel vahemaa võimalikult lühikese ajaga. Sõjalistel operatsioonidel on seevastu sageli märksa olulisem võime taluda suurt kehalist koormust võimalikult pika aja vältel. Need vastupidavusliku võimekuse aspektid on erinevate toidulisanditega mõjutatavad erineval määral ja eeldatava mõju ulatuse tuvastamiseks tuleb kasutada ka vastavaid testimismeetodeid. Näiteks kofeiini ergogeenne efekt tuleb kõige paremini esile vastupidavusaladel, mis nõuavad pikka või lausa suutlikkuseni pingutust. Seevastu naatriumtsitraati kasutavad sportlased valdavalt eesmärgiga suurendada keha puhversüsteemide mahutavust, mis parandab sooritusvõimet pigem lühiajalisel (kestusega 1–7 minutit) kõrge intensiivsusega tööl.

Kuigi kofeiini mõju kehalisele töövõimele ja kognitiivsetele funktsioonidele on teaduslikku tähelepanu ja praktilist huvi pälvinud juba pikka aega, on endiselt vähe andmeid kofeiini toimest kehalisele töövõimele kõrge temperatuuriga keskkonnas ning olemasolevad andmed on vastuolulised. Ka naatriumtsitraadi manustamise mõju jalgratturite vastupidavuslikule töövõimele on seni uuritud vaid normaalsetes keskkonnatingimustes ja nende uuringute tulemused on samuti vastuolulised. Samas kõrge temperatuuriga keskkonnas inimese kehaline töövõime langeb ning nii mõnegi toidulisandi mõju võib osutuda erinevaks võrreldes normaalse keskkonnaga.

Seega, käesoleva uurimistöö tulemused lisavad olulist teavet kahe toidulisandi (kofeiin ja naatriumtsitraat) manustamise mõjust vastupidavuslikule töövõimele kõrge temperatuuriga keskkonnas. Sellekohastel teadmistel on suur praktiline väärtus, kuna nii rahvusvahelised spordivõistlused kui ka sõjalised operatsioonid toimuvad sageli piirkondades, kus kõrge temperatuur on üheks oluliseks töövõimet mõjutavaks faktoriks.

Uurimistöö eesmärk

Uurimistöö peamiseks eesmärgiks oli välja selgitada kahe toidulisandi, kofeiini ja naatriumtsitraadi, manustamise mõju aklimatiseerumata treenitud vaatlusaluste vastupidavuslikule töövõimele kõrge temperatuuriga keskkonnas. Lisaks oli töö eesmärk võrrelda meeste ja naiste vahelisi erinevusi kofeiini akuutse manustamise mõjust kognitiivsele töövõimele ning füsioloogilistele funktsioonidele. Naatriumtsitraadi uurimise eesmärgiks oli lisaks veel hinnata toimeaine mõju veestaatusele, termoregulatsioonile ja stressihormoonide tasemele.

Uuritavad ja metoodika

Uuringus osales vabatahtlikkuse alusel nelikümmend kolm tervet, kehaliselt aktiivset mees- ja naissportlast. Uuritavate keskmine (±SD) vanus ja maksimaalne hapnikutarbimise võime (VO_2max) olid vastavalt: kofeiini uuringus meestel 24,9 ± 4,1 a ja 51,7 ± 2,7 $ml \cdot min^{-1} \cdot kg^{-1}$; naistel 22,5 ± 2,0 a ja 45,6 ± 4,0 $ml \cdot min^{-1} \cdot kg^{-1}$; ning naatriumtsitraadi uuringus meestel 30,8 ± 5,4 a ja 57,0 ± 5,9 $ml \cdot min^{-1} \cdot kg^{-1}$.

Kofeiini uuringu põhiosa koosnes kahest vastupidavusliku töövõime testist, mis seisnesid kõndimises liikuval jooksurajal intensiivsusega 60% VO_2max kuni kurnatuseni kõrge temperatuuriga kuivas keskkonnas (42 °C; RH 20%). Seevastu naatriumtsitraadi uuringu põhifaasis väntasid uuritavad veloergomeetril esmalt kõrge temperatuuriga keskkonnas (32 °C; RH 46%) eesmärgiga tekitada *ca* 4%-lise ulatusega dehüdratsioon. Seejärel pärast 16-h taastumisaega sooritasid uuritavad samades keskkonnatingimustes vastupidavusliku töövõime testi, mis seisnes 40 km läbimises võimalikult lühikese ajaga. Mõlema toimeaine uuringud teostati topeltpimeda, randomiseeritud, ristuva uuringukavandi järgi.

Kofeiini uuringus manustati uuritavatele 60 minutit enne töövõime testi algust 4 $mg \cdot kg^{-1}$ kofeiini või platseebot ning vahetult enne pingutuse algust veel 2 $mg \cdot kg^{-1}$ kofeiini või platseebot.

Naatriumtsitraadi uuringu 2 põhifaasi erinevus seisnes taastumisperioodil manustatud toidulisandis (naatriumtsitraat või platseebo). Naatriumtsitraati manustati kokku 600 $mg \cdot kg^{-1}$, mis jaotati 3 võrdseks osaks: esimene kogus kapsleid manustati laboris koos õhtusöögiga pärast dehüdratsioonikoormust, teine kogus võeti kodus 1 h enne magamaheitmist ning kolmas laboris koos hommikusöögiga (2 h enne töövõimetesti).

Tulemused

Tööeelne kofeiini manustamine võrreldes platseeboga vastupidavuslikku töövõimet ei mõjutanud ($p > 0.05$). Mehed suutsid platseebo- ja kofeiiniuuringus kõndida vastavalt 83,1 ± 16,6 min ja 81,6 ± 14,4 min ning naised vastavalt 82,1 ± 15,1 min ja 75,6 ± 11,2 min. Kofeiin võrreldes platseeboga kutsus esile ulatuslikuma ($p < 0,05$) südame löögisageduse ja vere laktaaditaseme tõusu nii meestel kui ka naistel. Samas naha- ja süvatemperatuuris erinevusi ei ilmnenud.

Kofeiin langetas pingutuse subjektiivselt tajutavat raskusastet meestel ($p = 0,022$), aga mitte naistel ($p > 0,05$). Mehed tajusid väsimust kofeiini manustamise foonil oluliselt väiksemana kui platseebo manustamisel ($p = 0,034$), naistel toimeainevahelist erinevust väsimuse tajumises ei ilmnenud ($p = 0,286$).

Naatriumtsitraadi manustamine 16-h taastumisperioodil põhjustas suurema veetarbimise, mistõttu ilmnes ka ulatuslikum kehamassi ja plasma mahu taastumine võrreldes platseeboga ($p < 0,001$). Taastumisperioodile järgneval 40-km ajasõidul tarbisid vaatlusalused naatriumtsitraadi uuringus samuti rohkem vett ja kehamassi tööaegne langus jäi väiksemaks kui platseebo uuringus ($p < 0,05$). Samas higistamises, plasma mahu muutuses, pingutuse subjektiivselt tajutavas

raskusastmes ega ka 40 km läbimise ajas (CIT 68,1 ± 3,3 min, PLC 68,1 ± 2,9 min) erinevusi ei täheldatud ($p > 0,05$). Organismi süvatemperatuur oli 40-km ajasõidu lõpus naatriumtsitraadi uuringus kõigest 0,1 °C madalam kui platseebo uuringus.

Naatriumtsitraadi uuringus ilmnes 72% madalam aldosterooni tase veres enne 40-km ajasõitu ja 39% madalam tase pärast sõitu võrreldes platseeboga ($p < 0,001$). Lisaks oli aldosterooni taseme tööaegne tõus veres oluliselt väiksem kui platseeboga ($p < 0,001$). Kortisooli tase veres oli sarnaselt aldosterooniga madalam enne (13%, $p = 0,039$) ja pärast (14%, $p = 0,001$) töövõime testi, aga tööaegses muutuses erinevusi naatriumtsitraadi ja platseebo vahel ei olnud ($p > 0,05$). Prolaktiini ja kasvuhormooni taset naatriumtsitraadi manustamine ei mõjutanud ($p > 0,05$).

Järeldused

1. Kofeiin võrreldes platseeboga tõstab südame löögisagedust ning kutsub esile ulatuslikuma tööpuhuse laktaadi kontsentratsiooni tõusu veres, aga ei mõjuta naha- ega süvatemperatuuri. Kofeiin vähendab pingutuse subjektiivselt tajutavat raskusastet ja väsimuse taju meestel, aga mitte naistel. Kofeiini tööeelne manustamine ei paranda noorte aklimatiseerumata meeste ega naiste vastupidavuslikku töövõimet kõrge temperatuuriga kuivas keskkonnas.
2. Naatriumtsitraadi manustamine taastumisperioodil pärast dehüdratsiooni soodustab veepeetust, stimuleerib organismi veestaatuse normaliseerumist, suurendab plasma mahtu ja hõlbustab kehamassi taastumist, kuid ei mõjuta termoregulatsiooni, pingutuse subjektiivselt tajutavat raskusastet ega 40-km läbimise aega kõrge temperatuuriga keskkonnas aklimatiseerumata jalgratturitel.
3. Naatriumtsitraadi manustamine taastumisperioodil pärast *ca* 4% dehüdratsiooni põhjustab madalamat aldosterooni ja kortisooli taset veres võrreldes platseeboga vahetult enne ja pärast 40-km ajasõitu kõrge temperatuuriga keskkonnas. Naatriumtsitraadi manustamine võrreldes platseeboga aeglustab aldosterooni, aga mitte kortisooli taseme tööaegset tõusu. Prolaktiini ja kasvuhormooni taset naatriumtsitraadi manustamine võrreldes platseeboga ei mõjuta.

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11.2016–04.2017	Tervise Arengu Instituut, Eesti laste isiksuse, käitumise ja terviseuuring, assistent
12.2015–04.2016	Tartu Ülikool, Meditsiiniteaduste valdkond, Sporditeaduste ja füsioteraapia instituut, assistent
08.2013–08.2014	Põlva Ühisgümnaasium, kehalise kasvatuse õpetaja

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Suvi, S., Mooses, M., Timpmann, S., Medijainen, L., Unt, E., Ööpik, V. (2019) Influence of sodium citrate supplementation after dehydrating exercise on responses of stress hormones to subsequent endurance cycling time-trial in the heat. *Medicina*, 55(4): 103. doi: 10.3390/medicina55040103

Pind, R., Mooses, K., Suvi, S., Purge, P., Viru, M., Pehme, A., Kaasik, P., Mooses, M. (2019). Better economy on indoor track compared to treadmill running with 1% inclination. *Research Quarterly For Exercise and Sport*, 8: (1−9).doi: 10.1080/02701367.2019.1618784

Suvi, S., Mooses, M., Timpmann, S., Medijainen, L.,Narõškina, D., Unt, E., Ööpik, V. (2018) Impact of sodium citrate ingestion during recovery after dehydrating exercise on rehydration and subsequent 40-km cycling time-trial performance in the heat. *Applied Physiology, Nutrition, and Metabolism*, 43(6): 571–579. doi: 10.1139/apnm-2017-0584

Suvi, S., Timpmann, S., Tamm, M., Aedma, M., Kreegipuu, K., Ööpik, V. (2017) Effects of caffeine on endurance capacity and psychological state in young females and males exercising in the heat. *Applied Physiology, Nutrition, and Metabolism*, 42(1): 68–76. doi: 10.1139/apnm-2016-0206

Suvi, S., Timpmann, S., Tamm, M., Aedma, M., Kreegipuu, K., Ööpik, V. (2016). Physical and cognitive performance effects of caffeine in women and men exercising in the heat. *Medicine and science in sports and exercise*, 48(5S Suppl 1): 62. doi: 10.1249/01.mss.0000485195.82151.29

Peamised uurimisvaldkonnad:
Sporditeadused, spordifüsioloogia, vastupidavuslik töövõime kõrge temperatuuriga keskkonnas

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