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

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In all papers, Ivi Vaher was responsible for study design development, recruiting the subjects, conducting measurements, data analysis, and writing manuscripts.

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BE	Base exess
[BE]	Base exess concentration
BM	Body mass
CIT	Sodium citrate
Cl^-	Chloride ion
$[Cl^-]$	Chloride ion concentration
GID	Gastrointestinal distress
H^+	Hydrogen ion
$[H^+]$	Hydrogen ion concentration
HCO_3^-	Bicarbonate ion
$[HCO_3^-]$	Bicarbonate ion concentration
HR	Heart rate
HS	Body heat storage
K^+	Potassium ion
$[K^+]$	Potassium ion concentration
MTC1;4	Monocarboxylate transporter 1;4
Na^+	Sodium ion
$[Na^+]$	Sodium ion concentration
$NaHCO_3$	Sodium bicarbonate
NH_4^+	Ammonium ion
OA^-	Organic anion
PLC	Placebo
Post-Ex	After 5000 m running time trial
Pre-Ex	Before 5000 m running time trial
PV	Plasma volume
RPE	Rating of perceived exertion
RPF	Rating of perceived fatigue
T_c	Rectal temperature
T_{sk}	Weighted mean skin temperature
TS	Thermal sensation
UOSM	Urine osmolality
USG	Urine specific gravity
VO_2 peak	Peak oxygen consumption
VO_2 max	Maximum oxygen consumption
5000-TT	5000 m running time trial

1. INTRODUCTION

Competitive sport is a cultural phenomenon that dates back to Homer's 8th-century BC epic poem *The Iliad* (Grivetti & Applegate, 1997). The ancient Olympic Games exemplified the importance of elite sport in ancient Greek society and continued uninterrupted for over a millennium (Grivetti & Applegate, 1997).

Innate predispositions and training are the primary factors affecting an individual's athletic performance (Guth & Roth, 2013). A comprehensive analysis, however, reveals numerous other factors that could affect the realization of innate athletic potential and the effectiveness of training to a greater or lesser extent. The specialists responsible for preparing athletes for the ancient Olympic Games were aware of the importance of attending to athletes' diet and nutrition, as Grivetti & Applegate (1997) and Skiadas & Lascaratos (2001) noted. In ancient Greek society, these specialists were considered authoritative figures on food and nutrition, on the same level as physicians, thus exemplifying their level of competence (Skiadas & Lascaratos, 2001).

Experimental studies on the use of food and nutrients in the body, including the human body, began in the 17th and 18th centuries and included attention to how food and nutrition affected muscle function (Saltin & Gollnick, 1983). The contemporary scientific comprehension of the correlation between food and nutrition and physical (as well as athletic) performance was chiefly established by Scandinavian sports physiologists during the 1960s. Their success was based on establishing and utilizing the needle biopsy technique to acquire tissue samples from human skeletal muscle. This enabled them to directly examine the impact of diet, nutrition, and various exercise regimes on muscle function (Bergström et al., 1967; Bergström & Hultman, 1967).

Over the last few decades, the use of dietary supplements among athletes and non-athletes has garnered significant attention, including from a scientific standpoint. Although a dietary supplement is yet to be accurately defined (Maughan et al., 2007, 2018), vitamins are regarded as the first such products that became widely accessible during the 1920s (Swann, 2016). The global dietary supplements market is currently estimated to be worth hundreds of billions of pounds annually, of which over £40 billion is made up of products for athletes and those who follow an athletic lifestyle (Binns et al., 2018; Garthe, 2019).

Regulations around the production and marketing of dietary supplements are generally lax and driven by business rather than public health interests (Binns et al., 2018). As a consequence of this, only a small number of dietary supplements available on the market have undergone a thorough scientific examination of their safety and efficacy (Burke et al., 2006; Kerksick et al., 2018; Maughan et al., 2018; Peeling et al., 2019).

Many authors have attempted to categorize dietary supplements using different approaches (Burke et al., 2006; Garthe & Maughan 2018; Kerksick et al., 2018; Maughan et al., 2018; Peeling et al., 2019). For instance, Maughan et al. (2018) differentiate between supplements that 1) aid in the mitigation or elimination of nutrient deficiencies, 2) serve as convenient sources of energy and

nutrients, 3) enhance physical performance directly, 4) improve physical performance indirectly; and 5) facilitate an increase in lean muscle mass and a reduction in body fat. Within this classification, β-alanine and sodium bicarbonate ($NaHCO_3$) are categorized as supplements having a proven direct effect on physical performance. The physiological mechanism of action of these two substances is based on increasing the capacity of the body's buffer systems (Brisola & Zagotta, 2019; Gilsanz et al., 2023; Grgic et al., 2021) Sodium citrate (CIT) administration also increases body's buffering capacity, but its effects on physical performance have been relatively less extensively studied (de Oliveira et al., 2022).

The effects of dietary supplements that increase extracellular buffering capacity, i.e., $NaHCO_3$ and CIT, have been studied mainly in the context of short-term, high-intensity physical exertions and much less in relation to aerobic endurance exercise (Carr et al., 2011). However, endurance athletes' ability to undertake intermediate spurts during a race and final acceleration before the finish is an essential factor affecting their performance (Potteiger et al., 1996b; Tucker et al., 2006). In such situations, work intensity increases to a level where the rate of H^+ accumulation is high, leading to metabolic acidosis (Robergs et al., 2004). Since acidosis is considered one of the main causes of muscle fatigue (Fitts, 2004; 2016; Finsterer, 2012; Robergs et al., 2004), increasing the capacity of buffering systems can improve performance. Usually, exercise-induced increases in blood lactate levels correlate with decreases in blood pH (Robergs et al., 2004). Although the 5000 m run is classified as an endurance event, evidence suggests a significant anaerobic contribution, particularly in the final stages. Blood lactate concentrations exceeding 20 $mmol \cdot L^{-1}$ and pH values as low as 6.95 have been reported post-race in trained runners (Osnes & Hermansen, 1972). Similarly, Ramsbottom et al. (1992) observed lactate levels of 10.1 ± 2.1 $mmol \cdot L^{-1}$ at 4700 meters, while Svedenhag and Sjödin (1984) reported post-race lactate concentrations of 13.8 and 15.0 $mmol \cdot L^{-1}$ at one and three minutes, respectively, in elite athletes. Thus, it is likely that nutritional supplements that have a positive effect on the body's buffering capacity can improve performance in this sport. Therefore, the main objective of this dissertation was to determine the effect of sodium citrate ingestion on 5000 m running performance in a temperate laboratory environment, in competitive conditions in the outdoor stadium, and in a warm laboratory environment.

2. REVIEW OF LITERATURE

2.1. Acid-base balance

Maintaining a stable internal environment is essential prerequisite for the normal functioning and survival of the human body. The acid-base balance is one of the many important parameters of the internal environment (Shier et al., 1996). The degree of acidity or alkalinity is estimated based on the pH scale, which inversely reflects the molar concentration of hydrogen ions ($[H^+]$) in a solution. The human body's cells release between 50 and 100 mmol of H^+ daily. Neverthless, the optimal pH of blood plasma, a major component of the internal environment, is approximately 7.4, corresponding to $[H^+]$ of about 40 $nmol \cdot L^{-1}$ (Crook, 2012). Thus, it is evident that acid-base homeostasis, i.e., maintaining an optimal acid-base balance, is a challenge in a physiological sense.

Acid-base homeostasis is based on the interaction of intra- and extracellular buffers and the function of the lungs and kidneys (Costanzo, 2018; Fox, 2016). A buffer could be defined as a system of molecules and ions that diminishes or prevents a change in pH when H^+ is added to or removed from a solution (Costanzo, 2018; Fox, 2016). Standard intracellular buffers are phosphates (equation 1) and proteins.

$$HPO_4^{2-} + H^+ \leftrightarrow H_2PO_4^- \qquad \text{(equation 1)}$$

However, a primary intracellular buffer is hemoglobin, found only in erythrocytes. In the physiological pH range, deoxyhemoglobin is a more efficient buffer than oxyhemoglobin (Costanzo, 2018).

The bicarbonate buffer system (equation 2) is essential in extracellular space. It is estimated that HCO_3^- accounts for more than 60% (Crook, 2012) or even over 85% (Poupin et al., 2012) of the blood buffering capacity (Crook, 2012).

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O \qquad \text{(equation 2)}$$

Nevertheless, the H^+ in the urine is mainly buffered by ammonium (equation 3) and phosphate buffers (Costanzo, 2018; Fox, 2016).

$$NH_3 + H^+ \leftrightarrow NH_4^+ \qquad \text{(equation 3)}$$

In a resting state, blood pH is usually kept within the narrow range of 7.35 to 7.45. However, metabolism continuously produces H^+, while most buffers can only bind H^+ but cannot excrete these ions from the body (Crook, 2012). The bicarbonate buffer incorporates H^+ into water, but only if the reaction is driven to the right by removing CO_2 (equation 2), which, on the other hand, reduces the HCO_3^- level (Crook, 2012). Therefore, without H^+ excretion, the capacity of buffer systems to stabilize pH would quickly be exhausted. Excretion of H^+ occurs mainly through the kidneys, where this biochemical mechanism is coupled with

the generation of HCO_3^- (Crook, 2012; Fox, 2016; Poupin et al., 2012). Thus, kidneys excrete H^+ and concomitantly regenerate the body's buffering capacity.

In resting skeletal muscle, intracellular pH is maintained close to 7.2 by various control mechanisms including intracellular physicochemical buffering as well as active and passive transport of H^+ into the surrounding interstitium and blood (Lancha Junior et al., 2015). The major intracellular buffers in skeletal muscle are proteins, phosphates and carnosine (Lancha Junior et al., 2015; Maclaren & Morton, 2012). Important transmembrane H^+ transport mechanisms include the Na^+/H^+ exchange system and lactate/H^+ cotransport (Juel, 2008; Thomas et al., 2012). Two lactate/H^+ cotransporter proteins (monocarboxylate transporters) MCT1 and MCT4 have been identified in the plasma membrane of skeletal muscle (Juel, 2008; Thomas et al., 2012). The MCT1 isoform is predominantly expressed in oxidative fibers, whereas the MCT4 isoform is present with large interindividual variations in all human muscle fiber types (Pilegaard et al., 1999; Juel, 2008; Thomas et al., 2012). Since the MCT1 transporter is present in the mitochondrial membrane, mitochondria also function as an intracellular buffering mechanism by facilitating the transport of lactate and H^+ into the mitochondrial matrix (Messonnier et al., 2007).

2.2. Acidosis and fatigue

Muscle fatigue occurring during exercise can be defined as a loss of force, velocity, and power output leading to reduced performance of a given task (Fitts, 2004; 2016). It is a complex phenomenon that can be caused by exercise-induced changes in the working muscle itself (peripheral fatigue) and/or in the central nervous system (central fatigue) (Taylor et al., 2016). One primary cause of muscular fatigue on the level of cross-bridge activity during short-duration and maximum-intensity activities is the accumulation of H^+ (Fitts, 2004; 2016). Intramuscular pH declines, due to an increase in $[H^+]$ during maximum-intensity activities, ranging from 7.2 to 6.5 (Horswill, 1995; Osnes & Hermansen, 1972; Poupin et al., 2012), with a most pronounced decline to 6.2 observed in fast glycolytic fibers (Kent-Braun et al., 2012). This physiological effect is mirrored by a drop in blood plasma acidity from 7.4 to 6.9 (Cairns, 2006). Intracellular acidosis has been shown to inhibit mitochondrial function and limit oxidative phosphorylation (Jubrias et al., 2003). Moreover, high $[H^+]$ inhibits sarcoplasmic reticulum ATPase (Fitts, 2004; Fuchs, 1969), phosphofructokinase, and subsequently the glycolytic rate (Sutton et al., 1981; Hollidge-Horvat et al., 1999), competitively inhibits Ca^{2+} binding to troponin C and reduces cross-bridge activation (Kent-Braun et al., 2012). A high $[H^+]$ contributes to the exit of K^+ from the cell (Bangsbo et al., 1996; Street et al., 2005), reducing the excitability of skeletal muscle (Clausen, 2003). Rapid accumulation of H^+ and K^+ causes activation of group III and IV afferents in skeletal muscle (Light et al., 2008), which limits central motor drive in exercising individuals (Cairns, 2006; Kay, 2008), thereby protects against excessive development of peripheral fatigue (Amann et al., 2011).

In conditions where significant acid-base disturbances occur, performance can be improved by the supplementation of substances that increase extracellular (Burke et al., 2006; Carr et al., 2011; Requena et al., 2005) or intracellular (Hobson et al., 2012; Quesnele et al., 2014) buffer capacity during high-intensity exercise.

2.3. Dietary supplements influencing acid-base balance and physical working ability

In addition to a well-structured exercise routine and diet plan, consuming specific dietary supplements can enhance training efficiency and competitive performance. Research dating back to the 1930s aimed to demonstrate how modifying the body's buffer system can improve physical performance (Horswill, 1995). One such modification employed the administration of beta-alanine (β-alanine) to facilitate intracellular carnosine production (Harris et al., 2006; Hill et al., 2007), which plays a critical physiological role in buffering pH in skeletal muscle cells (Sale et al., 2011). To modify the extracellular buffer status, various methods are employed, including $NaHCO_3$, CIT (Carr et al., 2011; Requena et al., 2005), sodium pyruvate (Olek et al., 2014), lactate (Morris et al., 2011; van Montfoort et al., 2004), or dietary manipulations (Ball & Maughan, 1997; Greenhaff et al., 1987). The ergogenic effects of commonly used extracellular alkalizing agents, such as $NaHCO_3$ and CIT, may be primarily based on their ability to increase interstitial and blood HCO_3^- levels, as stated by McNaughton (1990), Potteiger et al. (1996a, b), and Street et al. (2005). This allows for easier efflux of intracellular lactate and H^+ from contracting muscle cells, as mentioned by Bishop et al. (2004), Potteiger et al. (1996a, b), and McNaughton et al. (1999). Reducing extensive pH drops is crucial to maintaining undisturbed contractile function (Fitts, 2004) and enhancing muscle glycogenolytic ATP production during exercise (Hollidge-Horvat et al., 1999). Street et al. (2005) discovered that alkalosis alleviates the accumulation of H^+ in muscle cells and interstitial K^+ accumulation, which preserves the excitability of the cell membrane, delaying the onset of fatigue.

Most authors have concluded that acute pre-exercise supplementation of extracellular (Burke et al., 2006; Carr et al., 2011; Linderman & Gosselink, 1994; Requena et al., 2005) or intracellular alkalizers (Culbertson et al., 2010) inducing alkalosis, results usually in ergogenic effects during high-intensity exercises lasting between 1 and 10 min. The findings have not been deemed conclusive regarding longer-lasting efforts, particularly with extracellular alkalinization (Linderman & Gosselink, 1994; Requena et al., 2005), or have hinted at a weak impact (Carr et al., 2011). There is some evidence supporting the beneficial effects of extracellular alkalizing agents in long-term endurance exercise (Potteiger et al., 1996a; Shave et al., 2001). These effects are likely achieved through distance and finish sprints, which are highly intense (Tucker et al., 2006).

Other essential factors influencing potential performance improvement after supplementation with alkalizing compounds, in addition to exercise intensity, include the quantity of the compound administered, the duration of action between ingestion and the start of exercise (McNaughton, 1990), the degree of alteration in HCO_3^- levels in circulation, the type of exercise performed, and the potential adverse effects (Requena et al., 2005; van Montfoort et al., 2004).

2.4. Use of sodium citrate as a dietary supplement

CIT ($Na_3C_6H_5O_7$ or $Na_2C_6H_5O_7$) is the sodium salt of citric acid that possesses alkalinizing properties. Use of CIT as a dietary supplement may offer advantages over $NaHCO_3$ due to better tolerability (McNaughton, 1990; Requena et al., 2005; Peacock et al., 2021). Compared to $NaHCO_3$, CIT ingestion may reduce the risk of nausea and vomiting (Street, 2003). Furthermore, when CIT and $NaHCO_3$ are administered in equal osmotic amounts, CIT results in greater increases in blood [HCO_3^-] and pH are greater with CIT (van Montfoort et al., 2004).

2.4.1. Mechanism of action

Upon ingestion, CIT initially dissociates into Na^+ and citrate anion ($citrate^{3-}$) and then, under gastric acid action, citrate anion is converted to citric acid (Kowalchuk et al., 1989; Lindinger & Heigenhauser, 1991; Stewart, 1983). In the alkaline environment prevailing in the duodenum, citric acid is converted back to $citrate^{3-}$, which is swiftly absorbed from the small intestine into the bloodstream (Kowalchuk et al., 1989). The precise biochemical mechanism by which CIT ingestion leads to an increase in extracellular [HCO_3^-] is not entirely clear. However, hepatic oxidation of dietary organic anions (OA^-) including $citrate^{3-}$ is known to produce HCO_3^- (equation 4; Poupin et al., 2012). Thus, it is reasonable to assume that the metabolism of CIT administered as a dietary supplement follows the same pathway.

$$OA^- + H_2CO_3 \leftrightarrow CO_2 + H_2O + HCO_3^- \qquad \text{(equation 4)}$$

However, there is an alternative explanation for increases in plasma [HCO_3^-] and decreases in [H^+] following CIT ingestion that is based on the concept that changes in strong ion levels influence acid-base balance (Stewart, 1983). The key point of this concept is the fact that aqueous solutions always maintain electrical neutrality, that is, the sum of the concentrations of all negatively charged ions in them is equal to the sum of the concentrations of all positively charged ions (Stewart, 1983). Since after ingestion of CIT, $citrate^{3-}$—but not Na^+—is removed from the plasma, the electrical neutrality of the plasma is disturbed. Therefore, [H^+] is lowered and [HCO_3^-] is raised to restore it (Kowalchuk et al., 1989).

Whatever the details of the specific biochemical mechanism, the indisputable fact is that administration of CIT induces an increase in [HCO_3^-] in the blood, i.e. increases the capacity of blood buffer systems (Cunha et al., 2019; McNaughton

& Cedaro, 1992; van Montfoort et al., 2004; Schabort et al., 2000; Linossier et al., 1997; Tiryaki & Atterbom, 1995). Increased [HCO_3^-] reduces plasma [H^+] leading to increased gradient of muscle to plasma that is known to stimulate efflux of lactate and [H^+] from working muscles (Mainwood & Worsley-Brown 1975) most likely via lactate/H^+ cotransporters MCT1 and MCT4 (Juel, 2008). Thus, CIT ingestion, leading to facilitated H^+ outflow from active muscles during intense exercise may cause a delay in the reduction of intramuscular pH to a critical level that inhibits glycolytic energy system and the function of contractile apparatus. Therefore, improved control of intramuscular pH is considered the most likely mechanism for the ergogenic effects of both CIT and $NaHCO_3$ (Heibel et al., 2018; Requena et al., 2005).

K^+ is released from working skeletal muscle that may lead to significant increases in interstitial (Street et al., 2005) and venous plasma K^+ levels (Juel et al., 1990). An increase in interstitial [K^+] reduces muscle excitability (Clausen, 2003), which is considered a factor inducing fatigue during exercise (Fitts, 1994). Street et al. (2005) showed that CIT ingestion reduced both exercise-induced plasma [H^+] and interstitial [K^+] accumulation, which was accompanied by a tendency towards an increase in time to exhaustion. Thus, the performance-enhancing effect of CIT may partially result from changes in skeletal muscle [K^+] kinetics during exercise.

Finally, CIT ingestion favours water retention which is reflected in increases in plasma volume and body mass (Ööpik et al., 2008; Timpmann et al., 2012). Accute pre-exercise plasma volume expansion was shown to improve endurance capacity in untrained men in a moderate environment (Greenleaf et al., 1997), as well as in trained men (Sims et al., 2007b) and women (Sims et al., 2007a) who exercised in a warm environment. Thus, it appears that, under certain conditions, CIT can also improve performance by influencing the body's water balance.

2.4.2. Dosage, mode and timing of administration

It has been postulated that performance may improve when blood HCO_3^- concentration increases by at least 4 $mmol \cdot L^{-1}$ following the ingestion of alkalizing agents (de Oliveira et al., 2022). McNaughton (1990) evaluated the effect of five different doses of CIT (100, 200, 300, 400, and 500 $mg \cdot kg^{-1}$ BM) on performance in a 1-min maximal cycle ergometer test. The greatest amount of work was performed, and the highest peak power achieved in the trial where 500 $mg \cdot kg^{-1}$ BM CIT dose was ingested. Schabort et al. (2000) administered 200, 400 or 600 $mg \cdot kg^{-1}$ BM of CIT to trained male cyclists prior to 40-km cycling time-trial. The greatest increases in blood pH and HCO_3^- levels immediately before as well as during exercise were observed in the trial where the highest dose of CIT (600 $mg \cdot kg^{-1}$ BM) was used. Another research group (Urwin et al., 2016) examined the effect of three doses of CIT (500, 700, and 900 $mg \cdot kg^{-1}$ BM) on blood pH and HCO_3^- concentration in young men and women. All three CIT treatments resulted in significantly higher blood pH and HCO_3^- levels compared to placebo. However, the magnitude of increases in blood pH and HCO_3^- did not

differ across the three CIT ingestion trials (Urwin et al., 2016). Authors of relevant review articles have concluded that the most frequently used dose of CIT in original studies is 500 $mg \cdot kg^{-1}$ BM (Carr et al., 2011), that higher amounts of CIT may not induce greater increases in blood pH and HCO_3^- levels (Cerullo et al., 2020), and that 500 $mg \cdot kg^{-1}$ BM dose may be sufficient to improve physical performance (Requena et al., 2005).

The mode of administration of CIT can affect the ease of ingestion, the rate at which alkalosis develops, the level of peak alkalosis, and the gastrointestinal distress (GID) symptoms that may occur. CIT is often administered dissolved in flavored water. In different studies, the volume of administered solution has ranged from 400 ml (Ibanez et al., 1995; Schabort et al., 2000) to 1L (Potteiger et al., 1996a; Shave et al., 2001). Flavoring the water is important to mask the salty taste of CIT and to minimize the possibility that subjects will be able to distinguish CIT solution from placebo (Bracken et al., 2005). Some studies have used a cool sports drink as a solvent instead of flavored water (Urwin et al., 2016; 2019), and in one known study subjects administered CIT by eating soup (Parry-Billings & Maclaren 1986). Many studies have used gelatin capsules instead of an aqueous solution to administer CIT (Ball & Maughan, 1997; Cunha et al., 2019; Potteiger et al., 1996b; van Montfoort et al., 2004; Suvi et al., 2019). Gelatin capsules containing CIT have been usually administered with 750 ml (van Montfoort et al., 2004) to 1 L (Cunha et al. 2019; Potteiger et al. 1996b) of water. In one recent study (Urwin et al. 2023), participants took gelatin capsules with a standardized high-carbohydrate meal which also included 750 mL of a sports drink. When CIT is administered in an aqueous solution, alkalosis occurs more rapidly but is less pronounced compared to administration in capsule form (Urwin et al., 2019).

The timing of CIT administration prior to the onset of exercise may affect performance as alkalosis takes some time to develop. The time interval between the end of CIT ingestion and the start of performance test ranges from 60 min (Ball & Maughan 1997; Kowalchuk et al., 1989; Schabort et al., 2000; Shave et al., 2001) to 90 min (Ööpik et al., 2008; Potteiger et al., 1996a; van Montfoort et al., 2004; van Someren et al., 1998), 120 min (Cunha et al., 2019; Fernandez-Castanys et al., 2002; Potteiger et al., 1996b) 150 min (Parry-Billings & Mac-Laren, 1986), or 180 min (Ibanez et al., 1995). Positive effects of CIT on performance have been observed in cases with 60 min (Shave et al., 2001), 90 min (Potteiger et al., 1996a) and 120 min (Cunha et al., 2etween administration and test start. Urwin et al. (2019) have demonstrated that the mode of CIT administration significantly influences the time to reach the peak blood pH and $[HCO_3^-]$ values. After administration of a dose of CIT 500 $mg \cdot kg^{-1}$ BM in water solution, blood pH and $[HCO_3^-]$ reach peak levels after 175 min (95% CI 159–191 min) and 164 min (95% CI 148–180 min) and after capsule administration, the 199 min (95% CI 183–215 min) and 204 min (95% CI 188–220 min) respectively.

2.4.3. Adverse effects

Some researchers (McNaughton, 1990; Peacock et al., 2021; Requena et al., 2005), but not all (van Montfoort et al., 2004; Urwin et al., 2023), have observed better tolerability of CIT supplements compared to $NaHCO_3$. Nevertheless, symptoms of GID in association with CIT ingestion have been reported in many studies (Cox & Jenkins, 1994; Ööpik et al., 2008; Potteiger et al., 1996a; Schabort et al., 2000; Shave et al., 2001; van Someren et al., 1998). The incidence and severity of GID may depend on the dosage, mode of administration, and individual subject characteristics. Schabort et al. (2000) found that five out of their eight subjects experienced GID during a 40 km cycling time trial after administration of CIT at a dose of 600 $mg \cdot kg^{-1}$ BM, while only two subjects reported similar symptoms after a lower dose of 400 $mg \cdot kg^{-1}$ BM. Potteiger et al. (1996a) noted that three of their eight subjects experienced GID after consuming 500 $mg \cdot kg^{-1}$ BM of CIT. Urwin et al. (2016) reported that a 900 $mg \cdot kg^{-1}$ BM dose of CIT elicited a significantly greater total rating of GID when compared with a 500 $mg \cdot kg^{-1}$ BM dose. Urwin et al. (2019) compared the effect of 500 $mg \cdot kg^{-1}$ BM of CIT ingested in solution or capsules on occurrence of GID. They observed significantly elevated GID symptoms for both ingestion modes at each time point between 30 and 120 min after ingestion, with no difference between modes at any time point. Of note, in both studies (Urwin et al., 2016; 2019), peak GID occurred earlier than peak levels of blood pH and $[HCO_3^-]$. In some studies, in which subjects were administered CIT in amounts of 100–500 $mg \cdot kg^{-1}$ BM, no associated GID symptoms were observed (McNaughton & Cedaro, 1992; McNaughton, 1990) or they were mild (Cunha et al., 2019).

2.4.4. Impact on performance in high-intensity exercise

Glycolytic phosphorylation is one of the three major energy systems that contribute to regeneration of ATP in skeletal muscle during exercise (Baker et al., 2010; Hargreaves & Spriet, 2020; Robergs et al., 2004; Serresse et al., 1988). Glycolytic phosphorylation dominates the ATP supply to muscle during maximal intensity exercise lasting approximately 30–90 seconds, whereas the contribution of phosphagen system is of great importance in efforts lasting less than 30 s (Serresse et al., 1988). However, the high turnover rate of ATP regenerated by glycolytic phosphorylation quickly produces metabolic acidosis, leading to a decline in performance (Baker et al., 2010; Robergs et al., 2004). Therefore, the probability of achieving an ergogenic effect with CIT administration is greater, the more ATP resynthesis in muscle depends on glycolytic phosphorylation. Data from many experimental studies confirm the validity of this assumption (Linossier et al., 1997; McNaughton & Cedaro, 1992; McNaughton, 1990).

2.4.5. Impact on performance in endurance exercise

The anaerobic energy production system is clearly not the primary factor responsible for the outcome of the competition in endurance events. Still, it plays a vital role in various tactical situations, such as acceleration at the start, over the distance, or at the finish (Potteiger et al., 1996b; Tucker et al., 2006). 5000 m is a typical long-distance run where the performance time of elite athletes varies between 12–15 min, and most of the energy is produced aerobically (96%) and only 4% anaerobically (Weyand et al., 1993). The large amount of lactate produced (Ramsbottom et al., 1992) and the pH drop to 6.95 (Osnes & Hermansen, 1972) indicate a significant contribution of anaerobic metabolism to total energy production. In middle-distance running (400–5000 m), metabolic acidosis and neuromuscular fatigue can harm performance (Schubert & Astorino, 2013). It is, therefore, clear that ingesting buffering agents before high-intensity endurance exercise has the potential to improve performance during this type of exercise (Potteiger et al., 1996b).

Many studies have evaluated the use of CIT as an ergogenic aid for improving endurance performance (Martins et al., 2010; Potteiger et al., 1996a; Schabort et al., 2000; Jain et al., 2003), including in runners (Ööpik et al., 2010; 2008; Potteiger et al., 1996b; Shave et al., 2001), but the results are equivocal. CIT enhanced performance in 30 km cycling time trial (Potteiger et al., 1996a), supramaximal endurance cycling (Jain et al., 2003), and 3000 m run (Shave et al., 2001). Despite improved performance following CIT supplementation, Potteiger et al. (1996a) and Shave et al. (2001) reported increased GID disturbances among the subjects. On the other hand, CIT supplementation did not affect running time to exhaustion at different treadmill speeds (Ööpik et al., 2010; Potteiger et al., 1996b) or the performance of female middle-distance runners in the 1500 m race (Ööpik et al., 2008). CIT supplementation seems to create favorable conditions for improved performance (increased resting pH, BE and $[HCO_3^-]$)but for some reason, performance does not always actually improve (Ball & Maughan, 1997; Martins et al., 2010; Ööpik et al., 2010; Ööpik et al., 2008; Potteiger et al., 1996b; Schabort et al., 2000).

It may be that the inconsistency in results is due to differences in the type of test protocol, exercise intensity, and duration used (Potteiger et al., 1996b). Potteiger et al. (1996b) state that pre-exercise supplementation of CIT increased blood pH during exercise, but this was not reflected in performance because the subjects ran at a fixed intensity which they could not alter (30 min run at lactate threshold > 4 $mmol \cdot L^{-1}$, 87% VO_2max, then increased speed by 110% of lactate threshold speed, thus running to exhaustion) as a capacity test requires the individual to exert to the point of voluntary exhaustion, as opposed to a fixed cessation point in a performance test, resulting in maximal H^+ production (Potteiger et al., 1996b). The results obtained may also differ due to the different training levels of the participants. In athletes who are adapted to high-intensity activities, the administration of alkalosis-inducing compounds may have a smaller effect (Linossier et al., 1997).

Considering that muscle (Febbraio, 2001) and blood (Febbraio et al., 1994; McAnulty et al., 2005) lactate accumulation is augmented during exercise in warm environments, suggesting a more severe disturbance in the acid-base balance (Robergs et al., 2004), high ambient temperatures may allow the ergogenic effect of CIT to become more apparent. This presumption is further supported by the finding that CIT ingestion acutely increases PV (Ööpik et al., 2010; 2008; Timpmann et al., 2012) in a magnitude that has been demonstrated to be sufficient for improving endurance running capacity in the heat (Sims et al., 2007a).

2.5. Summary of the literature review

Metabolic acidosis as a fatigue factor in high-intensity exercise was identified many years ago (Fitts, 1994; Green, 1995). Early studies from the 1930s show that dietary manipulations that lower blood pH impair performance in high-intensity exercise, whereas alkalotic treatments improve such performance (Burke et al., 2006). Based on these initial observations, research from 1977 to 2003 focused on increasing the capacity of extracellular buffer systems by assessing the ergogenic effects of $NaHCO_3$ and CIT in various exercise modes (Requena et al., 2005). Of the 49 studies published during this period, 69% used $NaHCO_3$, showing benefits in short, high-intensity exercises. However, the effects on prolonged efforts were unclear due to the small number of relevant studies and conflicting results (Requena et al., 2005).

Nevertheless, in the 1990s and early 2000s three well-designed studies demonstrated positive impact of CIT (Potteiger et al., 1996a; Shave et al., 2001) and $NaHCO_3$ supplementation (McNaughton et al., 1999) on endurance performance in trained athletes. In these studies, the duration of exercise ranged from 10.2 minutes of running (Shave et al., 2001) to approximately one hour of cycling (McNaughton et al., 1999; Potteiger et al. 1996a). Notably, CIT supplementation improved performance in both 3000 m running (Shave et al., 2001) and 30 km cycling (Potteiger et al., 1996a), i.e. during exercises, where muscle energy needs can be met mainly by oxidative phosphorylation, with anaerobic glycolytic phosphorylation playing only a supporting role (Gastin, 2001). In 5000 m run the contribution of aerobic and anaerobic energy production has been estimated to be approximately 96% and 4%, respectively (Weyand et al., 1993). Despite that, blood lactate levels above 20 $mmol \cdot L^{-1}$ and pH as low as 6.95 have been observed in trained runners in the finish of this distance (Osnes & Hermansen, 1972). Therefore, it can be considered likely that nutritional supplements known to have a positive effect on the body's buffering capacity can improve performance in the 5000 m run.

CIT supplementation may enhance endurance performance in the heat, as exercise in such conditions leads to increased lactate accumulation in muscle and blood (Febbraio, 2001; Febbraio et al., 1994; McAnulty et al., 2005), indicating greater acid–base imbalance (Robergs et al., 2004). CIT has been shown to acutely expand plasma volume (Ööpik et al., 2008, 2010; Timpmann et al., 2012) to a level sufficient to improve endurance capacity in the heat (Sims et al., 2007b).

As endurance performance may decline in the heat due to reduced plasma volume (McDermott et al., 2017).

The experimental studies underlying this dissertation were conducted many years ago, but the topic is still highly relevant. Over the past 10–15 years, several meta-analyses (Carr et al., 2011; de Oliveira et al., 2022) and review articles (Cerullo et al., 2020; Heibel et al., 2018; Lancha Junior et al., 2015; McNaughton et al., 2018; Schubert & Astorino, 2013) have highlighted that inconsistent findings in CIT research may result from variations in dosage, supplementation protocols, the magnitude of induced alkalosis, exercise test design, and individual differences in fitness level and physiological state. These findings underscore the importance of controlling for confounding variables to ensure reliable and reproducible outcomes in future studies. A notable line of inquiry has focused on optimizing the CIT supplementation protocol. Urwin et al. (2016, 2019, 2023) and Tinnion et al. (2024a) have explored how to achieve optimal alkalosis while minimizing GID. According to Carr et al. (2023), further studies on extracellular buffering agents, including CIT, in hot conditions are needed to determine the combined effect of manipulating hydration status and buffering capacity on performance outcomes.

Studies examining the ergogenic potential of CIT have continued in recent years, investigating its effects across various types of physical activity and sports disciplines. For instance, Cunha et al. (2019) studied tennis players, Flueck et al. (2014) focused on paracyclists, Suvi et al. (2019) examined middle-distance runners, while Nabilpour et al. (2024) explored CIT's impact in CrossFit-style functional fitness. In adolescent swimmers, Russell et al. (2014) assessed the performance effects of acute versus chronic CIT supplementation. Tinnion et al. (2024b), in turn, concentrated on the reproducibility of CIT-induced alkalosis rather than sport-specific outcomes and concluded that blood pH and bicarbonate responses were not reliably reproducible, regardless of the dose.

3. RESEARCH AIMS AND HYPOTHESIS

The main objective of this dissertation was to determine the effect of sodium citrate ingestion on 5000 m running performance in a temperate laboratory environment, in competitive conditions in the outdoor stadium, and in a warm laboratory environment.

The specific aims and hypotheses of the studies on which the dissertation is based were:

1. To test the hypothesis that sodium citrate administered two hours before a 5000 m running time trial would improve performance in well-trained college runners in a temperate laboratory environment.
2. To assess the effects of sodium citrate supplementation shortly before exercise on metabolism and performance capacity in a 5000 m competitive outdoor stadium run in trained male runners.
3. To assess the impact of sodium citrate supplementation on 5000 m running performance in a warm environment in non-heat-acclimated endurance-trained males. It was hypothesized that sodium citrate supplementation, inducing alkalosis and an acute increase in plasma volume, reducing extracellular K^+ accumulation, and alleviating heat strain during exercise, improves running performance.

4. METHODS

4.1. Participants

Forty-three endurance-trained young male participants volunteered for three studies conducted under different environmental conditions (Tables 1 and 2). On each occasion, the participants completed two 5000-meter running time trials (5000-TT), following prior supplementation with either sodium citrate (CIT) or a placebo (PLC). The study protocols were approved by the Research Ethics Committee of the University of Tartu, Estonia (protocol no. 73/4; 21.06.1999 and 218/T-21; 02.11.2012). All participants provided written informed consent prior to engaging in any research procedures. They were all non-smokers and had been engaged in regular endurance training for approximately ten years. In the laboratory study conducted under warm climatic conditions (L-W), none of the participants had a history of heat-related illness, nor were they acclimatized to heat, as they had not been exposed to a warm climate for at least two months prior to the study.

Table 1. Description of the subjects

Study (environment)	n	Age (years)	Body mass (kg)	Height (cm)	VO_2 max/peak ($ml \cdot kg^{-1} \cdot min^{-1}$)
L-T	17	20.9 ± 1.9	75.6 ± 5.4	182.9 ± 5.5	61.3 ± 4.9
F-T	10	22.1 ± 2.5	74.1 ± 2.5	180.1 ± 5.7	60.8 ± 5.5
L-W	16	25.8 ± 4.4	76.7 ± 6.1	184.0 ± 5.0	56.9 ± 4.7

Data are expressed as mean ± SD. L-T: laboratory study in temperate climatic conditions; F-T: field study in temperate climatic conditions; L-W: laboratory study in warm climatic conditions.

4.2. Study design

The three studies were conducted under distinct environmental conditions (Table 2). The first laboratory study, conducted under temperate climatic conditions (L-T), took place in the Laboratory of Exercise Physiology at the University of Tartu. The second field study, also under temperate climatic conditions (F-T), was carried out in a near-real competition setting at the University of Tartu athletics stadium, where participants competed in pairs. The third laboratory study, conducted under warm climatic conditions (L-W), was performed in a climatic chamber (Design Environmental Ltd., Gwent, South Wales, UK) located within the Laboratory of Exercise Physiology at the University of Tartu. In this study, the air temperature and relative humidity (RH) were maintained at 32 °C and 50%, respectively. In all three studies, CIT and PLC were administered using a double-blind, randomized, counterbalanced, crossover design (Table 2).

Table 2. Description of the study conditions

Study	CIT	PLC	Beginning of suplementation	Supplementation duration	Rest before 5000- TT	Environment	Warm-up
L-T	500 mg·kg^{-1} dissolved in 1L mineral water	Flavored mineral water	130 min	Within 10 min	120 min	Laboratory 22 °C	Customary 5 min
F-T	500 mg·kg^{-1} dissolved in 1.5L mineral water	Flavored mineral water	180 min	Within 60 min; 250 ml after every ~12 min	120 min	Outdoor stadium 19°C	Customary 5 min
L-W	500 mg·kg^{-1} in gelatine capsules, bolus of plain water *ad libitum*	Wheat flour in gelatine capsules	150 min	Within 30 min	120 min	Climatic chamber 32°C, 50% RH	1km run on a treadmill at a moderate space

L-T: laboratory study in temperate climatic conditions; F-T: field study in temperate climatic conditions; L-W: laboratory study in warm climatic condition; RH: relative humidity 5000-TT: 5000 m running time trial.

4.3. Preparatory research procedures

4.3.1. Anthropometric measures

In each study, anthropometric measurements were taken during the participants' first visit to the laboratory. In the L-T and F-T studies, body height was measured to the nearest 0.5 cm using a stadiometer, and nude body mass was measured to the nearest 0.05 kg using an electronic scale. In the L-W study, body height was measured to the nearest 0.005 m using a stadiometer, and nude body mass was measured to the nearest 0.001 kg using an electronic scale (CH3G–150I Combics, Sartorius AG, Goettingen, Germany).

4.3.2. Measurement of maximal aerobic power (VO_2max/ VO_2peak)

In the L-T and F-T studies, maximal oxygen uptake (VO_2max) was assessed using a progressive treadmill exercise test (Runrace HC 1400; Technogym, Gambettola, Italy). The test commenced with a five-minute warm-up, followed by incremental increases in running speed—starting at 8 km·h^{-1} and increasing by 0.5 km·h^{-1} every 200 meters—until the participant reached volitional exhaustion. The graded exercise protocol employed in these studies was based on the principles originally described by Conconi et al. (1982). Expired gases were continuously sampled and analyzed using a calibrated online system (True Max 2400; Parvo Medics, East Sandy, Utah, USA) prior to each test session.

In the L-W study, peak oxygen uptake (VO_2peak) was determined under temperate environmental conditions (air temperature: 20 °C; relative humidity: 35%) using a breath-by-breath metabolic system (MasterScreen CPX, Viasys Healthcare GmbH, Hoechberg, Germany) and a motorized treadmill (Viasys/Jaeger LE300 C, Viasys Healthcare GmbH, Hoechberg, Germany), following the protocol described by Whitehead et al. (2012). The continuous graded exercise test consisted of multiple 3-minute stages. After a warm-up at 4 km·h^{-1}, the speed was increased to 8 km·h^{-1} (stage 1), then to 9.7 km·h^{-1} (stage 2). From stage 3 onward, the treadmill speed remained constant at 9.7 km·h^{-1}, while the incline was increased by 3% per stage until volitional fatigue.

4.3.3. Prescriptions

Participants were instructed to abstain from alcohol and caffeine consumption, as well as from engaging in strenuous physical activity, for 24 hours prior to each study day. They were also advised to maintain their habitual dietary patterns throughout the study period. To control for nutritional status, hydration, and physical activity, participants recorded their dietary intake and physical activity on the day preceding each 5000-TT trial and were asked to replicate the same routine before subsequent trials.

In the L-W study, participants received additional instructions to ensure adequate hydration by consuming approximately 1 liter of water two hours before bedtime on the evening prior to each laboratory visit.

4.3.4. Familiarization trial

In the L-W study, participants completed one familiarization trial prior to the 5000-TT. During this session, they practiced regulating treadmill belt speed while running and experienced all research procedures, except for blood and urine sample collection and the ingestion of CIT or PLC. The familiarization trial was conducted under temperate environmental conditions (air temperature: 20 °C; relative humidity: 35%).

4.4. Administration of dietary supplements

CIT or PLC was administered in a double-blind, counterbalanced, crossover manner. The dose of CIT used in all studies was 500 $mg \cdot kg^{-1}$ of body mass (Table 4). This dosage has been reported to be optimal for inducing a significant increase in bicarbonate concentration ($[HCO_3^-]$) (McNaughton, 1990) and achieving an alkalotic state approximately 100–120 minutes after ingestion (Potteiger et al., 1996b). In the L-T and F-T studies, CIT was dissolved in mineral water with a low mineral content (K^+: 90–120 $mg \cdot L^{-1}$; Na^+: 30–70 $mg \cdot L^{-1}$; Ca^{2+}: 35–70 $mg \cdot L^{-1}$; Mg^{2+}: 30–50 $mg \cdot L^{-1}$; Cl^-: 120–200 $mg \cdot L^{-1}$; HCO_3^-: 290–400 $mg \cdot L^{-1}$) and flavored with a very low-energy flavoring agent. In the PLC trials, either 1000 ml or 1500 ml of the same mineral water was used in L-T and F-T, with the taste masked by adding flavoring. The energy content of both drinks was less than 4.18 $kJ \cdot L^{-1}$. In the L-W study, participants ingested gelatin capsules containing either CIT (500 $mg \cdot kg^{-1}$; Caelo, Caesar & Loretz GmbH, Hilden, Germany) or PLC (wheat flour). The capsules were consumed within 30 minutes, accompanied by a bolus of plain water. In all studies, participants were regularly monitored for potential adverse side effects from CIT or PLC ingestion, from the supplementation period until the end of the trial.

4.5. Time trials

CIT or PLC was administered in a double-blind, counterbalanced, crossover design. The dose of CIT used in all studies was 500 $mg \cdot kg^{-1}$ of body mass (Table 4). This dosage has been reported to be optimal for eliciting a significant increase in bicarbonate concentration ($[HCO_3^-]$) (McNaughton, 1990) and for inducing an alkalotic state approximately 100–120 minutes post-ingestion (Potteiger et al., 1996b).

In the L-T and F-T studies, CIT was dissolved in mineral water with a low mineral content (K^+: 90–120 $mg \cdot L^{-1}$; Na^+: 30–70 $mg \cdot L^{-1}$; Ca^{2+}: 35–70 $mg \cdot L^{-1}$; Mg^{2+}: 30–50 $mg \cdot L^{-1}$; Cl^-: 120–200 $mg \cdot L^{-1}$; HCO_3^-: 290–400 $mg \cdot L^{-1}$) and

flavored with a very low-energy flavoring agent. In the PLC trials, either 1000 ml or 1500 ml of the same mineral water was used (depending on the study), with taste masked by the addition of flavoring. The energy content of both drinks was less than 4.18 $kJ \cdot L^{-1}$.

In the L-W study, participants ingested gelatin capsules containing either CIT (500 $mg \cdot kg^{-1}$; Caelo, Caesar & Loretz GmbH, Hilden, Germany) or PLC (wheat flour). Capsules were consumed within 30 minutes, accompanied by a bolus of plain water. Across all studies, participants were regularly monitored for potential adverse effects from CIT or PLC ingestion, from the supplementation period until the conclusion of the trial.

4.6. Measurements

4.6.1. Body mass

In the L-T study, participants' body mass was measured immediately after consuming the assigned drink, two hours later following a toilet visit just before the 5000-TT, and again immediately after completing the trial, after removing damp clothing. In the F-T study, body mass was measured prior to drink ingestion, immediately before the trial following a toilet visit, and immediately after the 5000-TT, also after removing damp clothing. During the 120-minute interval following ingestion of the solutions, participants were allowed to use the toilet but were not permitted to consume any food or drink. In the L-W study, a similar body mass measurement protocol was followed as in the F-T study, with the exception that participants were allowed to drink water ad libitum during the 120-minute rest period. The amount of water consumed was recorded.

4.6.2. Heart rate

Heart rate (HR) was measured during the 5000-TT in the L-T and L-W studies. In L-T, HR was recorded using the Polar PE 3000 cardio tester (Polar Electro Oy, Finland). In L-W, participants wore a Polar WearLink® transmitter paired with a Polar RS400sd running computer (Polar Electro Oy, Finland), which recorded HR continuously at 15-second intervals. HR was not monitored during the experimental trials in the F-T study.

4.6.3. Ratings of perceived exertion, fatigue, and thermal sensation

Ratings of perceived exertion (RPE), based on the 15-point Borg scale (Borg, 1982), were recorded in the L-T study after the first 2.5 km and at every subsequent 0.5 km interval. In the L-W study, RPE, ratings of perceived fatigue (RPF), and thermal sensation (TS) were recorded at the beginning of the 5000-TT and after each completed kilometer.

4.6.4. Thermoregulatory measurements

In the L-W study, participants inserted a rectal probe (REC-UU-VL5-0, Grant Instruments Ltd., UK) approximately 10 cm beyond the anal sphincter for core temperature (Tc) measurement. Tc was recorded every minute using an electronic data logger (SQ2020-1F8, Grant Instruments Ltd., UK). Skin temperature (Tsk) was simultaneously measured at four anatomical sites (chest, arm, thigh, and leg) using miniature temperature probe/data loggers (DS1922L, Maxim Integrated Products, Inc., USA), which were affixed to the same side of the body with adhesive plaster. Tsk was recorded at 1-minute intervals, and the data were transferred to a computer using the appropriate interface device (DS1401-4+, Maxim Integrated Products, Inc., USA) after the probes were removed following completion of the run. Weighted mean Tsk was calculated according to the method described by Ramanathan (1964). Tc and Tsk values were used to compute the Tc–Tsk gradient. Body heat storage (HS) was calculated using the equation by Adams et al. (1992): $HS\ (W \cdot m^{-2}) = 0.965 \cdot BW \cdot \Delta Tb \cdot AD^{-1}$, where $0.965\ W \cdot °C^{-1} \cdot kg^{-1}$ is the specific heat constant of body tissues, BW is the participant's body weight in kilograms, ΔTb is the change in mean body temperature in degrees Celsius, and AD is the body surface area in square meters (Du Bois & Du Bois, 1989). The rate of heat storage (HS rate, $W \cdot m^{-2} \cdot min^{-1}$) was calculated by dividing HS by the duration of the run in minutes.

4.6.5. Assessment of hydration status

In the L-W study, participants were instructed to collect urine in a container during each toilet visit. Water retention was calculated as the difference between the volume of water consumed and the volume of urine excreted during the 30-minute capsule ingestion period and the subsequent 120-minute rest period prior to the 5000-TT. Urine specific gravity (USG) and urine osmolality (UOSM) were measured using a digital clinical refractometer (PDX-CL, VeeGee Scientific Inc., Kirkland, WA, USA) and a freezing point depression osmometer (Model 3250, Advanced Instruments Inc., USA), respectively.

4.6.6. Blood sampling and biochemical analyses

In the L-T and F-T studies, venous blood samples (4.5 mL, 7.0 mL, and 11.5 mL) were drawn from the median cubital vein. To facilitate sampling, a tourniquet was applied for a few seconds prior to needle insertion. Blood was collected into Vacutainer tubes (7.0 mL, without additives) and tubes containing ethylenediaminetetraacetic acid (EDTA; 4.5 mL). In L-T, the first sample was taken before the standardized warm-up (Pre-Ex) and the second five minutes after completing the 5000-TT (Post-Ex). In F-T, samples were collected at three time points: before CIT or PLC ingestion (baseline), before the standardized warm-up (Pre-Ex), and five minutes post-trial (Post-Ex). In the L-W study, fingertip capillary blood samples were collected in both CIT and PLC trials at three time points:

in the morning before ingestion (baseline), immediately before the warm-up run (Pre-Ex), and immediately after completing the 5000-TT (Post-Ex). Prior to the first and second sampling, participants stood for 20 minutes with an electric heating pad wrapped around the palm to induce arterialization of fingertip blood (Timpmann et al., 2012). In L-T and F-T, EDTA-treated samples were used to determine hemoglobin concentration (cyanmethemoglobin method; Boehringer Mannheim GmbH, Mannheim, Germany; diagnostic kit No. 124729) and packed cell volume (via spun hematocrit). In L-W, hemoglobin concentration and hematocrit were measured using a Celltac α MEK-6108K blood analyzer (Nihon Kohden, Japan). These values were used to calculate relative changes in plasma volume according to Dill & Costill (1974). In L-T and F-T, EDTA-containing samples were cooled in ice water, centrifuged, and the plasma stored at –25 °C for subsequent lactate and glucose analysis. Lactate and glucose concentrations were determined enzymatically using diagnostic kits from Biocon (Vöhl-Marienhagen, Germany): No. 301 (lactate) and No. 458 (glucose). The intra-assay coefficients of variation in the laboratory were 1.6% (n = 27) for hemoglobin, 0.68% (n = 22) for packed cell volume, 1.0% (n = 10) for lactate, and 1.2% (n = 10) for glucose. In the L-W study, lactate concentration was measured enzymatically using the Dr. Lange Cuvette Test LKM 140 and a mini photometer LP 20 Plus (Dr. Lange, Germany). In F-T, baseline and Pre-Ex blood samples were collected into Vacutainer tubes (7.0 mL, no additives) and used to measure blood pH with a Sentron 2001 pH meter (Federal Way, WA, USA). In L-W, blood gases, pH, and electrolyte concentrations (Na^+, K^+, Cl^-) were measured immediately after sampling using an ABL™77 series blood analyzer and ABL77 SCi sensor cassette (Radiometer, Copenhagen, Denmark). This device also calculated bicarbonate concentration ($[HCO_3^-]$) and base excess (BE) from pH and pCO_2 values. All measured lactate and glucose concentrations were corrected for individual changes in plasma volume across all studies.

4.7. Statistical analysis

The distribution of the data was assessed using the one-sample Kolmogorov–Smirnov test in all studies. In the L-T and F-T studies, conventional statistical methods were used to calculate the mean and standard deviation (SD) for each variable, and results are presented as mean ± SD. A paired-samples t-test was used to determine differences in 5000-TT completion times. For all other dependent variables, repeated-measures analysis of variance (ANOVA) was employed to identify treatment effects. Statistical significance was set at $P \leq 0.05$, while $P \leq 0.1$ was reported to indicate trends. Relationships between variables were assessed using Pearson's correlation coefficient (r).

In the L-W study, statistical analyses were performed using Statistica version 10. A two-factor (time × trial) repeated-measures ANOVA was used to evaluate within- and between-trial differences. When a significant main effect was observed, Tukey's HSD post hoc test was applied to identify pairwise differences. Regarding running performance, Cohen's d was calculated based on trial

averages (CIT minus PLC, divided by the pooled standard deviation), and effect sizes were interpreted as small = 0.2, moderate = 0.5, and large = 0.8 (Cohen, 1992). The likelihood that CIT ingestion would have a beneficial, trivial, or negative effect on running performance was evaluated using Hopkins' spreadsheet (Hopkins, 2007), with the smallest worthwhile change in performance defined as half the within-subject variability (Hopkins, 2005), i.e., 0.6%.

5. RESULTS

5.1. Running performance

Treatment significantly affected 5000-TT running time in the L-T (Table 3). Notably, 13 of the 17 subjects achieved faster rinning times after CIT, and only four were faster after PLC ingestion in the L-T study.

There was no significant effect of the treatment on the 5000-TT in the F-T study. Although the faster result was observed after PLC ingestion, it was not statistically significant (Table 3). Notably, only 3 of the ten subjects achieved a better result after CIT, whereas seven were faster after PLC ingestion.

In the L-W study, the subjects ran faster after the ingestion of CIT in comparison with PLC, although it was not statistically significant ($P = 0.183$; d = −0.09) (Table 3). Nevertheless, ten out of the 16 subjects ran faster with CIT, while six ran faster with PLC.

Table 3. 5000-TT times (s) in different conditions

Study	Treatment	
	PLC	CIT
L-T (n = 17)	1183.8 ± 91.4	1153.2 ± 74.1*
F-T (n = 10)	1082.7 ± 62.0	1100.0 ± 79.1
L-W (n = 16)	1147.0 ± 143.0	1135.0 ± 123.0

Data are expressed as mean ± SD. L-T: laboratory study in temperate climatic conditions; F-T: field study in temperate climatic conditions; L-W: laboratory study in warm climatic condition. Significantly different ($P \leq 0.05$): *from PLC.

Thus, out of 43 subjects, 26 ran faster after CIT and 17 after PLC ingestion.

In the L-T study, the average speed of running in the CIT trial exceeded that measured in the PLC trial during the 2nd and 4th kilometer of the distance (Figure 1): 15.8 ± 1.2 *vs* 15.4 ± 1.4 km·h^{-1} ($P = 0.01$) and 16.2 ± 1.1 *vs* 15.7 ± 1.2 km·h^{-1} ($P = 0.002$). A trend towards a better performance in the CIT trial than the PLC trial was evident during the 3rd kilometer: 16.0 ± 1.2 *vs* 15.7 ± 1.2 km·h^{-1} ($P = 0.06$). In both trials, the fastest average speed was achieved during the 5th kilometer, but there was no difference between the trials: 16.9 ± 1.3 km·h^{-1} for the CIT trial and 16.7 ± 1.2 km·h^{-1} for the PLC trial, $P = 0.43$. The maximum speed achieved in the two trials did not differ: 17.7 ± 1.4 km·h^{-1} for the CIT trial and 17.7 ± 1.3 km·h^{-1} for the PLC trial.

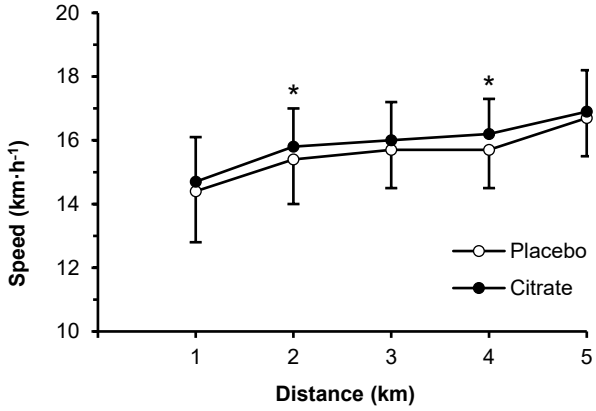

Figure 1. The running speed of the subjects at each kilometer of the 5000-TT (mean ± SD) in the L-T study, n = 17. Significantly different ($P \leq 0.05$): *from the PLC.

In the F-T study, average running speed (Figure 2) was comparable between the CIT and PLC trials across most segments of the 5000-TT. The only exception was the fourth kilometer in the CIT trial, which was completed significantly faster than the third kilometer: 17.5 ± 2.3 km·h^{-1} versus 15.3 ± 1.3 km·h^{-1} (P = 0.03). This increase in pace was primarily attributed to four runners who accelerated following a relatively slower third kilometer. However, only two of these participants achieved marginally faster overall finish times in the CIT trial compared to the PLC trial.

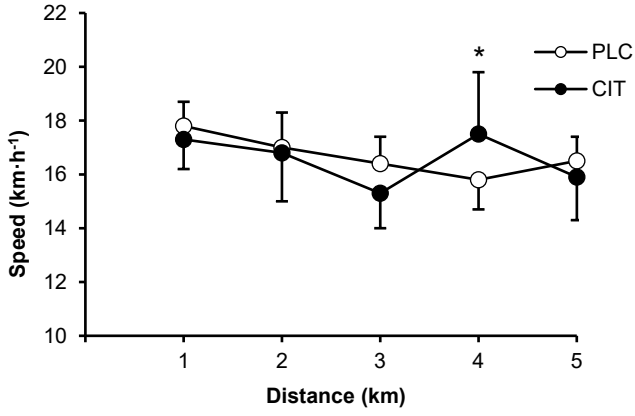

Figure 2. Running speed of the subjects at each kilometer of the 5000-TT in outdoor stadium (mean ± SD) in the F-T study, n = 10. Significantly different ($P \leq 0.05$): *from the preceding kilometer.

In the L-W study, in both trials, the mean running speed was the slowest during the first kilometer (15.3 ± 1.9 and 15.4 ± 1.8 km·h^{-1} in PLC and CIT, respectively; P = 0.536) and the fastest during the fifth kilometer (16.6 ± 1.8 and 16.9 ± 1.7 km·h^{-1} in PLC and CIT, respectively; P = 0.277). Using magnitude-based

inferences, the ingestion of CIT represented a 66% possibly beneficial chance of improving 5000-TT running performance by 0.6% with a very unlikely chance of harm (2%) and a 32% possible chance that the effect was trivial, compared to PLC.

5.2. Body mass and hydration status

Baseline body mass, measured either immediately before or after ingestion of CIT or PLC, did not differ significantly between the two trials in any of the studies (Table 4). However, during the two-hour period between ingestion and the start of the 5000-TT, body mass was consistently higher in the CIT trial compared to the PLC trial across all studies (Table 4). This difference in body mass between trials persisted after the run in both the F-T and L-W studies.

Table 4. Body mass (kg) in all studies

Study	Treatment	
	PLC	CIT
L-T (n = 17)		
Baseline	78.7 ± 5.9	78.9 ± 5.5
Pre-Ex	77.5 ± 6.1	78.2 ± 5.6*
F-T (n = 10)		
Baseline	73.7 ± 6.2	73.8 ± 6.3
Pre-Ex	73.4 ± 6.2	74.2 ± 6.1*
Post-Ex	72.6 ± 6.1	73.5 ± 6.1*
L-W (n = 16)		
Baseline	76.8 ± 6.0	76.8 ± 5.7
Pre-Ex	76.7 ± 5.9	77.2 ± 5.8*
Post-Ex	75.9 ± 5.9	76.4 ± 5.7*

Data are expressed as mean ± SD. L-T: laboratory study in temperate climatic conditions; F-T: field study in temperate climatic conditions; L-W: laboratory study in warm climatic condition Significantly different ($P \leq 0{,}05$): *from PLC.

The calculated relative change in PV between ingestion the solution and pre-exercise in the F-T study was −1.99 ± 3.49% in the PLC and 9.75 ± 6.51% in the CIT trial ($P = 0.001$) (Table 5). The relative increase in PV was significantly greater in CIT compared to PLC (P = 0.005) in the L-W study (Table 5). At the same time, the calculated relative decrease in PV during exercise was similar in the L-T and F-T studies in the two trials (Table 5). In the F-T study, the relationship between the differences in body mass and performance times in the two trials was not statistically significant ($r = -0.19$; $p \geq 0.05$). Similarly, PV shift differences were unrelated to performance times ($r = -0.06$; $p \geq 0.05$).

Table 5. Changes in plasma volume (%) in all studies

Study	Treatment	
	PLC	CIT
L-T (n = 17)		
Baseline → Pre-Ex		
Pre-Ex → Post-Ex	−4.5 ± 3.1	−4.9 ± 3.8
F-T (n = 10)		
Baseline → Pre-Ex	−1.99 ± 3.43	9.75 ± 6.51*
Pre-Ex → Post-Ex	−6.11 ± 4.53	−7.45 ± 5.97
L-W (n = 16)		
Baseline → Pre-Ex	−0.80 ± 4.34	2.92 ± 4.36*
Pre-Ex → Post-Ex	−1.46 ± 5.28	−1.87 ± 5.2

Data are expressed as mean ± SD. L-T: laboratory study in temperate climatic conditions; F-T: field study in temperate climatic conditions; L-W: laboratory study in warm climatic condition; *Pre-Ex*: immediately before 5000 m run; *Post-Ex*: immediately after 5000 m run. Significantly different ($P < 0.05$): *from PLC.

In the L-W study subjects' urine-specific gravity and urine osmolality measured in the morning upon arrival in the laboratory were similar in the two trials (Table 6). During 120 min time interval before the 5000-TT, water intake did not differ in the two trials ($P = 0.106$), but urine volume passed during the same period was significantly smaller ($P = 0.0001$) and apparent water retention was substantially greater ($P = 0.002$) in CIT compared to PLC trial (Table 6). A strong correlation was seen between body mass change and apparent water retention in both PLC ($r = 0.875$) and CIT trial ($r = 0.957$).

Table 6. Body hydration status in the L-W study

	PLC	CIT
Urine-specific gravity	1.0222 ± 0.0061	1.0226 ± 0.0051
Urine osmolality (mOsmol·kg^{-1})	880 ± 208	891 ±1 74
Water intake (mL)	772 ± 206	907 ± 272
Urine volume (mL)	626 ± 325	294 ± 194*
Water retention (mL)	145 ± 445	613 ± 298*

Data are expressed as mean ± SD. n = 16. Significantly different ($P < 0.05$): *from PLC.

During the run, body mass loss and relative decrease in PV did not differ in the two L-W trials (P = 0.215 and P = 0.739, respectively).

5.3. Blood pH, HCO_3^- and base excess

Blood pH, bicarbonate (HCO_3^-), and base excess (BE) were not measured in the L-T study. In the F-T study, HCO_3^- and BE were also not measured, but the blood pH increased from 7.34 ± 0.007 to 7.49 ± 0.07 (P = 0.002) following CIT ingestion, while it remained stable after consumption of the placebo drink (Figure 3). The extent of the pH decrease during the 5000-TT was similar in both the PLC (−0.10 ± 0.06) and CIT trials (−0.10 ± 0.11).

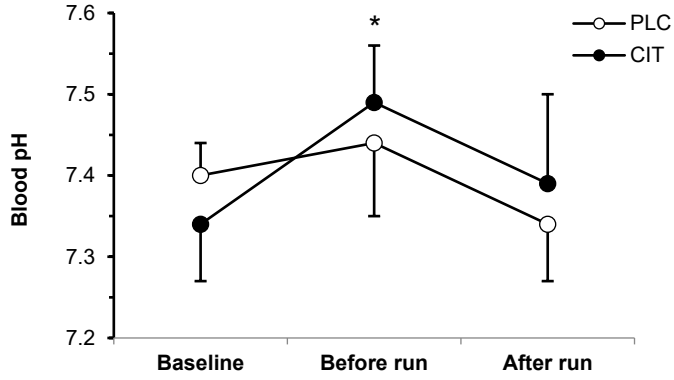

Figure 3. Changes in blood pH (mean ± SD) under PLC and CIT treatment in the F-T study, n = 10. Significantly different (P < 0,05): *from the baseline value.

Significant main effects of the trial (F = 51.49, P < 0.0001), time (F = 55.82, P < 0.0001), and trial-by-time interaction (*F* = 10.37, *P* = 0.0004) were observed for blood pH in the L-W study. Baseline blood pH did not differ in the two trials (*P* = 0.994). Still, under both Pre-Ex and Post-Ex conditions, this parameter was significantly higher in the CIT trial compared to the PLC trial (P = 0.0001 and P = 0.0002, respectively; Figure 4).

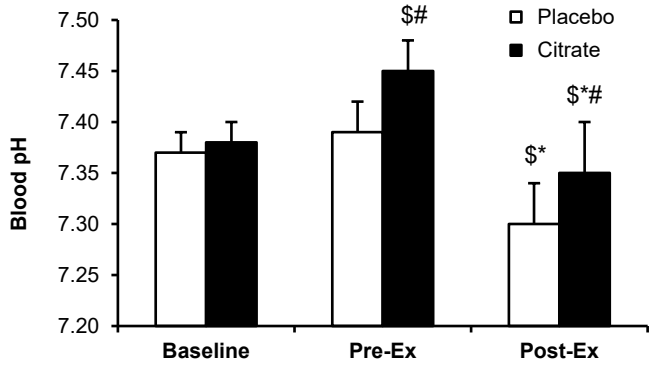

Figure 4. Blood pH under PLC and CIT treatment in the L-W study. Data are presented as mean ± SD, n = 16. *Baseline*: before treatment; *Pre-Ex*: immediately before 5000-TT; *Post-Ex*: immediately after 5000-TT. Significantly different (*P* < 0.05): $from baseline; *from Pre-Ex; #from PLC.

In addition to blood pH, blood [HCO_3^-] and [BE] were measured in the L-W study. Significant main effects of the trial ($F = 71.00$, $P < 0.0001$), time ($F = 280.21$, $P < 0.0001$), and trial-by-time interaction ($F = 64.76$, $P < 0.0001$) were observed for blood [HCO_3^-]. Similarly to pH, baseline blood [HCO_3^-] did not differ in the two trials ($P = 0.996$). Significantly higher levels were observed in the CIT trial compared to the PLC trial under both Pre-Ex and Post-Ex conditions ($P = 0.0001$ for both; Figure 5).

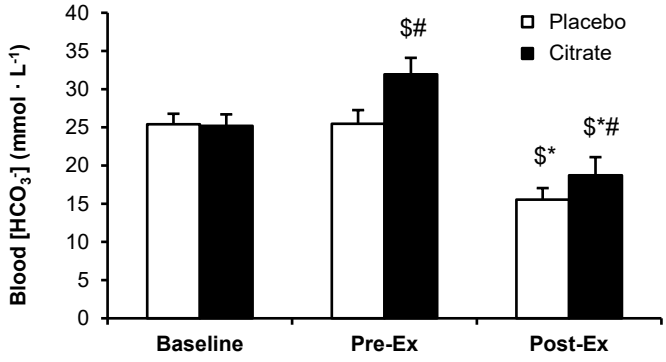

Figure 5. [HCO_3^-] under PLC and CIT treatment in the L-W study. Data are presented as mean ± SD, n = 16. *Baseline*: before treatment; *Pre-Ex*: immediately before 5000-TT; *Post-Ex*: immediately after 5000-TT. Significantly different ($P < 0.05$): $^{\$}$from baseline; *from Pre-Ex; $^{\#}$from PLC.

There were also significant main effects of the trial ($F = 105.28$, $P < 0.0001$), time ($F = 186.05$, $P < 0.0001$), and trial-by-time interaction ($F = 44.73$, $P < 0.0001$) for [BE]. Baseline [BE] values did not differ between the two trials (P = 1.0), whereas both Pre-Ex and Post-Ex values were significantly higher in the CIT trial compared to the PLC trial (P = 0.0001 for both comparisons).

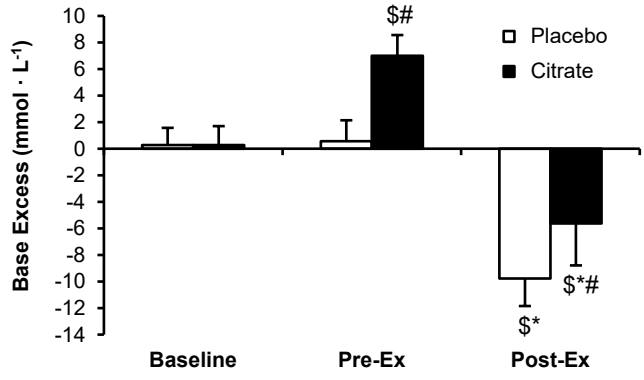

Figure 6. BE under PLC and CIT treatment in the L-W study. Data are presented as mean ± SD, n = 16. *Baseline* before treatment, *Pre-Ex* immediately before 5000-TT, *Post-Ex* immediately after 5000-TT. Significantly different ($P < 0.05$): $^{\$}$from baseline; *from Pre-Ex; $^{\#}$from PLC.

5.4. Blood lactate and glucose concentrations

There were no differences in plasma lactate concentration before the 5000-TT between the CIT and PLC trials across all three studies (Table 7). A significant increase in lactate concentration was observed as a result of the 5000-TT in all three studies. However, the plasma concentration of lactate was significantly higher after the CIT in comparison PLC ingestion in the L-T and L-W studies (Table 7). These significant between CIT and PLC differences were also evident after the correction of the measured concentrations of lactate for the individual changes in plasma volume (data not shown).

Table 7. Changes in blood (plasma) lactate and glucose concentrations in the three studies

Study	L-T (n = 17)		F-T (n = 10)		L-W (n = 16)	
Treat-ment	PLC	CIT	PLC	CIT	PLC	CIT
Lactate ($mmol \cdot L^{-1}$)						
Baseline			1.74 ± 0.58	1.72 ± 0.43		
Pre-Ex	2.0 ± 0.9	2.2 ± 1.1	1.53 ± 0.35	1.56 ± 0.37	1.09 ± 0.18	1.30 ± 0. 34
Post-Ex	9.8 ± 2.8*	11.9 ± 3.0$^{*\#}$	11.5 ± 1.5*	12.5 ± 2,6*	8.22 ± 2.64*	11.1 ± 3.2$^{*\#}$
Change	7.8 ± 2.4	9.7 ± 2.3$^{\#}$	9.9 ± 1.2	10.9 ± 2.2	7.3 ± 2.62	9.8 ± 3.2$^{\#}$
Glucose ($mmol \cdot L^{-1}$)						
Baseline			4.8 ± 0.6	5.0 ± 0.8		
Pre-Ex	5.0 ± 0.6	4.9 ± 0.7	5.2 ± 0.3	6.0 ± 0.4$^{\#}$		
Post-Ex	8.8 ± 1.7*	8.3 ± 1.9$^{*\#}$	9.7 ± 1.5*	8.3 ± 2.4$^{*\#}$		
Change	3.8 ± 1.8	3.4 ± 1.9$^{\#}$	4.5 ± 1.6	2.3 ± 2.1$^{\#}$		

Data are expressed as mean ± SD. L-T: laboratory study in temperate climatic conditions; F-T: field study in temperate climatic conditions; L-W: laboratory study in warm climatic condition. *Pre-Ex* immediately before 5000-TT, *Post-Ex* immediately after 5000-TT. Significantly different ($P < 0.05$): *from Pre-Ex; #from PLC.

In the F-T study, the measured concentration of lactate in plasma was significantly higher after the 5000-TT in the CIT (13.6 ± 3.0 $mmol \cdot L^{-1}$) than in the PLC (12.3 ± 1.5 $mmol \cdot L^{-1}$, $P = 0.04$). However, this significant between-trial difference was not evident after correcting for the measured concentrations of lactate for the individual changes in plasma volume (11.5 ± 1.5 $mmol \cdot L^{-1}$ in the PLC and 12.5 ± 2.6 $mmol \cdot L^{-1}$ in the CIT trial, $P = 0.16$) (Table 7).

Plasma glucose concentration was measured in the L-T and F-T studies but not in the L-W study. In two studies plasma glucose concentration did not differ before 5000-TT between the CIT and PLC groups. A significant increase in glucose level was observed as a result of the 5000-TT in both studies; however, the extent of the increase was significantly higher after PLC vs CIT administration (Table 7). The values of plasma glucose concentration measured after the PLC or CIT drink and after the 5000-TT are presented as corrected for the individual changes in PV.

5.5. Heart rate

HR was measured in the L-T and L-W studies. HR in the L-T study did not differ during 5000-TT between CIT and PLC trials, except that measured after three minutes (173.2 ± 12.0 beats·min^{-1}) in the CIT *vs* in the PLC trial (169.5 ± 13.5 beats·min^{-1}; $P = 0.03$). The maximum HR measured during 5000-TT did not differ: 194.8 ± 10.3 beats·min^{-1} in the CIT and 193.7 ± 9.2 beats·min^{-1} in the PLC.

A significant main effect of time ($F = 201.28$, $P < 0.0001$) but not of trial ($F = 0.0036$, $P = 0.953$) was evident for HR in the L-W study. Pre-Ex HR did not differ in the two trials (157 ± 13 beats·min^{-1} in PLC and 156 ± 11 beats·min^{-1} in CIT, respectively; $P = 0.556$). HR increased significantly during the 5000-TT with no statistical difference between the trials at any point in time. The highest HR values were registered at the finish of the run (195 ± 8 beats·min^{-1} in PLC and 195 ± 9 beats·min^{-1} in CIT, respectively; $P = 1.0$).

5.6. Ratings of perceived exertion, fatigue, and thermal sensation

RPE was measured in the L-T and L-W studies. During the L-T study, the participant's perception of effort remained consistent between the two trials throughout the exercise session. The RPE during the run varied from 14.9 ± 1.9 to 18.7 ± 0.8 for the CIT trial and from 15.4 ± 1.6 to 18.7 ± 1.3 for the PLC trial.

In the L-W study, a significant main effect of time and trial was evident for RPE (F = 459.85, P < 0.0001 and $F = 4.87$, $P = 0.043$ recpestively) (Figure 7). Overall, RPE was higher in PLC (14.0 ± 4.2) compared to CIT (13.6 ± 4.2, $P = 0.043$), but post hoc analysis revealed no significant between-trial difference at any time point. No effect of time-by-trial interaction was observed for RPE (F = 1.97, P = 0.092).

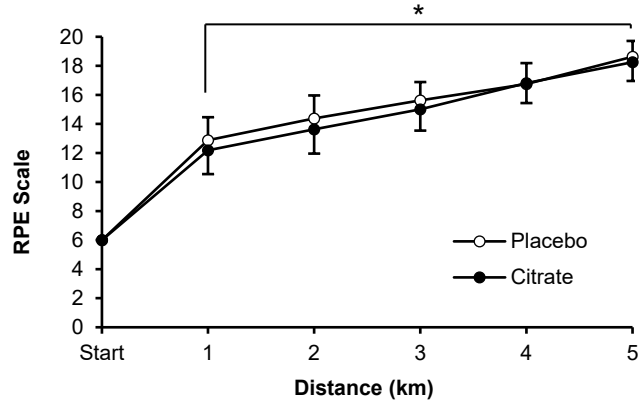

Figure 7. Ratings of perceived exertion during 5000-TT in the L-W study. Data are presented as mean ± SD, n = 16. Significantly different ($P < 0.05$): *from the start.

In addition to RPE the RPF and TS were measured in the L-W study, where significant main effect of time was evident for RPF (F = 196.04, P < 0.0001) (Figure 8), and TS (F = 138.34, P < 0.0001) (Figure 9) but no significant main effect of the trial for RPF (F = 1.54, P = 0.233) and TS (F = 0.016, P = 0.901). No effect of time-by-trial interaction was observed for RPF (F = 1.37, P = 0.245), and TS (F = 0.50, P = 0.778).

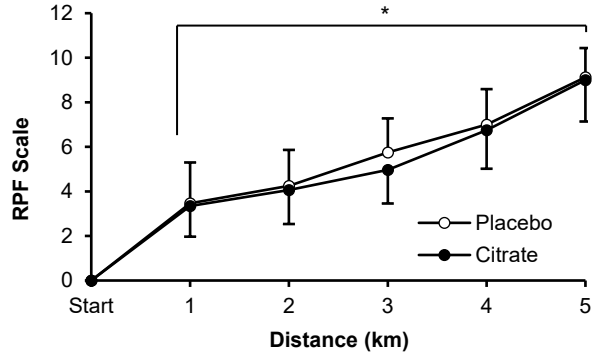

Figure 8. Ratings of perceived fatigue during the 5000-TT in the L-W study. Data are presented as mean ± SD, n = 16. Significantly different (P < 0.05): *from the start.

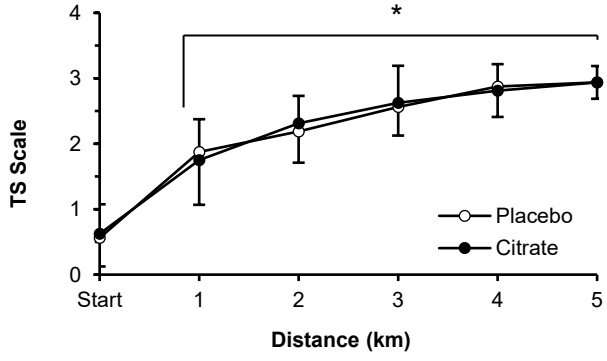

Figure 9. Ratings of thermal sensation during 5000-TT in the L-W study. Data are presented as mean ± SD, n = 16. Significantly different (P < 0.05): *from the start.

5.7. Gastrointestinal symptoms and other side effects

The side effects of CIT observed in the L-T study included nausea and thirst in 12 subjects combined with headache in two. All 17 subjects reported an urge to defecate or diarrhea after CIT supplementation.

In the F-T study, seven out of the ten subjects who participated reported mild nausea and diarrhea following ingestion of the solution containing CIT. In contrast, there were no such complaints in the PLC trial.

In the L-W study, four men complained of mild nausea or diarrhea within 30-minute time interval before the start of the run in the CIT trial. Three subjects reported indefinite unusual sensations (weird whirrs or feelings of dryness) in CIT before the 5000-TT. In PLC, one subject complained of an indefinite lousy feeling during 5000-TT, and one reported nausea after finishing 5000-TT.

5.8. Thermophysiological parameters measured in the L-W study

There was a significant main effect of time ($F = 555.04$, $P < 0.0001$) but not of trial ($F = 0.802$, $P = 0.385$) or trial by time interaction ($F = 1.66$, $P = 0.051$) for T_c. T_c increased with the duration of exercise, with no significant difference between the trials at any point in time (Figure 10).

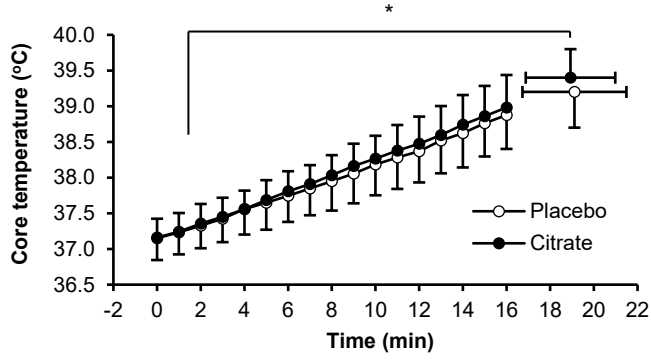

Figure 10. The core temperature during the 5000-TT in the L-W study. Data are presented as mean ± SD, n = 16. Significantly different ($P < 0.05$): *from time point 0.

Significant main effects of trial ($F = 6.73$, $P = 0.020$) and time ($F = 71.61$, $P < 0.0001$), but not time by trial interaction ($F = 0.498$, $P = 0.952$), were observed for T_{sk}. Overall, T_{sk} was higher in CIT (35.0 ± 0.8 °C) compared to PLC (34.8 ± 0.8 °C; $P = 0.020$), but post hoc analysis revealed no significant difference between PLC and CIT at any time point (Figure 11).

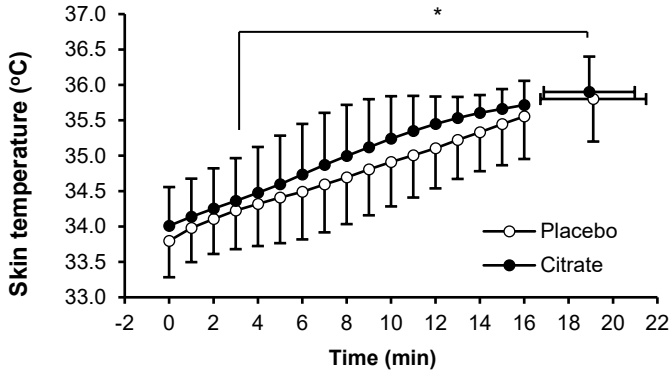

Figure 11. Skin temperature during 5000-TT in the L-W study. Data are presented as mean ± SD, n = 16. Significantly different ($P < 0.05$): *from time point 0.

There was no significant main effect of the trial ($F = 3.51$, $P = 0.081$), time ($F = 0.974$, $P = 0.488$), or trial by time interaction ($F = 0.358$, $P = 0.992$) for T_c to T_{sk} gradient, which did not change during exercise (Figure 12). There was no significant difference in HS (76.9 ± 8.9 and 79.9 ± 8.3 $W \cdot m^{-2}$ in PLC and CIT, respectively, $P = 0.298$) or HS rate (4.06 ± 0.53 $W \cdot m^{-2} \cdot min^{-1}$ in PLC and 4.26 ± 0.55 $W \cdot m^{-2} \cdot min^{-1}$ in CIT; $P = 0.196$) in the two trials.

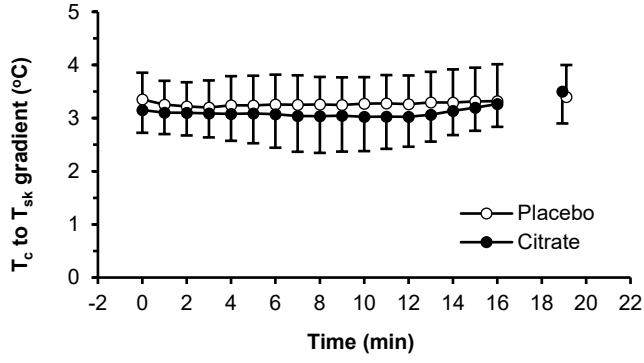

Figure 12. Core-to-skin temperature gradient during 5000-TT in the L-W study. Data are presented as mean ± SD, n = 16.

5.9. Blood electrolyte concentrations measured in the L-W study

There were significant main effects of trial (F = 17.55, P = 0.0008), time (F = 22.88, P < 0.001), and trial-by-time interaction (F = 15.71, P < 0.001) on blood [Na^+]. During the 5000 m run, blood [Na^+] increased equally (by 1.8 mmol·L^{-1}) in both the CIT and PLC trials (Table 8).

No significant main effect of trial (F = 3.41, P = 0.085) was observed for blood [K^+]. However, there was a significant main effect of time (F = 4.05, P = 0.028) and a significant trial-by-time interaction (F = 3.94, P = 0.030) on blood [K^+]. Pre-Ex blood [K^+] was significantly lower in the CIT trial than in the PLC trial (P = 0.011), but Post-Ex blood [K^+] levels did not differ between the two trials (Table 8).

Table 8. Blood [Na^+], [K^+], and [Cl^-] in the L-W study.

Electrolyte	Treatment	
	PLC	CIT
Na^+ (mmol·L^{-1})		
Baseline	140.4 ± 1.5	140.0 ± 1.7
Pre-Ex	139.3 ± 1.2	141.6 ± 1.7$^{\$\#}$
Post-Ex	141.1 ± 1.7*	143.4 ± 1.8$^{\$*\#}$
K^+ (mmol·L^{-1})		
Baseline	4.5 ± 0.4	4.5 ± 0.3
Pre-Ex	5.0 ± 1.0	4.3 ± 0.4$^{\#}$
Post-Ex	4.9 ± 0.5	4.9 ± 0.8*
Cl^- (mmol·L^{-1})		
Baseline	114.5 ± 2.3	115.0 ± 1.7
Pre-Ex	114.3 ± 2.1	113.0 ± 1.7$^{\$}$
Post-Ex	116.4 ± 2.4$^{\$*}$	114.8 ± 2.0$^{*\#}$

Data are expressed as mean ± SD; n = 16 Significantly different (P < 0.05): $^{\$}$from baseline; *from Pre-Ex; $^{\#}$from Placebo. *Pre-Ex* immediately before 5000 m treadmill run, *Post-Ex* immediately after 5000-TT.

Regarding blood [Cl^-], no significant main effect of trial (F = 2.49, P = 0.135) was observed, but there was a significant main effect of time (F = 24.98, P < 0.001) and a significant trial-by-time interaction (F = 6.98, P = 0.003). Pre-Ex blood [Cl^-] did not differ significantly between the two trials (P = 0.052), but Post-Ex blood [Cl^-] was significantly higher in the PLC trial compared to the CIT trial (P = 0.009) (Table 8).

6. DISCUSSION

The main objective of the three double-blind crossover studies forming the basis of this dissertation was to determine the effect of pre-exercise CIT ingestion on performance in endurance-trained young men during a 5000 m time trial (5000-TT) under different environmental conditions. A statistically significant performance-enhancing effect of CIT ingestion was observed only in a temperate laboratory environment (L-T study), where subjects completed the distance on average 2.6% (30.6 seconds) faster in the CIT trial compared to the PLC trial. In a simulated competition setting at an outdoor stadium under temperate conditions (F-T study), no ergogenic effect of CIT ingestion was observed. Similarly, in a warm laboratory environment (L-W study), there was no statistically significant difference in 5000-TT finish times between the CIT and PLC trials. However, participants completed the distance on average 12 seconds (1%) faster in the CIT trial, and calculations using Hopkins' (2007) method indicated that the probability of beneficial, trivial, or negative effects of CIT ingestion on performance under these conditions was 66%, 32%, and 2%, respectively.

6.1. Running performance in temperate environmental conditions

The main mechanism through which CIT ingestion may enhance physical performance is considered to be an increase in the capacity of extracellular (blood) buffering systems, as reflected in elevated blood pH, [HCO_3^-], and [BE] (de Oliveira et al., 2022). Unfortunately, due to lack of equipment, we did not measure any of these parameters in the L-T study.

However, increased extracellular buffering capacity facilitates lactate efflux from intensely working skeletal muscle (Lancha Junior et al., 2015). Thus, in the L-T study, the significantly higher plasma lactate levels observed after completion of the 5000-TT in the CIT trial compared to the PLC trial indirectly confirm the presence of greater extracellular buffering capacity in the former. Consequently, it is likely that this contributed to the improved performance observed in the CIT trial.

In the F-T study, both blood pH and plasma lactate levels were measured. CIT ingestion induced significant increases in blood pH, whereas PLC administration had no such effect. However, no significant between-trial differences in blood pH were observed at any time point—before supplement administration, prior to the 5000-TT, or after its completion. In other words, blood pH data did not provide clear evidence of increased buffering capacity in the CIT trial compared to the PLC trial.

The similar plasma volume-corrected lactate levels observed in both trials after the 5000-TT suggest that an increase in blood buffering capacity was likely not achieved in the CIT trial of the F-T study. Consequently, the failure to induce a sufficient increase in extracellular buffering capacity may have been one reason

why performance in the 5000-TT did not improve in the CIT trial. In fact, participants completed the 5000-TT in the CIT trial on average 17.3 seconds (1.6%) slower than in the PLC trial.

Research conducted under conditions resembling actual sports competition, as opposed to tightly controlled laboratory experiments, can offer more practical insights into the benefits of nutritional supplements (Schubert & Astorino, 2013). However, such studies inherently involve numerous uncontrollable variables. For example, although weather conditions (temperature, sunshine, wind) during the CIT and PLC trials in the F-T study were similar, they were not identical.

Additionally, certain study design elements may have influenced the results. Generally, competing head-to-head in matched pairs is thought to motivate athletes to complete the distance as quickly as possible (Ramsbottom et al., 1992). However, it cannot be ruled out that some pairs competed tactically to win their race rather than to achieve the fastest possible time. Thus, in addition to the failure to induce a sufficient increase in extracellular buffering capacity, some uncontrolled factors may explain why 5000-TT performance was not improved in the CIT trial compared to the PLC trial in the F-T study.

A sodium load comparable to that used in the L-T and F-T studies has been shown to significantly increase plasma volume (PV), with the effect potentially lasting more than three hours after ingestion of a sodium-containing solution (Lindinger et al., 2000). Unfortunately, we did not assess the relative changes in PV between CIT and PLC ingestion and the onset of the 5000-TT in the L-T study. Nevertheless, the significantly lower packed cell volume and hemoglobin concentration in the CIT trial compared to the PLC trial at the beginning of the 5000-TT suggest that PV had increased in the CIT trial at that time point (Dill & Costill, 1974).

In the F-T study, CIT ingestion induced a 9.8% increase in PV, whereas a 2% decrease occurred following PLC administration. Notably, Sawka et al. (2000) reported a 6% increase in VO_2max as a result of an 11% expansion in PV, with no changes in erythrocyte volume. A likely explanation for the improvement in VO_2max under these conditions is improved skeletal muscle perfusion during exercise (Mitchell et al., 1990). Moreover, Mitchell et al. (1990) demonstrated that, during exercise performed at 80% VO_2max, intravenous infusion of both $NaHCO_3$ and NaCl improved endurance performance compared to control conditions (no infusion), although only $NaHCO_3$ prevented the development of acidosis. In this context, the ergogenic effect may be attributed not only to enhanced buffering capacity but also to increased PV resulting from sodium-containing fluid infusion. However, since CIT-induced PV expansion occurred before the start of the 5000-TT in both the L-T and F-T studies, this does not explain why the positive effect of CIT supplementation on performance was observed only in the L-T study.

In the F-T and L-T studies, CIT was consumed over 60 and 10 minutes, beginning 180 and 130 minutes before the 5000-TT, respectively. Positive effects of CIT on performance have been observed when the time between ingestion and performance testing was 60 minutes (Shave et al., 2001), 90 minutes (Potteiger

et al., 1996a), or 120 minutes (Cunha et al., 2019). Thus, it is possible that in the F-T study, CIT ingestion was initiated too early before the start of the 5000-TT, and the alkalotic effect—if achieved—may have partially dissipated during the run. However, Urwin et al. (2019) recently reported that when CIT was administered in solution at a dose of 500 $mg \cdot kg^{-1}$ body mass, peak blood pH and [HCO_3^-] occurred at 175 minutes (95% CI: 159–191 min) and 164 minutes (95% CI: 148–180 min) after ingestion, respectively.

Considering these new data (Urwin et al., 2019), the timing of CIT administration in both the F-T and L-T studies may have been suboptimal—possibly more so in the F-T study due to the longer consumption period. In the CIT trial of the F-T study, our subjects began the 5000-TT with, on average, 0.8 kg greater body mass (BM) than in the PLC trial. Theoretically, the need to carry additional body mass may reduce running speed (Gigou et al., 2012). However, changes in BM and 5000-TT times did not correlate in our participants across the two trials.

Furthermore, participants in the L-T study also had greater BM before the 5000-TT in the CIT trial than in the PLC trial, yet they still completed the 5000-TT faster in the CIT trial. Notably, the findings of Gigou et al. (2012) suggest that carrying an additional load of approximately 1 kg is unlikely to impair running speed in trained runners. Thus, increased BM in the CIT trial is not a likely explanation for the lack of an ergogenic effect of CIT supplementation in the F-T study.

Alkalizing substances, including CIT, may induce gastrointestinal discomfort (GID) (Cox & Jenkins, 1994; Ööpik et al., 2008; Potteiger et al., 1996a; Schabort et al., 2000; Shave et al., 2001; van Someren et al., 1998). In total, 7 out of 10 participants in the F-T study reported mild nausea and diarrhea following CIT ingestion, while no such complaints were reported after PLC administration.

Nevertheless, this finding does not explain the lack of an ergogenic effect of CIT, as in the L-T study, all 12 participants reported some symptoms of GID within the first hour after CIT administration, yet their 5000-TT performance improved. Similarly, other researchers have observed improvements in endurance performance in cycling (Potteiger et al., 1996a) and running (Shave et al., 2001) following acute CIT administration, despite the presence of GID symptoms.

During intense exercise, metabolic acidosis becomes increasingly pronounced as the effort continues. Therefore, it can be assumed that the negative impact of acidosis on performance increases with exercise duration, and that the potential ergogenic effect of CIT may be more evident in the presence of relatively stronger acidosis—i.e., in the later stages of intense exercise.

Indeed, in multidisciplinary elite athletes, CIT ingestion compared to placebo significantly improved performance time in the 3000 m run due to a higher pace in the fifth and sixth 400 m segments (Shave et al., 2001). Based on this, we hypothesized that CIT could help maintain a higher pace, especially toward the end of the 5000-TT.

Our results partially support this assumption: running pace was significantly higher in the CIT trial than in the PLC trial at kilometers 2 and 4 in the L-T study, and at kilometer 4 in the F-T study. However, in the study by Potteiger et al.

(1996a), cyclists completed a 30-km ride significantly faster (57.7 minutes) in the CIT trial than in the PLC trial (59.4 minutes), but their power output in the CIT trial was higher only during the first 25 minutes—not in the later stages of the ride.

In the L-T and F-T studies, blood glucose levels increased during the 5000-TT, but significantly less so in the CIT trial compared to the PLC trial. It can be speculated that in the L-T study, this difference between trials may be explained by increased muscle glucose uptake due to slightly higher average exercise intensity.

On the other hand, results from a study using perfused rat hindquarters suggest that metabolic alkalosis itself may enhance glucose uptake in contracting skeletal muscle (Spriet et al., 1986). Alternatively, metabolic alkalosis has been shown to suppress the catecholamine response to exercise (Bouissou et al., 1988), which may reduce hepatic glucose release into the bloodstream. However, Bouissou et al. (1988) used $NaHCO_3$, while Bracken et al. (2005) induced metabolic alkalosis using CIT and observed no effect on epinephrine levels during exercise.

In addition to metabolic alkalosis, an acute increase in PV may blunt the exercise-induced rise in catecholamine levels (Grant et al., 1996; Green et al., 1989). In the L-T and F-T studies, CIT ingestion induced greater increases in PV than PLC administration prior to the 5000-TT, but changes in PV during the 5000-TT did not differ between trials.

Thus, due to the lack of catecholamine data in our L-T and F-T studies and the inconsistent findings in the literature (Bouissou et al., 1988; Bracken et al., 2005), it remains unclear whether the differences in blood glucose responses between the CIT and PLC trials were caused by differences in catecholamine responses to exercise.

Athletes with superior training status exhibit greater anaerobic glycolytic capacity, which may enhance the ergogenic effects of alkalizing agents in this population (Heibel et al., 2018; Requena et al., 2005). On the other hand, Peart et al. (2013) argue that better training status is associated with higher MCT protein density and muscle buffering capacity, and therefore enhancing extracellular buffering capacity may provide fewer performance benefits in highly trained individuals.

Linossier et al. (1997) found that VO_2peak correlated with time to exhaustion, with less fit athletes showing greater performance improvements following CIT ingestion than fitter ones. This may explain why some studies (Martins et al., 2010; Ööpik et al., 2008, 2010; Potteiger et al., 1996b) found no CIT-related performance gains in well-trained subjects.

Based on aerobic capacity (Table 1), the L-T group had the highest training status, the F-T group moderate, and the L-W group the lowest. Thus, the performance gains observed in the L-T group may be attributed to their higher training status. However, the lack of improvement in the F-T and L-W groups does not support the claim by Peart et al. (2013) or the findings of Linossier et al. (1997) that lower training status enhances responsiveness to extracellular buffering agents.

The highly individual response to CIT supplementation has prompted calls for further research into personalized dosing strategies (Heibel et al., 2018; Schubert & Astorino, 2013). Although blood acid–base responses following CIT ingestion show limited reproducibility—making individualized timing based on peak alkalosis difficult to recommend (Tinnion et al., 2024b)—our findings suggest that a tailored approach may still be beneficial.

Of the 43 subjects across our three studies, 26 improved their performance after CIT ingestion, while 17 ran faster after taking the placebo (PLC). Notably, two out of five participants who took part in both the L-T and F-T studies improved their 5000 m performance under CIT in both conditions. Similarly, Potteiger et al. (1996b) reported that four out of seven subjects improved performance by approximately 30% during CIT trials compared to PLC.

6.2. Running performance in a warm environment

Our L-W study was the first to investigate whether acute pre-exercise ingestion of CIT, in a quantity previously shown to enhance endurance capacity under controlled temperate conditions, is effective in improving endurance running performance in a warm environment. The main findings of the study indicate that CIT induced metabolic alkalosis, water retention, and an increase in plasma volume (PV), but did not alleviate heat strain or significantly improve performance in the 5000 m time trial (5000-TT). Nevertheless, ten of the sixteen participants performed better in the CIT trial, while six were faster in the placebo (PLC) trial. The likelihood of benefit from CIT supplementation was 66%, with only a 2% chance of harm.

Increasing blood buffering capacity, thereby improving the regulation of intramuscular pH, is considered the most likely mechanism behind the ergogenic effect of CIT (Heibel et al., 2018; Requena et al., 2005). During exercise in the heat, muscle energy supply relies more heavily on glycolytic phosphorylation compared to equivalent exercise in temperate conditions, resulting in greater increases in muscle and blood lactate levels (Febbraio, 2001; Febbraio et al., 1994; McAnulty et al., 2005) and [H^+] concentration (Robergs et al., 2004). Therefore, it can be hypothesized that a warm environment favors the manifestation of the ergogenic effects of alkalizing agents such as CIT during intense exercise. However, our findings do not support this assumption.

Several factors may have attenuated the potential ergogenic effect of CIT in our participants. For instance, the exercise intensity may have been too low, inducing only mild acidosis. Indeed, Ibanez et al. (1995) noted that the likelihood of performance improvement due to induced alkalosis is greater in exercise tasks where blood lactate levels rise to 9–18 mmol·L^{-1}, compared to tasks producing a rise to only 6 mmol·L^{-1}. In the PLC trial of the L-W study, the mean post-5000-TT blood lactate concentration was 8.2 mmol·L^{-1}—lower than the 9.8 mmol·L^{-1} observed in our L-T study and the 15.9 mmol·L^{-1} reported by Shave et al. (2001) for subjects completing a 3000-m run in temperate conditions. In both cases (our

L-T study and Shave et al., 2001), CIT ingestion significantly improved performance.

However, Potteiger et al. (1996) reported mean blood lactate levels of only ~4 mmol·L^{-1} and pH values around 7.35 at the end of a 30-km cycling trial with prior PLC administration, yet still observed significant performance improvement due to CIT ingestion. In the PLC trial of the L-W study, our participants' mean blood pH was 7.3. Thus, the blood lactate and pH data suggest that exercise intensity was likely not the primary factor limiting the potential ergogenic effect of CIT in our L-W study.

In addition to exercise intensity, the degree of induced metabolic alkalosis is an important factor influencing the performance effects of CIT (McNaughton, 1990). In the L-W study, CIT administration led to mean increases of 26.7% in blood HCO_3^- concentration, 0.07 units in pH, and a 12.3-fold increase in base excess (BE). A similar increase (21.3–27.4%) in pre-exercise blood HCO_3^- levels has been associated with markedly improved performance in high-intensity ergometer exercise (Robertson et al., 1987).

In cyclists who showed improved performance with CIT compared to PLC ingestion, blood pH remained unchanged during the first 90 minutes after CIT ingestion but increased by 0.02–0.09 units during the subsequent 30-km ergometer time trial (Potteiger et al., 1996). Furthermore, CIT compared to PLC increased post-exercise blood lactate concentration by 19.5–40% in studies demonstrating its ergogenic effect on endurance performance (our L-T study; Potteiger et al., 1996; Shave et al., 2001). A similar effect was observed in our L-W study, where post-5000-TT blood lactate levels were 35.4% higher in the CIT trial compared to PLC.

Altogether, these data suggest that the degree of metabolic alkalosis induced by CIT ingestion in our L-W study participants was within the range shown to be sufficient for eliciting performance-enhancing effects.

Hydration status is considered an important factor influencing endurance performance in warm environments (Cheuvront et al., 2010; McDermott et al., 2017; Sawka et al., 2012). Similar values for body mass (BM), urine specific gravity (USG), and urine osmolality (UOSM) confirm that our subjects arrived at the laboratory equally hydrated in both the PLC and CIT trials of the L-W study.

CIT ingestion, compared to PLC, reduced urine output and increased apparent water retention, resulting in expanded plasma volume (PV) and increased BM just before the start of the 5000-TT. A pre-exercise acute PV increase in the range of 4.4–4.8% has been shown to improve endurance capacity in both trained women and men exercising in the heat (Sims et al., 2007a, b), and in untrained men under temperate conditions (Greenleaf et al., 1997).

In the L-W study, the increase in PV in the CIT trial was greater than in the PLC trial, but modest in magnitude (mean change 2.9%) and therefore possibly physiologically insufficient to induce any ergogenic effect. On the other hand, a much greater acute PV expansion (9.8%) following CIT ingestion had no impact on 5000-TT performance in our F-T study.

Thus, the impact of acute pre-exercise PV expansion on endurance capacity may vary depending on the type of test protocol employed. In studies demonstrating a positive effect of acute PV expansion on endurance performance (Greenleaf et al., 1997; Sims et al., 2007a, b), time to volitional exhaustion was measured during constant-intensity exercise.

On the other hand, the combined effects of exercise type and intensity may influence the impact of acute plasma volume (PV) expansion on endurance capacity in warm environments. Sims et al. (2007a) administered equal volumes of either a concentrated-sodium or low-sodium drink to their subjects prior to constant-load cycling to volitional exhaustion in the heat. The former beverage, compared to the latter, induced acute pre-exercise PV expansion along with a significant reduction in the rate of core temperature rise (0.02 vs. 0.03 °C·min^{-1}) during exercise and prolonged time to exhaustion.

In our L-W study participants, CIT ingestion increased PV but had no effect on the rate of core temperature rise during exercise (0.11 °C·min^{-1} in PLC and 0.12 °C·min^{-1} in CIT) or on 5000-TT performance. The higher average heart rate during exercise in our participants (178 beats·min^{-1} in both trials), compared to 156–165 beats·min^{-1} reported in Sims et al. (2007a), suggests that our subjects exercised at a higher intensity.

Thus, although not conclusive, the data from Sims et al. (2007a) and our L-W study collectively imply that modest acute PV expansion may be sufficient to alleviate thermoregulatory strain and improve endurance capacity during constant-load, moderate-intensity exercise in warm environments. In contrast, during high-intensity exercise (e.g., time trials), the high rate of body heat generation may exceed any potential thermoregulatory and performance benefits provided by modest pre-exercise PV expansion.

According to the concept of critical core temperature (Tc), physical performance in the heat declines when Tc exceeds 39.5–40.1 °C (Gonzalez-Alonso et al., 1999; Nielsen et al., 1993). However, Cheuvront et al. (2010) and Sawka et al. (2012) have presented strong evidence that hot skin (defined as skin temperature, Tsk > 35 °C), rather than high Tc, is the primary factor limiting aerobic exercise performance in the heat.

In our L-W study participants, mean Tsk reached 35 °C after 8 and 11 minutes of running in the CIT and PLC trials, respectively, and overall Tsk was slightly (0.2 °C) higher in the CIT trial. Moreover, there were no between-trial differences in the Tc–Tsk gradient, heat storage (HS), or HS rate during exercise. Collectively, these data suggest that CIT ingestion induced only marginal changes in thermoregulation, which did not favor endurance performance in a warm environment.

Overall, our data from the L-W study suggest that CIT ingestion induced only minor changes in thermoregulation and a modest acute pre-exercise PV increase, neither of which favored endurance performance in a warm environment. In previous studies, acute PV expansion was associated with significantly lower mean (Sims et al., 2007a) or final (Sims et al., 2007b) ratings of perceived exertion

(RPE) and prolonged time to exhaustion during constant-load, moderate-intensity exercise in the heat.

In our study, there were no significant differences between conditions in ratings of perceived fatigue (RPF) or thermal sensation during the 5000-m run. Overall RPE in the L-W study was lower in the CIT trial than in the PLC trial, but the absolute difference was marginal (0.4 units).

In temperate conditions in our L-T study, however, CIT ingestion improved endurance performance without affecting RPE, similar to the findings of Potteiger et al. (1996). According to Cheuvront et al. (2010), heat stress increases RPE, and any factor that alters RPE may influence motivation-driven motor-neural activation. Therefore, the weak impact on perception may partially explain the lack of ergogenic effect of CIT in our subjects under warm conditions.

Alkalosis induced by $NaHCO_3$ (Sostaric et al., 2006) or CIT ingestion (Ööpik et al., 2010; Street et al., 2005) has been shown to lower circulating potassium (K^+) levels and extend time to fatigue (Sostaric et al., 2006) during dynamic forearm exercise. Reduced blood K^+ accumulation is thought to reflect less exercise-induced membrane depolarization, which may be a key mechanism behind improved performance with induced alkalosis (Sejersted & Sjøgaard, 2000; Sostaric et al., 2006).

Conversely, Broch-Lips et al. (2007) found that extracellular alkalosis did not affect K^+ efflux from contracting muscle or muscle fatigue in an isolated rat muscle model. In our study, pre-5000-TT blood [K^+] was slightly lower in the CIT trial compared to the PLC trial, but no differences were observed post-5000-TT. Therefore, alkalosis did not reduce K^+ release from active muscles during the 5000-TT in our subjects. The significance of this finding in explaining the lack of ergogenic effect of CIT remains unclear.

In the L-W study, CIT was administered in gelatin capsules, and only four out of sixteen subjects (25%) reported any gastrointestinal discomfort (GID). Three of them described an unusual sensation (dryness), yet all performed better in the CIT trial. Previous (van Montfoort et al., 2004) and more recent studies (Peacock et al., 2021; Urwin et al., 2019, 2023) show that consuming CIT in gelatin capsules with a water bolus is less likely to cause GID than ingesting an equivalent amount in aqueous solution.

6.3. Strength and limitations

One of the key strengths of this dissertation is that it draws on data collected from three randomized, double-blind, placebo-controlled crossover studies. This study design generally enhances the internal validity of experimental research. Furthermore, the shared methodological framework across the three studies strengthens consistency and improves the comparability of the findings. The participants were well-trained endurance athletes, ensuring a high level of physiological preparedness and relevance to competitive performance. Although none were specifically specialized in the 5000 m event, their training backgrounds were nonetheless well-aligned with the demands of the study protocol.

Notably, our L-W study was the first to investigate the potential ergogenic effect of CIT supplementation on endurance running performance in a warm environment, representing a novel contribution to the field of exercise physiology.

One limitation worth noting is that the first two studies employed a relatively limited set of methods for assessing biochemical parameters. While the core outcomes remained clearly measurable and relevant to the research questions, a broader range of biochemical markers could have provided a more comprehensive understanding of the underlying physiological mechanisms. Nevertheless, the methodological consistency and focus on performance-related outcomes ensured that the findings remain meaningful and applicable.

CONCLUSIONS

Acute sodium citrate supplementation before exercise at a dose of 500 $mg \cdot kg^{-1}$ body mass, compared to placebo treatment:

1. Improves performance in well-trained athletes during a 5000 m treadmill time trial in a temperate laboratory environment. However, in a simulated competition setting—either in an outdoor stadium or during a treadmill run in a warm laboratory environment—the ergogenic effect of sodium citrate supplementation on performance does not occur.
2. Induces metabolic alkalosis, reflected by increases in blood pH, bicarbonate (HCO_3^-) concentration, and base excess, apparently facilitating lactate efflux from intensively contracting skeletal muscles and increasing post-exercise blood lactate levels.
3. Induces pre-exercise water retention, increases plasma volume and body mass, but does not affect changes in plasma volume or attenuate increases in blood potassium (K^+) concentration during the 5000 m run.

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SUMMARY IN ESTONIAN

Naatriumtstraadi manustamise mõju ainevahetusele ja 5000 m jooksu tulemusele hästi treenitud vastupidavusalade sportlastel

Sissejuhatus

Intensiivse kehalise tööga kaasnev kiire ja ulatuslik ATP hüdrolüüs ning selle jätkuv taastootmine glükolüütilsel ainevahetusrajal, tingib töös osalevate lihaste ja vere ulatusliku hapestumise. Vabade vesinikioonide kontsentratsiooni suurenemist sarkoplasmas ning rakkudevahelises vedelikus ja veres peetakse üheks väsimusseisundi tekkimise ja süvenemise põhjuseks kõrge intensiivsusega kehalisel tööl (Fitts, 2016). Vesinikioonide hulga tõusu suhtes on tundlikud eelkõige anaeroobse glükolüütilise ainevahetusraja regulaatorensüümid (Jubrias et al., 2003) ning ka kontraktiilaparaat (Kent-Braun et al., 2012). Organismi puhversüsteemide mahutavuse kunstliku suurendamise idee, töövõime hõlbustamiseks, pärineb juba möödunud sajandi neljandast kümnendist (Carr et al., 2011; 2023). Kõnealuses valdkonnas on otsese toimeainena kõige enam tähelepanu pälvinud naatriumvesinikkarbonaat, väiksemal määral naatriumtsitraat (CIT) ja mõningad fosfaatsoolad. Teoreetiliselt peaks viimati mainitud ühendite tööeelne manustamine kutsuma esile ulatusliku alkaloosi, suurendades veres ringlevate bikarbonaat ioonide kontsentratsiooni ja tõstma vere pH väärtust (McNaughton, 1990; Requena et al., 2005). Sel moel valmistatakse organism justkui ette võitluseks vabade vesinikioonidega, mille hulk kasvab kiiresti kõrge intensiivsusega tegevuse käigus ning häirib kontraktiilse aparaadi tööd, ATP taastootmist, ioonest tasakaalu. Lisaks võib CIT soodustada vedelikupeetust organismis (Ööpik et al., 2008; Timpmann et al., 2012), mis võib parandada vastupidavuslikku töövõimet (McDermott et al., 2017).

Hoolimata peamise toimemehhanismi mõistetavusest jääb aga paljugi arusaamatuks CIT kui toidulisandi tarbimisel seoses tegevuse erineva iseloomu, spordiala spetsiifilisuse ja kõrge temperatuuriga keskkonnas.

Uurimistöö eesmärk

Uurimistöö peamine eesmärk oli tuvastada CIT manustamise mõju ainevahetusele ja 5000 m jooksu tulemusele mõõduka ja sooja temperatuuriga laboratoorses keskkonnas ning võistlustingimustes välisstaadionil.

Uuritavad ja metoodika

Kolmes, erinevatel keskkonna tingimustel, läbiviidud uuringuseerias osales vabatahtlikkuse alusel kokku 43 hea treenitusega vastupidavusalade mees sportlast. 5000 m distants läbiti aja peale kas, jooksulindil siseruumi temperatuuril (22 °C; L-T), jooksulindil kuumas (32 °C ja õhuniiskus 50%) keskkonnas (L-W) või staadionil (19 °C), tõelisele võistlusele lähedastes tingimustes (F-T). Uuritavate arv (n), keskmine (±SD) vanus ja maksimaalne aeroobne võimekus (VO_2 *max;*

peak) olid vastavalt: L-T uuringus n=17, 20,9 ± 1,9 a ja 61,3 ± 4,9 ml·kg^{-1}·min^{-1}; F-T uuringus n = 10, 22,1 ± 2,5 a ja 60,8 ± 5,5 ml·kg^{-1}·min^{-1} ning L-W uuringus n = 16, 25,8 ± 4,4 a ja 56,9 ± 4,7 ml·kg^{-1}·min^{-1}.

Kõigi 3 uuringu korral kasutati juhuslikult määratud, platseeboga (PLC) kontrollitud, topeltpimedat ristuvat uuringukavandit ning manustatud CIT koguseks oli 500 mg·kg^{-1}. L-T ja F-T uuringus manustasid uuritavad selle lahustatuna kas 1L või 1,5L mineraalvees, 10 või 60 minuti vältel ning puhkasid seejärel 120 min. PLC manustati vastavalt 1 või 1,5L maitsestatud, madala kalorsusega mineraalvett. L-W uuringus manustasid uuritavad naatriumtsitraadi želatiinkapslites 30 min vältel koos piiramatu koguse veega, misjärel puhkasid 120 min. Platseebona manustati želatiinkapslitesse pakitud nisujahu koos piiramatu koguse veega.

Pärast CIT või PLC manustamist hinnati uuritavate töövõimet 5000 m läbimiseks kulutatud aja alusel. Päev enne testi sooritamist paluti uuritavatel jätta treeninguvabaks ning teisele testile eelnenud päeval paluti neil hoida sarnast kehalise koormuse ja toitumise režiimi nagu oli esimesele testile eelnenud päeval. 5000 m töövõime test algas kõigis kolmes uuringuseerias umbes 5 minutilise individuaalse soojendusega, testimise ajal ergutati uuritavaid verbaalselt parima võimaliku tulemuse saavutamisele, siiski oli uuritavatel lubatud jooksu kiirust muuta igal ajal vastavalt oma jõuvarude tunnetamisele.

Hindamaks CIT mõju veestaatusele fikseeriti vaatlusaluste kehamass vahetult enne (F-T, L-W), koheselt CIT või PLC manustamise järel (L-T), vahetult enne töövõime testimist, pärast tualetis käimist ning vahetult pärast töövõime testi lõpetamist, eemaldades higised riided. Täpsemaks keha hüdratatsiooni staatuse määramiseks, L-W uuringus, koguti vaatlusalustelt uriini proovid igakordsel tualetikülastusel uuringu ajal. Mõõdeti uriini erikaal ning osmolaalsus.

Kirjeldamaks CIT manustamise mõju südame-veresoonkonnale fikseeriti uuritavate südamelöögisagedus L-T ja L-W uuringus. Samuti andsid uuritavad töövõime testi vältel subjektiivse hinnangu tajutavale väsimuse astmele L-T ja L-W uuringus ning L-W uuringus anti hinnang ka temperatuuri tunnetusele.

Vereanalüüsides määrati hemoglobiini (Hgb), hematokriti (Ht), laktaadi ja glükoosi kontsentratsioonid L-T ja F-T uuringutes. F-T ja L-W uuringus lisaks eelpool loetletutele ka vere pH ning L-W uuringus lisaks veel vere gaasid, et kalkuleerida HCO_3^- kontsentratsioon ning puhveraluste nihe (BE) ning ka elektrolüütide Na^+, K^+ ja Cl^- kontsentratsioonid. L-W uuringus glükoosi kontsentratsiooni ei mõõdetud.

L-W uuringus mõõdeti ka keha rektaaltemperatuur (T_c) ja naha temperatuur (T_{sk}), mille põhjal kalkuleeriti T_c/ T_{sk} gradient.

Tulemused

L-T uuringu tingimustes läbiti 5000 m distants oluliselt kiiremini CIT manustamise foonil (p = 0,01). F-T uuringus 5000 m läbimise aeg ei erinenud CIT või PLC manustamise järgselt: 1100,0 ± 79,1 ja 1082,7 ± 62,0 s CIT ja PLC tingimustes, vastavalt (P = 0,09). L-W uuringus 5000 m läbimise aeg (18,92 ± 2,05 min CIT, 19,11 ± 2,38 min PLC; 66 % edu tõenäosus, d = −0.09) oli sarnane (P > 0,05) kahe menetluse tingimustes.

F-T uuringus vere pH tõusis 7,34 ± 0,07 kuni 7,49 ± 0,07 (P = 0,002) CIT manustamise järgselt, jäädes PLC manustamise järgselt stabiilseks 7,40 ± 0,04 ja 7,44 ± 0,09. Samuti L-W uuringus 5000 m jooksu eelne ja järgne HCO_3^- kontsentratsioon, BE ja pH olid oluliselt kõrgemad (P < 0,001) CIT manustamise järgselt.

Laktaadi kontsentratsioon 5000 m läbimise järgselt oli kõrgem CIT manustamise tingimustes võrreldes PLC manustamisega (11,9 ± 3,0 mmol·L^{-1} *vs* 9,8 ± 2,8 mmol·L^{-1}; P < 0,001) L-T uuringus ning ka L-W uuringus, kus 5000 m jooksu järgne vere laktaadi kontsentratsioon oli kõrgem (P < 0,001) CIT manustamise järgselt (11,05 ± 3,22 mmol·L^{-1}) võrreldes PLC manustamise järgsega (8,22 ± 2,64 mmol·L^{-1}). Nii L-T kui F-T uuringu 5000 m jooksu järgne glükoosi kontsentratsioon oli madalam CIT manustamise järgselt võrreldes PLC manustamise järgse kontsentratsiooniga (8,3 ± 1,9 mmol·L^{-1} *vs* 8,8 ± 1,7 mmol·L^{-1}; P = 0,02 L-T ja 9,0 ± 1,7 mmol·L^{-1} vs 10,3 ± 1,5 mmol·L^{-1} F-T uuringus). Kõigi 3 uuringu tingimustes, manustamise eelne kehamass kahe menetluse tingimustes ei erinenud, enne starti olid uuritavad oluliselt raskemad CIT manustamise järgselt võrreldes PLC manustamisega 78,2 ± 5,6 *vs* 77,5 ± 6,1 kg (P = 0,03) L-T, 74,2 ± 6,1 kg *vs* 73.4 ± 6,2 kg, P = 0,048) F-T ning 77,2 ± 5,8 *vs* 76,7 ± 5,9 kg, (P = 0,03) L-W uuringus.

Võrreldes PLC manustamisega oli CIT manustamise järgne 5000 m jooksu eelne ja järgne Ht ja Hgb kontsentratsioon madalam L-T uuringus, kuid suhteline plasmamahu muutus kahe menetluse tingimustes ei erinenud. F-T uuringus oli suhteline plasmamahu muutus PLC manustamise järgselt −1,99 ± 3,49% ja CIT manustamise järgselt 9,75 ± 6,51% (P = 0.001) ning L-W uuringus vastavalt −0,8 ± 4,34% ja 2,92 ± 4,36% (P = 0,001). L-T ja L-W uuringus subjektiivselt tunnetatud väsimuse aste ja südame löögisagedus ning väsimuse ja kuuma tunnetus, aga ka rektaaltemperatuur, keha soojuse salvestamine, selle ulatus L-W uuringus olid sarnased kahe menetluse tingimustes (P > 0.05).

Järeldused

Võrreldes platseeboga, naatriumtsitraadi manustamine enne 5000 m jooksu koguses 500 mg kehakaalu kilogrammi kohta:

1. lühendab distantsi läbimiseks kuluvat aega liikuval jooksurajal mõõduka temperatuuriga laboratoorses keskkonnas, kuid simuleeritud võistlustingimustes välistaadionil või liikuval jooksurajal kuumas laboratoorses keskkonnas sooritusvõimet ei paranda
2. põhjustab metaboolset alkaloosi, mis avaldub vere pH, HCO_3^- kontsentratsiooni ja eelisreservi tõusus, ning soodustab seeläbi laktaadi väljutamist töötavatest lihastest, mis tõstab laktaadi taset veres 5000 m jooksu lõpuks
3. põhjustab puhkeolekus enne jooksu algust vee peetust, suurendab plasma mahtu ja kehamassi kuid ei mõjuta plasma mahu muutuse ulatust ega pidurda vere K^+ kontsentratsiooni tõusu 5000 m jooksu ajal.

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