Hypervolemia, thermoregulation, and exercise performance under severe heat stress

by

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Abstract

The purpose of this study was to determine if the ingestion of sodium citrate (CIT), and the subsequent expansion of plasma volume, would have a direct effect on thermoregulation and physiological function during a simulated 1-hour time trial (TT) under sever heat stress. Eight subjects, aged 24.9 years (SD $\pm$ 4.4), were studied under the following three conditions: (1) High Sodium Citrate (HCIT; 0.2 g/kg sodium citrate), (2) Low Sodium Citrate (LCIT; 0.1 g/kg sodium citrate), and (3) Control (Gatorade). Blood samples, taken before and during exercise, were analyzed for hematocrit (Hct), haemoglobin (Hb), bicarbonate ($HCO3^-$), base excess (BE), pH, sodium (Na), potassium (K), and glucose (GLU). Rectal temperature (T_r) and skin temperature (T_{sk}) was also recorded. Heart rate and psychophysical strain were also measured throughout each TT. Plasma volume significantly increased by 7.1% after ingesting the HCIT compared to the other two trials. There were no differences in mean body temperature, physiological strain, cardiovascular strain or psychophysical perception. The ingestion of HCIT appears to improve cycling performance despite no difference in physiological measurements. HCIT reduced split times, better maintained power output over the TT, and improved time total cycling time.

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Dedication

To my Mom and Dad, Bonnie and Doug, and my brother, Sean, who have always supported my aspirations without question and believed in me when the times were tough. Your love and support has made this possible. Thank you.

Chapter 1

Sodium citrate (CIT) is used, as an ergogenic aid, with the intent of improving athletic performance. Once ingested, CIT dissociates into Na^+ and $citrate3^-$ ions forcing the blood to lose its acid-base balance due to the influx of the three negatively charged citrate ions. In an attempt to maintain the electrical neutrality, the body naturally decreases the concentration of hydrogen [H^+] ions and increases the amount of bicarbonate [$HCO3^-$] within the blood. $HCO3^-$ is one of the body's natural buffers. The subsequent reduction in H^+ induces alkalosis and causes an efflux of H+ and lactate [Lac] via a pH sensitive monocarboxylate transporter from the working muscles into the venous blood (Kowalchuk et al. 1989; Cho et al. 1992; Van Montfoort et al. 2004; Requena et al. 2005). During intense exercise, where the human body relies heavily on glycolysis for fuel, large concentrations of H^+ accompany the accumulation of Lac^-; a coupling which has been associated with the onset of fatigue (Robergs, 2005; van Someren et al. 1998; Van Montfoort et al. 2004). Artificially buffering the concentrations of H^+ produced during exercise through CIT ingestion has received a great deal of attention throughout the athletic community. A large body of work is available focusing on CIT ability to buffer Lac^- and H^+.

In an attempt to improve buffering capacity, athletes will often ingest large quantities of water (500-1000 ml) mixed with relatively high sodium citrate concentrations (0.5 g/kg body mass). The intent is to promote rapid efflux of Lac^- and H^+ from the working muscles creating a more favourable environment for the working muscles to function. It is possible that the ingestion of high sodium solutions prior to

exercise will expand plasma volume and improve fluid retention (Greenleaf et al. 1998; Coles and Luetkemeler, 2004). Very little work has been done to investigate the effects of high sodium ingestion prior to athletic competition. Expanding plasma volume prior to exercise could potentially improve the distribution of blood to working muscles and to the skin (Kargotich et al. 1998). Dehydration and hyperthermia have a negative effect on plasma volume; heat stress combined with exercise, can reduce plasma volume by as much as 27% (Fortney et al. 1988). Following impaired plasma volume, stroke volume and cardiovascular function are reduced, while heart rate and core temperature increase (Gonzalez-Alonso et al. 1997). More work is therefore needed to determine if sodium citrate ingestion prior to exercise can improve cardiovascular function and thermoregulation.

Purpose of the Study

The purpose of this study was as follows:

1) Determine if CIT ingestion provides a significant ergogenic benefit during competitive endurance exercise under varying dosages. Specifically, to monitor the impact on CIT ingestion on of hypervolemia on cardiovascular and thermoregulatory function as well as to distinguish or separate these changes from the potential buffering capacity of ingesting CIT.
2) Investigate if acute hypervolemia improves intense aerobic cycling performance under severe heat stress.

Need for the Study

It is evident throughout the literature that sodium citrate has the potential to improve the buffering capacity of an athlete when ingested under controlled

environments and variables. Almost all the studies involving sodium citrate and sodium bicarbonate have monitored this buffering mechanism. Less work has been conducted to investigate the potential for high sodium solutions to improve cardiovascular and thermoregulatory function during exercise. Even fewer studies have specifically monitored the effects *sodium citrate* has on the above mechanisms.

Athletes often compete under stressful circumstances; the stress of competition, physical and mental, as well as varying environmental stresses, like heat and humidity. Substrate metabolism is an inefficient process; where 80% of energy production is lost in the form of heat (Cheuvont and Haymes, 2001; Wilmore and Costill; 1999). Exercise is therefore one of the primary contributors to heat production in an athlete. This heat must then be controlled via two main mechanisms in an attempt to maintain optimal working temperature: (1) increased blood flow to the skin, and (2) evaporation (sweating) (Morimoto et al. 1998). Following environmental heat stress, athletes must further reduce this build-up of heat in an attempt to avoid hyperthermia. Thermoregulatory sweating eventually results in excessive body water loss, especially when evaporation and heat convection at the skin no longer significantly lowers core temperature (Armstrong et al. 1998). Dehydration has been shown to reduce plasma volume; a consequence which will severely limit skin blood flow in an attempt to supply adequate blood (O_2 and nutrients) to the working muscles and the core (Gonzalez-Alonso et al. 1997).

Very little work has been done to investigate this phenomenon. In a study conducted by Grant et al. (1997), plasma volume was expanded by infusing three different concentrations of a 6% saline solution: (1) 277 ml (low condition), (2) 554 ml

(high condition), and no infusion. Plasma volume was found to remain higher throughout rest and exercise in the hypervolaemic states (control < low < high). Cardiac function was also improved following the infusion, evidenced by an increase in cardiac output (~1.4 L/min) at the first exercise measurement (15 min) which persisted throughout the remainder of exercise. Stroke volume increased by approximately 14% and heart rate was reduced by 7 beats/minute. It was therefore concluded that the expansion of plasma volume increases cardiac output and stroke volume, and decreases heart rate and core temperature (Grant et al. 1997).

Athletes are not likely to have access to the infusion techniques employed above; therefore, Greenleaf et al. (1998) had subjects orally ingest one of four solutions. Sodium concentrations ranged from low (55.2 mEq/L) to high (163 mEq/L). The high sodium solution significantly ($p < 0.05$) increased plasma volume by ~7.9% and maintained the highest plasma volume during exercise. Although plasma volume was improved, rectal temperature and thermoregulatory parameters were not significantly improved during 70 minutes of sub-maximal exercise. This was also the case in a more recent study conducted by Coles and Luetkemeier (2004) who found that a 3.1% increase in plasma volume resulted in a performance improvement in a 15 minute time-trial. In this study, participants orally ingested solutions equal to that employed by Greenleaf et al. (1998). Coles and Luetkemeier (2004) found the high sodium solution did not negatively influence thermal regulatory or cardiovascular variables; however, no influence was observed in core temperature, heart rate or total body sweat rate regardless of plasma volume expansion.

To the best of our knowledge, only one study (Sims et al. 2007) has investigated the effects of plasma volume expansion under heat stress (exercise in high ambient temperatures). Most work has been done in temperature ranges between 21.0 – 24.0°C. More work, therefore, needs to be done to investigate the effects of plasma volume expansion on cardiovascular and thermoregulatory strain under severe heat stress. Previous studies have failed to relate plasma expansion to improvements in cardiac output or thermoregulation when exercise was performed at room temperature. It is possible that environmental heat stress ($\geq$ 30°C) may provide the necessary amount of stress to produce significant results; that is, the expansion of plasma volume under severe circumstances may better maintain blood flow to the working muscles and to the skin. It is also unclear if plasma expansion will improve athletic performance. Coles and Luetkemeier (2004) are the only known study to monitor performance following plasma expansion. More work therefore needs to be done to investigate the effects of hypervolemia on athletic performance.

Delimitations

1) 8 male subjects were chosen to participate in the study. Subjects were limited to triathletes and/or cyclists with competitive experience. Due to the nature of the workload and intensity of exercise it is unlikely that any population, other than the one chosen, would have been able to complete the study.
2) The environment was kept constant (~30°C dry bulb, ~60% humidity) throughout the trials.
3) Subjects with a Urine specific gravity > 1.020 g/ml were not allowed to participate in the study until they are adequately hydrated.

4) The research was a randomized, blind, quasi-experimental design. All subjects were randomly assigned an order in which they would complete the three trials. The contents of the treatment were withheld from both the participant and the primary investigators until the study was complete.
5) The following independent variables were measured: (1) heart rate; (2) whole blood: plasma volume (hemoglobin and hematocrit), Na^+, K^+; HCO3-; pH; and base excess, (3) core and skin temperature; and (5) sweat rate.
6) Subjects ingested 12 ml/kg body weight of the following solutions: (1) 0.2 g/kg body weight $Na_3C_6H_5O_7$ $2H_20$, 0.1 g/kg body weight $Na_3C_6H_5O_7$ $2H_20$, and Gatorade. Glucose was kept constant for all three solutions (Rehrer, 1994; Maughan and Leiper, 1999). Osmolality of the solutions was < 260 $mOsm \cdot L^{-1}$ in order to promote rapid uptake and absorption (Gisolfi and Duchman, 1992).
7) Subjects were given 20 minutes to consume each solution. Exercise commenced 100 minutes after ingestion.
8) A repeated measures analysis of variance (ANOVA) was used to identify differences between trials for the dependent variables. Paired t-tests were used to identify differences between means. Significance was set at $p \leq 0.05$. All values will be reported as a mean $\pm$ standard deviation (SD).

Limitations

1) Female subjects were not tested during this study. Because of the need to test female athletes during the early follicular phase of their menstrual cycle, especially during thermoregulatory studies, in order to avoid the subsequent increase (0.3-0.5°C) in core temperature, the primary investigator chose to avoid

testing this population. Highly trained female athletes are less likely than the normal female population to have a regular menstrual cycle.

2) The group of athletes selected for this study were highly trained. The results of this study are not likely to apply to the average population.
3) It is possible that subjects became acclimatized to the high ambient temperatures after two or three trials. Athlete exposure to heat stress was limited between trials; all testing took place during the late fall and it is unlikely that the subjects were still heat acclimatized from the summer. A minimum of 7 days was used to separate each trial.
4) No direct measure of cardiac output was taken. An increase or decrease in heart rate was used to predict changes in stroke volume.

Assumptions

The major assumption of this study was that high sodium concentrations, orally ingested prior to exercise, would expand plasma volume (Coles and Luetkemeler, 2004; Greenleaf et al. 1998). Furthermore, it was also assumed that the small percent (6%) of glucose added to the solution would increase, or assist with, gastric emptying and intestinal absorption (Rehrer, 1994).

Subjects were asked to provide a urine sample prior to beginning each trial. Urine specific gravity was used to gauge hydration status. It is assumed that a Usg >1.020 g/ml is an indication of dehydrated state (Armstrong et al. 1994).

Hypothesis

The ingestion of high concentrations of sodium citrate prior to exercise will significantly expand resting plasma volumes, translating into increased cardiovascular and thermoregulatory function.

The ingestion of sodium citrate prior to exercise will have a positive effect on athletic performance. Specifically, athletes will significantly reduce their exercise time to completion during a 1-hr time-trial following plasma volume expansion.

Definition of Terms

1) Thermoregulation: A measure of body temperature (core and skin). A variable dependent on the body's ability to dissipate heat through its two primary homoeothermic mechanisms: (a) increases skin blood flow – heat convection at the skin, and (b) evaporative mechanisms – sweating.
2) Cardiovascular: A measure of cardiac function. Commonly measured using heart rate (beats/minute) and stroke volume (ml/beat). As stress and intensity increases heart rate will tend to increase; stroke volume will naturally tend to decrease.
3) Ingestion: The act of swallowing a bolus.
4) Absorption: Ingestate moves from the stomach to the duodenum (proximal intestine) where it is transported across the intestinal membrane and up-taken by the body.
5) Trained: Competitive cyclists and/or triathletes who competed in the previous season with a $VO_{2peak} > 50$ ml/kg/min.

Chapter 2

During high-intensity exercise an athlete's main energy source is from anaerobic glycolysis (Requena et al. 2005; McNaughton, 1990; Cho et al. 1992; Faff et al. 1996; Ibanez et al. 1995; Jain et al. 2003; Linossier et al. 1997; Parry-Billings and MacLaren, 1986). Through this pathway, the body utilizes glucose/glycogen in a series of 10 enzymatic steps to form 3-carbon molecules of pyruvic acid and 12 enzymatic steps to form lactic acid (Wilmore and Costill, 1999). Lactic acid dissociates to form hydrogen [H^+], an ion which decreases muscle pH (van Someren et al. 1998; Van Montfoort et al. 2004). An athlete's ability to sustain high-intensity exercise depends on their ability to buffer or minimize increases in cellular and blood [H^+] ion concentrations (Robergs, 2005). The inability to buffer or minimize decreases in pH is detrimental to athletic performance (Shave et al. 2001). The use of artificial buffers such as sodium bicarbonate ($NaHCO_3$) and sodium citrate (CIT) have gained popularity within the athletic community. Both substances have been shown to increase alkalosis during exercise (Bracken et al. 2005; Ball and Maughan, 1997; Oopik et al. 2004); however, their ability to enhance performance remains unclear. $NaHCO_3$ has been shown to produce considerable gastro-intestinal (GI) distress (McNaughton, 1990; Jain et al. 2003; Linossier et al. 1997; Hausswirth et al. 1995); leading researchers and athletes to use CIT, a substance which has been credited with less GI distress and complications (Faff, 1996).

Very little work has been done, however, exploring the potential hydrating capabilities and thermoregulatory effects of sodium rich beverages. Sodium has been shown to increase fluid retention and decrease urine production, as well as increase plasma volume during rehydration (Rehrer, 1994). In a study performed by Oopik et al.

(2004) plasma volume was found to increase after the administration of CIT. In an attempt to increase the bodies buffering capacity, athletes often ingest large quantities (500-1000 ml) of water containing electrolytes (CIT). It would seem obvious then to explore the potential benefits, both hydrating and thermoregulatory, which CIT ingestion could provide endurance athletes.

Buffering H^+ through HCO_3^-

High-intensity exercise relies on anaerobic glycolysis for fuel (Requena et al. 2005). The end-product of anaerobic glycolysis is pyruvate, which diffuses into the mitochondria from the cytoplasm to undergo further aerobic metabolism. If pyruvate is not degenerated at a great enough rate, and begins to accumulate, it is then further reduced to lactate which later diffuses into the blood (Tiryaki and Atterbom, 1995). The accumulation of lactic acid results in the elevation of the intra- and extra-cellular H^+ concentration and the subsequent decrease of blood and muscle pH (Requena et al. 2005; McNaughton, 1990; McNaughton and Cedaro, 1992; Faff et al. 1996; Ibanez et al. 1995; Jain et al. 2003; Parry-Billings and MacLaren, 1986; Tiryaki and Atterbom, 1995). H^+ ions are naturally excreted through three primary processes: via the kidneys, through hyperventilation, and through blood bicarbonate [HCO_3^-] (Robergs, 2005). Athletes cannot naturally continue to buffer H^+ ions indefinitely; rather, the energy demands of an athlete will eventually exceed its H^+ buffering capacity and allow for the accumulation of H^+ ions at a greater rate than buffering can occur (Oopik et al. 2003). The accumulation of H^+ ions will in turn decrease the pH in the blood and muscles resulting in fatigue (Robergs, 2005; Potteiger et al. 1995; Jain et al. 2003; Ibanez et al. 1995; Kowalchuk et al. 1989; Linossier et al. 1997; McNaughton and Cedaro, 1992; Oopik et al. 2003; Parry-

Billings and MacLaren, 1986). Arterial pH has been shown to decrease from 7.4 to 6.9 during high-intensity, short duration exercise; where, blood pH can drop as low as 6.5 (McNaughton and Cedaro, 1992).

Muscular contraction and anaerobic glycolysis are pH sensitive; therefore, acidity is believed to contribute to decreased athletic performance through the inhibition of key glycolytic enzymes, the inhibition of calcium release from the sarcoplasmic reticulum and its binding to troponin C, and the reduced contractility of muscle fibres (Oopik et al. 2003; Shave et al. 2001; Parry-Billings and MacLaren, 1986; Ibanez et al. 1995). Elevated concentrations of H^+ ions limit glycolysis, glycogenolysis, and the re-phosphorylation of adenosine diphosphate (ADP) (Faff et al. 1996). Phosphofructokinase (PFK), the primary enzyme of anaerobic glycolysis, is believed to be inhibited when functioning in an environment with a decreased pH (Cho et al. 1992; Faff, 1993; Ibanez et al. 1995). Phosphorylase is also inhibited by elevated H^+ concentrations; thereby, further inhibiting glycolysis (Schabort et al. 2000; van Someren et al;. 1998).

H^+ accumulation is also believed to affect the interaction of actin and myosin through the inhibition of calcium release from the sarcoplasmic reticulum (Requena et al. 2005; Jain et al. 2003). Decreases in pH are therefore believed to reduce the contractile ability of muscle and impair muscle tension via impaired cross-bridge cycling (McNaughton, 1990; Jain et al. 2003, McNaughton and Cedaro, 1992; Ibanez et al. 1995). Muscular contraction relies on the formation of an actin-myosin complex; the inhibition of such a complex limits muscular contraction (Schabort et al. 2000). Increased H^+ concentrations are therefore assumed to be one of the primary contributors of fatigue during exercise (Cho et al. 1992). Fatigue can be defined as: (1) "a decrease in

force production in the presence of increased perception of effort" (Requena et al. 2005); (2) "a failure to maintain the expected power output" (Hausswirth et al. 1995); or (3) the "inhibition of performance" (Cho et al. 1992).

H^+ ions accompany lactate during intense exercise and are first neutralized by intra-cellular buffering mechanisms and by ammonium ions generated through AMP dissemination; diffusion then becomes the main mechanism by which excess H^+ leaves the cell. Approximately 15 to 18% of the [H^+] ions generated during intense exercise are buffered by blood $HCO3^-$ (Faff, 1993). HCO_3^- is, therefore, one of the main buffers in the body and acts by neutralizing protons to form carbons dioxide (CO_2) and water (H_2O) (Crowe, 2001). Extra-cellular pH also increases in the presence of HCO_3^- which in turn raises the H^+ and lactate [Lac^-] efflux from active muscles via the Lac^-/ H^+ cotransporter (Requena et al. 2005; Fernandez-Castanys et al. 2002; Hausswirth et al. 1995; Ibanez et al. 1995; Jain et al. 2003; Van Montfoort et al. 2004). Lac^- and H^+ diffuse from working muscles through a monocarboxylate transporter; one which is pH sensitive. The maintenance of a higher pH during exercise, through HCO_3^-, may allow this transport mechanism to facilitate greater lactate flux from the muscle (Jain et al. 2003; Potteiger et al. 1995; Potteiger et al. 1996; Teryaki and Atterbom, 1995). A greater efflux of Lac^- through the monocarboxylate transporter could potentially enhance the contractile performance of muscle and delay the onset of fatigue (Requena et al. 2005; Hausswirth et al. 1995; Ibanez et al. 1995). Extra-cellular alkalosis may therefore promote lactate transport through muscle cell membrane and delay the onset of critical intra-cellular acidosis (Hausswirth et al. 1995). Isolated observations of the working gastrocnemius

muscle of dog and frog serratus muscle show faster rates of lactate efflux when extra-cellular pH and $HCO3^-$ are increased (Kowalchuk et al. 1989).

Alkalosis, therefore, increases the rate of proton and lactate release from the muscle to the blood which potentially delays the fall in muscle pH and thus its inhibitory effect on PFK. By reducing the accumulation of H^+ ions in active muscles, and decreasing muscle pH, HCO_3^- delays the inhibition of glycolysis and promotes the formation of lactate (Faff et al. 1996; Linossier et al. 1997). Enhanced glycogenolysis will then contribute to improved muscle contraction and athletic performance during intense exercise (Oopik et al. 2004). The maintenance of an optimal intramuscular pH will therefore improve glycolysis and promote higher blood lactate formation (Potteiger et al. 1995). Through this buffering mechanism, the inhibition of key glycolytic enzymes would be less prominent, which would allow greater energy turnover through anaerobic glycolysis and would promote greater force production over a greater period of time (Shave et al. 2001; van Someren et al. 1998).

Efficacy of artificial buffering

The human body, though very effective, cannot buffer the accumulated H^+ at a great enough rate so that athletic performance can be maintained infinitely. In an attempt to improve upon the body's natural buffering capacity, many athletes and sport practitioners have began to ingest artificial buffers designed to increase the buffering capacity of the blood and improve athletic performance (Potteiger et al. 1995). The ingestion of alkalizing agents increases the extra-cellular HCO_3^- reserve, and enhances the efflux of H^+ and Lac^- from the working muscles through the monocarboxylate transporter discussed above. The increased HCO_3^- also works to reduce the deceleration

of intra-cellular pH and the inhibition of key glycolytic enzymes during glycolysis (Requena et al. 2005; Ibanez et al. 1995; Kowalchuk et al. 1989). Therefore, alkalizing agents increase the content of HCO_3^- in the body, one of the principle buffering mechanisms in blood and muscle, producing significant elevations in pH (Faff, 1993).

Sodium-citrate (CIT), an alkaliser used to improve athletic performance, works by offsetting the increase in H^+ prior to exercise by increasing the buffering gradient, HCO_3^- and significantly increasing resting pH (McNaughton and Cedaro, 1992; Fernandez-Castanys et al. 2002). Ingestion of CIT has been shown to increase resting blood pH, HCO_3^-, and base excess (Ball and Maughan, 1997; Potteiger et al. 1996; Schabort et al. 2000; Faff et al. 1996); thus, a more favorable pH gradient may promote the efflux of intra-cellular H+ and Lac- via the pH sensitive monocarboxylate transporter (Oopik et al. 2003; Potteiger et al. 1996). Increased endurance and work performance following citrate ingestion has been attributed to its alkalizing (buffering) capacity (Greenleaf et al. 1997). Following CIT ingestion, an increase in venous lactate may be attributed to the: (1) enhanced efflux of lactate from working muscles; (2) the maintenance of an optimal pH for anaerobic glycolysis; or (3) a combination of the above (Shave et al. 2001). Increased extra-cellular buffering capacity through CIT ingestion has the potential to improve athletic performance (van Someren et al. 1998).

Citrate increases blood HCO_3^- and pH due to its three negative charges; therefore, metabolism of ingested citrate, through the consumption of hydrogen ions, generates more HCO_3^- and elevates pH (VanMontfoort et al. 2004; Teryaki and Atterbom, 1995). Once CIT is ingested it dissociates into Na^+ and citrate$^-$ ions; in turn, citrate anion is removed from the plasma, altering the sum of cations and anions. The ratio of strong

cations to strong anions increases and there is an electrical imbalance. Such an unstable electrical equilibrium causes H^+ ions to be removed and replaced with $HCO3^-$ ions; triggering an alkalosis in an attempt to restore the bloods acid-base balance (Requena et al. 2005; Cho et al. 1992; Kowalchuk et al. 1989). Alkalization, due to CIT ingestion, facilitates the efflux of H^+ and Lac^- from the muscle cytosol into the blood, reducing muscle acidosis (Crowe, 2001; Schabort et al. 2000; Shave et al. 2001). In other words, CIT ingestion has been shown to increase venous lactate concentrations, associated with increased efflux of Lac^- and H^+ from the muscle cell, across the interstitium, and into the venous circulation. Venous HCO_3^-, after CIT ingestion, has been shown to increase by as much as 15% (Street et al. 2005; Oopik et al. 2004).

Sodium-citrate Ingestion

Many studies have previously been conducted using sodium citrate. There remains inconsistency among the literature regarding citrates efficacy as an ergogenic aid. Most of the work done with CIT has been non-uniform; that is, until recently little work had been completed regarding optimal absorption time, exercise type and/or duration, and training history of an athlete. Moreover, the mode of exercise has been predominantly with a cycle ergometer (Bracken et al. 2005; Crowe, 2001; McNaughton and Cedaro, 1992), with only several studies using different modes of exercise (Oopik et al. 2003; Shave et al. 2001; Ibanez et al. 1995). As mentioned previously, much of the work completed using CIT has produced varying results; with some reporting no significant benefit and others reporting positive results.

The ingestion of CIT before exercise appears to produce dose-dependent changes in pH and HCO_3^- (Faff, 1996; Oopik et al. 2004); following the ingestion of CIT these

changes become significant approximately 50 minutes after ingestion and peak approximately 100-120 minutes post-ingestion (Schabort et al. 2000; van Someren et al. 1998; Crowe, 2001; Jain et al. 2003; Potteiger et al. 1996; Oopik et al. 2004; Oopik et al. 2003). Furthermore, doses as low as 0.1 g/kg body mass have been reported to bring about significant changes in blood HCO_3^-; where doses as high as 0.5 g/kg body mass bring about optimal elevations in pH and HCO_3^- (Faff, 1996; Ibanez et al. 1995; Jain et al. 2003;). Due to the buffering mechanism of CIT, exercise relying upon the glycolytic energy system has produced the most favorable results. Exercise protocols which are too short (30-40 sec) or are not stressful enough will fail to produce significant performance improvements following CIT ingestion (Ibanez et al. 1995; Jain et al. 2003; Linossier et al. 1997; McNaughton and Cedaro, 1992). Exercise of very short, high intensity, will favor hydrolysis of creatine phosphate; likewise, if the exercise is not stressful enough, lactate and H^+ will not be produced and citrates buffering capacity will be negated (McNaughton and Cedaro, 1992).

Exercise Considerations

In order to capitalize on CIT buffering capacity, the mode of exercise must target the glycolytic energy system. As previously discussed, the major bi-product of anaerobic glycolysis is H^+ (Van Montfoort et al. 2004). Anaerobic glycolysis is typically associated with brief intense bouts of exercise lasting approximately two minutes (Wilmore and Costill, 1999). Many studies have attempted to maximize anaerobic glycolysis by exhausting subjects within two to four minutes (Fernandez-Castanys et al. 2002; Bracken et al. 2005; Tiryaki and Atterbom, 1995; Van Montfoort et al. 2004, McNaughton and Cedaro, 1992). The theory behind maximizing the anaerobic glycolytic system is to

allow maximal production of both Lac^- and H^+; thus allowing CIT buffering mechanism (HCO_3^-) to promote rapid efflux of Lac^- and H^+ from the working muscle and allow for performance improvements (Requena at al. 2005). Competitive endurance events, however, produce significant amounts of blood and muscle Lac^-; the use of CIT for such events which operate above lactate threshold may provide the potential for enhanced performance (Potteiger et al. 1995; Potteiger et al. 1996; Ibanez et al. 1995; Oopik et al. 2003). At power outputs >70-80% maximal aerobic capacity (VO_{2max}) energy production relies on the oxidation of creatine phosphate and anaerobic glycolysis (Kowalchuk et al. 1989). Fatigue experienced during high-intensity aerobic exercise is mainly attributed to the accumulation of H^+ ions in the working muscles (Schabort et al. 2000; van Someren et al. 1998). Only a few studies have investigated CIT ergogenic effect on aerobic exercise (Howalchuk et al. 1989; Oopik et al. 2004; Potteiger et al. 1996; Schabort et al. 2000; Oopik et al. 2003; Potteiger et al. 1996).

Potential Risks

The primary reason this investigator chose CIT is due its reduced gastro-intestinal (GI) distress associated with its ingestion (Faff, 1996). Another artificial buffer which is used by athletes, $NaHCO_3^-$, has been shown to produce considerable GI disturbance with doses >0.3 g/kg body mass (McNaughton, 1990; Jain et al. 2003; Linossier et al. 1997; Hausswirth et al. 1995; Faff, 1996). The use $NaHCO_3^-$ has produced such symptoms as: vomiting, diarrhea, and GI disturbance (Faff, 1996; Crowe, 2001). In an attempt to avoid such negative effects, many studies have advocated Na-citrates buffering capacity with little to no GI distress (Crowe, 2001; van Someren et al. 1998). In a study conducted by Oopik et al. (2004), 7 subjects using CIT (0.5 g/kg) experienced GI distress. Oopik et al.

(2003) reported that all 17 subjects complained of urges to defecate following citrate ingestion; other symptoms included nausea and thirst. In a study conducted by Schabort et al. (2000) a dose, the highest reported, of 0.6 g/kg body mass induced stomach cramps and GI distress in 5 of 8 subjects. Other studies have reported such symptoms as: GI distress, diarrhea, increased flatulence, and feelings of bloating (Shave et al. 2001; Potteiger et al. 1995; van Someren et al. 1998).

Following CIT ingesting there exists the possibility for increased osmolality of the GI tract; water shifts from the plasma to the intestine to avoid hypertonicity. The increased presence of HCO_3^- in the blood requires more water to be in the intestine to maintain its isotonic balance. An increase in gastric dumping could be the cause of the reported GI distress by subjects (Requena et al. 2005; Shave et al. 2001).

There appears to be no visible relationship between GI distress, dose and concentration, and the amount of time awarded to ingest each solution. Although Oopik et al. (2004) reported GI distress in seven subjects after ingesting 0.5 g/kg body mass CIT in 1.5 L solution over 60 minutes, Bracken et al. (2005) reported no GI disturbance after ingesting the same concentration of CIT with only 500 ml solute in a five minute period. Similarly, Oopik et al. (2003) ingested 0.5 g/kg body mass of CIT in a 1 L solute in 10 minutes reporting that all subjects experienced several negative symptoms; where, Potteiger et al. (1996) followed the exact same protocol and reported to symptoms or distress.

Hydration and Sodium Citrate

Dehydration can be defined as water loss exceeding 2-3% body mass; such a loss compromises an athlete's ability to perform physically, dissipate heat, and impairs

cardiovascular function (Armstrong et al. 1998; Shirreffs, 2000). Exercise performance can be reduced after a body mass loss of only 1.0 – 1.8% (Greanleaf et al. 1997). It has further been shown that a loss of body mass of approximately 2.5% results in a 45% decrease in performance capacity during intense exercise (Shirreffs, 2000). Heat dissipation is reduced during dehydration due to the reduced skin blood flow during exercise; typically resulting in an increase in core body temperature. Cardiovascular strain is evidenced by a reduced stroke volume; a body mass loss of 1% has been shown to increase heart rate (HR) by 5 – 8 beats/minute and cardiac output declines significantly (Coyle, 2004). The increase in HR and subsequent decrease in cardiac output occurs due to the heart's inability to compensate for the reduced stroke volume (Rehrer, 2001).

Fluid deficits which occur during exercise reduce plasma volume and stroke volume. Sport drinks with high sodium content have been shown to restore plasma volume (Rehrer, 2001; Coyle 2004). Oopik et al. (2004) found a significant increase in plasma volume after administering CIT compared to a placebo. A reduction in plasma volume may reduce performance by impairing blood flow to working muscles. Blood flow to working muscles must be maintained at a high level during exercise in order to supply oxygen and substrates at a high rate. Blood flow must also be supplied to the skin in order to aid in heat convection and thermoregulation. Therefore, reduced skin blood flow allows central venous pressure to be maintained and muscle blood flow to be maintained; reducing peripheral blood flow essential for thermoregulation (Shirreffs, 2000).

Sodium has also been shown to increase gastric emptying which is particularly beneficial since decreases in pH and intense exercise are believed to decrease gastric

emptying rate. The amount of fluid and solutes that can be released from the stomach and made available to the intestines for absorption can severely impact athletic performance (Rehrer, 2001). Absorption of ingested solutions occurs primarily in the proximal segment of the small intestine (duodenum). Availability of the ingestate and delivery to the duodenum directly effects athletic performance (Maughan and Leiper, 1999). Gastric emptying is dependent on several variables: (1) volume, increasing the volume consumed will promote emptying; (2) energy density, high density slows emptying; (3) osmolality, high solution osmolality (hypertonic) slows emptying; and (4) pH, fluctuations in either direction of neutrality will slow emptying. Exercise (>~70-75% VO_{2max}) and dehydration also reduces gastric emptying (Maughan and Leiper, 1999).

Sport drinks, therefore, often contain water (solvent) and varying concentrations of glucose and sodium (solutes) to promote fluid absorption and retention. Glucose, once ingested, is transported across the intestinal membrane via the active transport of sodium. When these two solutes are transported across the intestinal membrane, water molecules are osmotically pulled across the membrane (Figure 1). The presence of glucose and sodium in a sport drink are likely to increase the net intestinal absorption of a solvent (Rehrer, 2001; Maughan and Leiper, 1999). Therefore, the role of a hydrating sport drink is threefold: (1) stimulate rapid fluid absorption, (2) speed rehydration, and (3) reduce the physiological strain of exercise (Shirreffs, 2000).

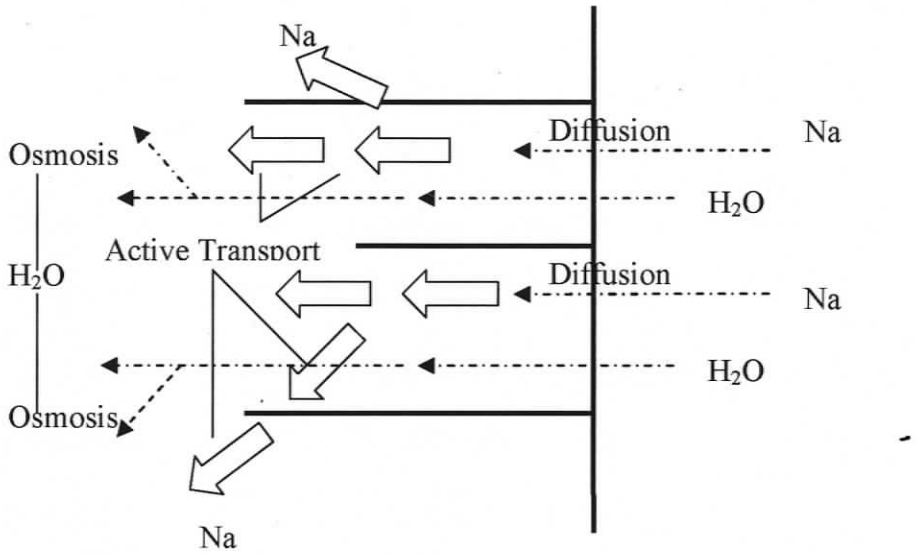

Figure 1 Absorption of sodium through the intestinal epithelium. Sodium is actively transported to the paracellular spaces. Water is osmotically pulled across the epithelium membrane into the paracellular spaces due to the elevated concentration.

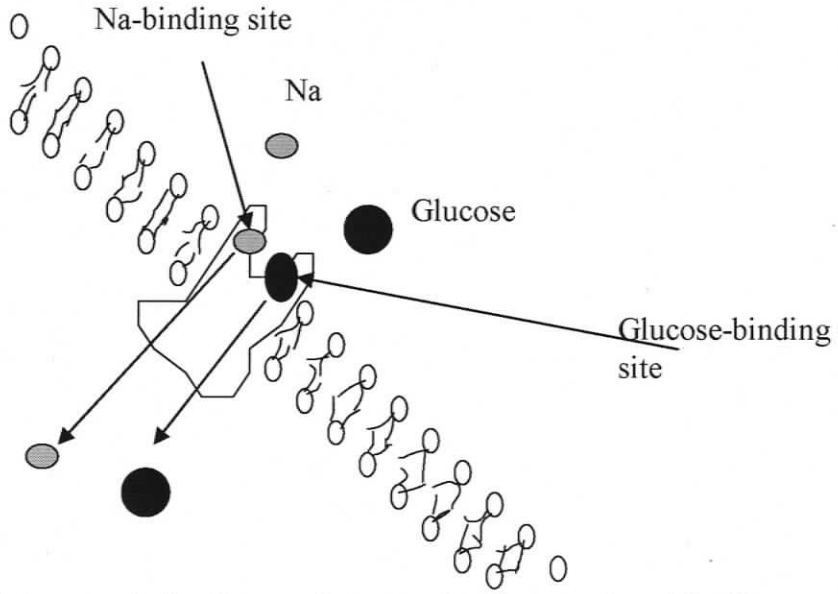

Figure 2 Co-transport of sodium and glucose via a transport protein. Because the transport protein has two binding sites (one for sodium, one for glucose), sodium movement into the interior cell will not occur without the presence of glucose. The low concentration of sodium within the cell "drags" sodium along with glucose into the cell.

Athletes typically will ingest approximately 1.0 – 1.5 L of fluid when supplementing a buffering agent like CIT (Potteiger et al. 1996; Oopik et al. 2003; Oopik et al. 2004). With the relatively large amount of fluid intake prior to exercise, along with the large concentration of sodium ingested, CIT ingestion may provide a hydrating effect along with its primary buffering effect. Few studies have been conducted to investigate CIT hydrating capability.

Hyperthermia and Hypohydration

Athletes often exercise in hot, humid environments. Thermoregulatory sweating results in body water losses in excess of those normally experienced in temperate climates (~21° C) (Armstorng et al. 1998). An increase in ambient temperature and humidity has been shown to increase sweat rate by approximately 1L/hr (Rehrer, 2001) and can be as high as 2L/hr (Armstrong et al. 1998). Montain and Coyle (2001) found a high correlation ($r = 0.79$ to 0.98) between esophageal temperature and body weight loss; suggesting that as body weight decreases thermoregulation is linearly impaired. Acute changes in body mass during exercise will generally be due to sweat loss; respiratory water loss and substrate oxidation are negligible in this case (Shirreffs, 2000). During exercise in the heat, fatigue has been related to elevated internal body temperatures (Gonzalez-Alonso et al. 1999). As mentioned above, even the slightest decrease in body weight (i.e. ~1%) can impair athletic performance, a 7% loss can lead to physical collapse, and a body water loss in excess of 10-15% has been associated with mortality (Armstrong et al. 1998; Shireffs and Maughan, 1998; Greenleaf et al. 1997).

Dehydration, the acute change in fluid stores from that of a steady-state condition (Casa, 2003), has been associated with a decrease in plasma volume (Shirreffs et al.

2004). A reduced plasma volume may influence work capacity by reducing the amount of blood flow to the skin, impairing the body's ability to convect heat to the body surface where it can be dissipated (Gonzalez-Alonso et al. 1997). High ambient temperatures promote rapid sweat loss and a reduced plasma volume (Shirreffs, 2000). Hypohydration will, therefore, increase core temperature (hyperthermia) and cardiovascular strain; evidenced by the vasoconstriction of skin blood vessels, tachycardia, decreased venous return, and reduced stroke volume (Armstrong et al. 1997; Gonzalez-Alonso et al. 1999; Gonzalez-Alonso et al. 1997). In a study conducted by Heaps et al. (1994) body water loss of 0.9% significantly elevated heart rate by 10 bt/min and reduced stroke volume by 9 ml/bt; when hypohydrated by 2.8% body weight, subjects showed an elevated heart rate by 18 bt/min and a decline in stroke volume by 18 ml/bt. Therefore, for every 1% loss of body weight due to dehydration, heart rate is elevated by ~7 bt/min and stroke volume is decreased by ~8 ml/bt (Heaps et al. 1994).

As dehydrated individuals exercise in high ambient temperatures they experience hyperthermia due to the reduced heat dissipation; a consequence of impaired skin blood flow and sweating response. Subsequent hyperthermia (39.3° C) during dehydration encourages a low cardiac output and blood pressure and elevated vascular resistance, making athletes more prone to ischemic injury (Coyle, 2004; Gonzalez-Alonso et al. 1997). Hyperthermia causes a redistribution of blood volume from the central circulation to cutaneous veins, reducing myocardial filling and subsequently stroke volume, triggering an increase in heart rate (Heaps et al. 1994; Gonzalez-Alonso et al. 1997). The coupling of dehydration and hyperthermia during exercise in the heat causes further reductions in stroke volume and cardiovascular function; in turn, making athletes less

able to cope with hyperthermia (Gonzalez-Alonso et al. 1997). An increase in heart rate along with reductions in stroke volume (cardiovascular drift) during exercise in high ambient temperatures is directionally proportional to the level of dehydration experienced by athletes (Heaps et al. 1994; Montain and Coyle, 1992).

Hypervolemia and pre-exercise sodium ingestion

The ingestion of large quantities of sodium has been shown to significantly increase plasma volume above baseline measures. Sodium citrate ingestion prior to an athletic competition may therefore promote the acute expansion of plasma volume in athletes (Coles and Luetkemeler, 2004). Following the ingestion of 163.7 mEq/L sodium, where consumption was equivalent to 10 ml/kg body mass, Coles and Luetkemeler (2004) showed a 3.1% increase in plasma volume before commencing exercise. This increase in plasma volume was associated with the subsequent 0.97 km improvement during a 15-min time trial. The high sodium drink also helped maintain plasma volume at 15 and 30 minutes of exercise (Coles and Luetkemeler, 2004). Greenleaf et al. (1998) conducted a similar study with many of the same findings presented by Coles and Luetkemeler (2004). Drinks equivalent to 10 ml/kg body mass with varying sodium concentrations (55.2-163.7 mEq/L) were ingested over 60 minutes, with a 90 minute rest prior to exercise. Plasma volume was significantly ($P < 0.05$) increased by 7.9% in the high sodium trial; and was better maintained than the low sodium trials or the no fluid ingestion trials upon the commencement of exercise. Although both studies ingested similar sodium concentrations prior to exercise, the difference in plasma volume expansion is likely a result of ingestion time. Greenleaf et

al. (1998) allowed almost twice the absorption time than Coles and Luetkemeler (2004); producing a considerably higher elevation in plasma volume.

The maintenance of plasma volume may improve thermoregulation and reduce heart rate during exercise. During exercise blood flow is increased to the skin and working muscles. When blood volume is compromised, during dehydration for instance, blood flow to the skin and exercising muscles is compromised (Deschamps et al. 1998; Kargotich et al. 1998). Hyperthermia and cardiovascular strain are increased due to the reduced heat convection at the skin; evidenced by an elevated heart rate, increase in core temperature and reduced stroke volume (Fortney et al. 1988). It has been estimated that for every 1% body weight loss due to dehydration, rectal core temperature increases by about 0.3°C (Morimoto et al. 1998). Under well-controlled conditions Popowski et al. (2001) demonstrated that for every 2% loss of body mass, plasma osmolality increased by ~5 mOsm/kg (an increase in plasma osmolality suggests a decrease in plasma volume). Homoeothermic animals respond to heat loading by cutaneous vasodilation in an attempt to transfer heat from the body's core to the body's surface; circulation and evaporative heat loss are therefore the primary mechanisms utilized to maintain optimal core temperature. This redistribution of blood to the skin lowers central venous pressure, while evaporative heat loss eventually leads to dehydration. Cardiac output must therefore be maintained in order to respond to the increased demand for skin blood flow (Morimoto et al. 1998).

Acute expansion of plasma volume increases cardiac output prior to exercise commencement; continuous blood flow should theoretically increase, attenuating any increases in core temperature and heart rate and decreases in stroke volume and central

venous pressure. Any improvement in skin blood flow, a response which should follow plasma volume expansion, should improve evaporative heat loss and reduce core temperature (Grant et al. 1997). An elevated plasma volume prior to exercise may allow blood volume to be better maintained throughout exercise. Presumably resulting in a relatively stable core temperature, heart rate and stroke volume; therefore, a lower body temperature may reduce heart rate by allowing better maintenance of peripheral blood flow (Greenleaf et al. 1998; Fortney et al. 1988).

Conclusion

There exists a potential for CIT ingestion to improve athletic performance through its apparent buffering mechanism. Na-citrate dissociates into Na^+ and $Citrate3^-$; thus, blood bicarbonate is elevated due to citrates three negative charges (van Someren et al. 1998). The elevation of extra-cellular HCO_3^- promotes a more alkolitic state and enhances the efflux of Lac^- and H^+ via a pH sensitive monocarboxylate transporter from the working muscle into the blood (Ibanez et al. 1995). Evidence of an ergogenic effect is limited; however, it is accepted that CIT ingestion does significantly enhance HCO_3^- and pH with doses as low as 0.1 g/kg (Faff, 1996). Many studies have been conducted but have produced conflicting results regarding CIT ability to enhance performance through it potential buffering capability (Table 1 and 2).

Very few studies have been conducted which have monitored the effects of high sodium intake prior to athletic performance as it relates to blood plasma volume, fluid retention, and thermoregulation. There exists the potential for athletes to expand their plasma volume (hypervolemia) prior to exercise (Greenleaf et al. 1998; Oopik et al. 2004; Coles and Luetkemeler, 2004). Hypervolemia could potentially reduce the impact of

high ambient temperatures and exercise on cardiovascular strain by sufficiently maintaining blood flow to the working muscles and to the skin. Moderate to intense exercise, coupled with heat stress, results in a decrease in plasma volume as water from the plasma compartment is redistributed to both the working muscles and to assist with evaporative heat loss (Kargotich et al. 1998). When heat stress and exercise are severe, plasma volume has been shown to decrease by as much as 27% (Fortney et al. 1988). The ability for athletes, especially those exercising in high ambient temperatures, to maintain plasma volume is physiologically significant as it effects both the cardiovascular and thermoregulatory systems. Dehydration and hyperthermia during exercise in the heat reduces stroke volume and cardiovascular function due to the redistribution of blood volume from the central circulation to cutaneous veins (Heaps et al. 1994; Gonzalez-Alonso et al. 1997). Plasma volume may indirectly alter heart rate through body temperature changes; a lower core temperature will reduce the body's reliance on blood flow to the skin and evaporative heat loss, better maintaining blood distribution and myocardial filling (Fortney et al. 1988). If cardiac output is better maintained (heart rate and stroke volume) throughout exercise, it should follow that performance will be improved. More robust and better controlled studies are needed in order to test this theory.

Chapter 3

Methodology

Subjects

8 trained male competitive cyclists and triathletes, between the ages of 20 and 30 years, were recruited for the study. All subjects provided informed consent and were advised of their right to withdraw from the study at any time without future implications. Ethics approval was obtained from the Human Research Ethics Committee at the University of Victoria.

Methods

Pre-experimental protocol

Each subject reported to the laboratory, 3-7 days prior to the beginning of the experiment. This session was used to introduce the subjects to the expectations and requirements of the study, to gain informed consent, and to collect anthropometric measurements (height, weight, and sum of seven skin folds). Following a standardized 5 minute warm-up, the participants completed an incremental test to exhaustion (Appendix C) on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to test for peak oxygen consumption (VO_{2peak}), maximum heart rate (HR_{max}), and maximum aerobic power (W_{max}). Expired gas samples were collected into a mounted face mask; a True One metabolic cart (Parvo Medics, USA) was used to measure expired gases (Ve, VO2, VCO2, RER). The metabolic cart was calibrated before each trial, according to standard laboratory procedures. Heart rate (HR) was also continuously monitored using a telemetric heart rate monitor (Polar, Finland) throughout the incremental test. VO_{2peak} was defined as the maximum minute oxygen consumption average attained during the incremental exercise test. The investigator was unable to

meet two of the following criteria for $VO2_{max}$: (1) attainment of predicted maximum heart rate (220-age); (2) a rise in VO_2 of less than 2 mL $kg^{-1} \cdot min^{-1}$ with an increase in workload; and/or (3) a respiratory exchange ration (RER) greater than 1.15. Oxygen consumption was therefore reported as a one minute peak average (VO_{2peak}).

Experimental protocol

The treatment was administered in a blind, randomized fashion. Upon arrival to the laboratory subjects provided a urine sample; a urine specific gravity (Usg) > 1.020 g/ml was chosen to define a dehydrated state and subjects were not allowed to participate until adequately hydrated. All subjects participated in three 1-hour time-trials (TT) on a cycle ergometer according to a previously validated endurance performance test by Jeukendrup et al. (1996) (Appendix I). All three trials were completed in a controlled environment (30.5 $\pm$ 1.1°C and 62.5 $\pm$ 6.6% humidity and 27.2 $\pm$ 0.8 WBGT). A thermal environmental monitor (Questemp° 36, Quest Technologies, Oconomowoc, WI) was used to measure the internal environment of the heat chamber. The subjects randomly ingested one of the following solutions dissolved into tap water equal to 12 ml/kg body weight:

Table 1 Treatment solution composition and formula. All calculations based on 1000 ml solution; equivalent to an 83.3 kg individual.

	HCIT 0.2 g/kg body mass	LCIT 0.1 g/kg body mass	CNT Gatorade
Sodium (g/L)	16.7	8.3	0.4
Carbohydrate (g/L)	60	60	66
Sodium (mEq/L)	170.3	84.7	18.3
Carbohydrate (%)	6.0	6.0	6.6

HCIT stands for the treatment solution using 0.2 g/kg sodium citrate. LCIT is the treatment solution using 0.1g/kg sodium citrate. Sodium Citrate Dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) has a molecular weight of 294.11 g/mol. All calculations for the treatments (HCIT and LCIT) were based on the molecular weight of sodium citrate dehydrate as a tri-sodium species. Gatorade was assumed to use single-sodium species and therefore was calculated based on the molecular weight of sodium (22.99 g/mol).

Glucose (Annie's Natural Foods, Richmond, B.C.) was kept constant at 6% for the two CIT solutions (Murray et al. 1999; Ryan et al. 1998). The subjects were given 20 minutes to ingest each solution. The 90-minutes of absorption time took place outside of the environmental chamber. Exercise commenced 100 minutes post-ingestion following a standardized 5-minute warm-up at 100 W. Subjects were given 125 ml of tap water to ingest every 15 minutes throughout the TT (15-, 30-, and 45-minutes) for each condition.

Anthropometric Measures

The sum of seven skin fold measurements was used to predict body fat percentage. The seven sites used were as follows: chest, triceps, subscapular, axilla, abdominal, suprailiac, and the anterior thigh. All measures were taken in triplicate using skin fold callipers (Harpenden, John Bull British Industries Ltd., England). The median value was used for each of the seven sites. Percent body fat was calculated, as a function of body density, using the seven-site skin fold formula by Jackson and Pollack (1978). Nude Bodyweight was measured to the nearest 0.02 kg using a digital scale (Model HL120, Avery Berkel, Taiwan) before and immediately after exercise.

Body Fluid Analysis

Capillary blood samples (~95 µl) were taken from a finger tip prior to fluid ingestion (baseline sample); after fluid ingestion (90 min); and during exercise (every 20 minutes). Approximately 95 µl of whole blood was collected directly into an i-STAT ECG 8+ cartridge. Baseline blood samples were taken after 20 minutes of seated stasis to ensure splenic replenishment of red blood cells (Stewart et al. 2003). The whole blood was immediately analyzed for glucose, sodium, potassium, hematocrit (Hct),

haemoglobin (Hb), pH, bicarbonate (HCO_3^-), and base excess (BE) using an I-STAT clinical analyzer (Abbott Point of Care, East Windsor, N.J.). Hematocrit (Hct) and haemoglobin (Hb) were used to calculate the %ΔPV according to the formula by Dill and Costill (1974):

$$\%PV = 100[(Hb_x/Hb_b)x((1-Hct_x)/(1-Hct_b))]-100\%$$

where subscripts x and b denote measurements at time (t) and at baseline (b) (-130 min). Hb is in grams per liter and Hct is a fraction.

The i-STAT hand held clinical analyzer uses approximately 65-95 µl of whole blood to run between 1 to 10 tests in 3 minutes or less. Whole blood samples are directly transferred into cartridges composed mainly of thin-film electrodes microfabricated on silicon chips (2.7 x 4.5 x 0.5 cm, w x l x h). Both venous blood samples and capillary blood samples can easily be transferred to the cartridge. Furthermore, the i-STAT clinical analyzer is a new and innovative system which has previously been validated against haematology analyzers in accredited medical laboratories (Gault and Harding, 1996; Mock, Morrison and Yatscoff, 1995; Jacobs et al. 1993). Photographs of the analyzer and the cartridges have also been previously been published (Erickson and Wilding, 1993; Lauks 1998).

Urine specific gravity was measured prior to exercise to verify all participants began each trial in a euhydrated state. The cut-off chosen to represent euhydration was a urine specific gravity < 1. 020 g/ml (Cheuvront and Sawka; 2005). Urine specific gravity was measured via a hand held clinical refractometer (Model PAL-10S, ATAGO, Tokyo, Japan).

Thermal Strain

Core temperature and skin temperature were measured using rectal thermometers (Mon-a-Therm General Purpose, Mallinckrodt, St. Louis, MO, USA) and biomedical ceramic chip thermisters (MA 100, 10KO negative temperature coefficient, Thermometrics, NJ, USA), respectively. Core temperature was continuously measured and recorded prior to warm-up, every five minutes throughout the exercise protocol, and every five minutes for 30 minutes after exercise using an 8-channel data logger (SmartReader 8 Plus, ACR Systems, Surrey, BC, Canada). Subjects self-inserted the rectal thermistor into the anal canal to a depth of 10 cm. The same thermistor was used for each participant for all three conditions (sterilized between uses). The ceramic chip thermistors were placed on the left side of the body and secured using adhesive tape. Skin temperature was continuously measured and recorded at the same intervals as core temperature at four sites (chest, upper arm, thigh, and calf):

1. Upper arm – lateral aspect of the left arm, approximately half way between the olecranon process and the acromion process.
2. Chest – Between the left axilla (armpit) and the left nipple
3. Quadriceps – anterior aspect of the quadriceps midway between the inguinal crease (groin; between torso and thigh) and the base of the patella.
4. Calf – posterior aspect of the lower left leg, at maximal calf girth

Mean skin temperature (T_{sk}) and total body temperature was calculated using the following formulas (Ramanathan, 1964):

$$T_{sk} = 0.3\ (T_{chest} + T_{left\ arm}) + 0.2\ (T_{left\ thigh} + T_{left\ leg})$$

$$T_b = (T_{core} * 0.85) + (T_{sk} * 0.15) * 0.5$$

Cardiovascular and Physiological Strain

Heart rate was continuously measured and recorded (Polar heart rate monitor) every five minutes throughout the time-trial. Physiological strain experienced by the subjects during each time-trial was calculated (Moran et al. 1998) using the physiological strain index (PSI). Combining both heart rate and rectal temperature the index starts at 0 (no/little strain) and progresses to a maximum of 10 (high physiological strain).

Psychophysical Strain

Thermal comfort and thermal sensation were measured based on the work of Gagg, Stolwijk and Hardy (1967). Subjects also provided their rating of perceived exertion using the Borge Scale (Burdon et al. 1982). Psychophysical strain was recorded every five minutes in a consistent order. All subjects were instructed how to use each scale prior to the commencement of exercise.

Sweat rate and Sweat loss

Nude bodyweight was recorded immediately before exercise, and immediately after exercise in order to determine %Δ in bodyweight; later used as an estimate of total sweat rate and sweat loss. Fluid ingestion and excretion were accounted for before calculating sweat rate. Subjects towelled dry prior to being weighed. A digital scale (Model HL120, Avery Berkel, Taiwan) was used to record body weight; accurate to 0.02kg.

Sweat loss = deficit + fluid intake

Deficit = (body $mass_{before}$ – body $mass_{after}$) x 1000

Sweat rate = sweat loss / time

Nude body mass is assumed to be nude body mass prior to exercise and/or immediately after exercise taken with a precision weight scale. Time is not a constant and can be substituted with a fraction of 60-min or as a rate/min.

Analysis

A repeated measures analysis of variance (ANOVA) was used to identify differences between trials for the dependent variables (heart rate, core temperature, skin temperature, body temperature, RPE, thermal comfort, thermal sensation and PSI). LSD Post Hoc test were also used to define each difference. Paired t-tests were used to identify differences among the repeated measures if such differences were found. One-way ANOVA with LSD post hoc tests were used to identify differences between fluid losses, blood parameters, and time-trial performance indices. Significance was set a $p \leq 0.05$. All values were reported as a mean $\pm$ standard deviation (SD). All statistical analysis was performed using SPSS 14.0 software for windows (SPSS, Chicago, IL).

Chapter 4

4.1 Subject Characteristics

Eight trained competitive male cyclists and/or triathletes completed all aspects of the study. Of the 14 subjects initially recruited for the study, one subject was asked to leave the study due to his fitness level, two subjects voluntarily withdrew from the study due to scheduling conflicts, and two of the subjects completed the first TT and voluntarily withdrew from the study due to the rigorous protocol. The physical characteristics of the subjects are described in Table 2.

None of the eight subjects who participated in the study completed any of the TT's. Two trials were voluntarily aborted, five trials were stopped due to the inability of the subject to maintain a power output $\geq$ 150 W, and the remaining TT was stopped by the primary investigator due to a core body temperature $\geq$ 39.5 °C. Due to the fact that none of the subjects completed the target amount of work; data was only analyzed up to 40-minutes; a time which the majority of subjects achieved.

Table 2 Physical characteristics, maximal aerobic power, and peak rate of oxygen consumption

Subject	Age (yrs)	Height (cm)	Weight (KG)	%Body Fat	Wmax (W)	W/kg	VO_{2peak} (ml/kg/min)
1	23.0	169.2	64.0	6.1	310.0	4.8	63.0
2	21.0	179.9	74.1	5.8	346.0	4.7	59.5
3	26.0	176.2	74.7	11.2	340.9	4.6	53.1
4	23.0	185.4	79.6	10.1	341.8	4.3	55.5
5	22.0	178.8	73.1	4.2	412.1	5.6	65.8
6	35.0	179.7	74.3	13.3	413.4	5.6	65.5
7	25.0	175.6	75.6	7.6	340.0	4.5	60.1
8	24.0	183.2	77.5	7.4	410.0	24.0	65.5
Mean	**24.9**	**178.5**	**74.1**	**8.2**	**364.3**	**24.9**	**61.0**
SD	**4.4**	**5.0**	**4.6**	**3.1**	**40.9**	**4.4**	**4.8**

Wmax denoted the maximal aerobic power (W) achieved during the step test.
W/kg denotes the fraction of maximal aerobic power (W) divided by body weight (kg).

4.2 Body Fluids

4.2.1 Hydration level

All subjects were tested in a euhydrated state. Mean urine specific gravity (Usg) was < 1.020 g/ml for all three trials. Two subjects arrived to the study with an Usg > 1.020 g/ml. Approximately 30 minutes was provided for the ingestion on 1 L of tap water before Usg was re-tested. A second urine sample verified a euhydrated state (Usg < 1.020 g/ml).

4.2.2 Hypervolemia

Plasma volume was significantly ($P < 0.05$) expanded by 7.1% from resting baseline samples in the HCIT group. PV was unchanged in the LCIT and CNT trials. PV was better maintained in the HCIT group compared to the LCIT and CNT throughout the TT as shown in Figure 3. PV was reduced by 7.6% in the HCIT trial and 7.8% in the LCIT trial in the first 20-min. Between time 20-min and 40-min an opposite trend occurred, with PV slightly expanding by 3.2% and 1.2% in the LCIT and CNT trials, respectively. PV was significantly reduced from baseline at minute 20 and 40 in the CNT trial; where, PV was not significantly different from baseline or pre-exercise values in both sodium trials. The PV trends are illustrated in Figure 3.

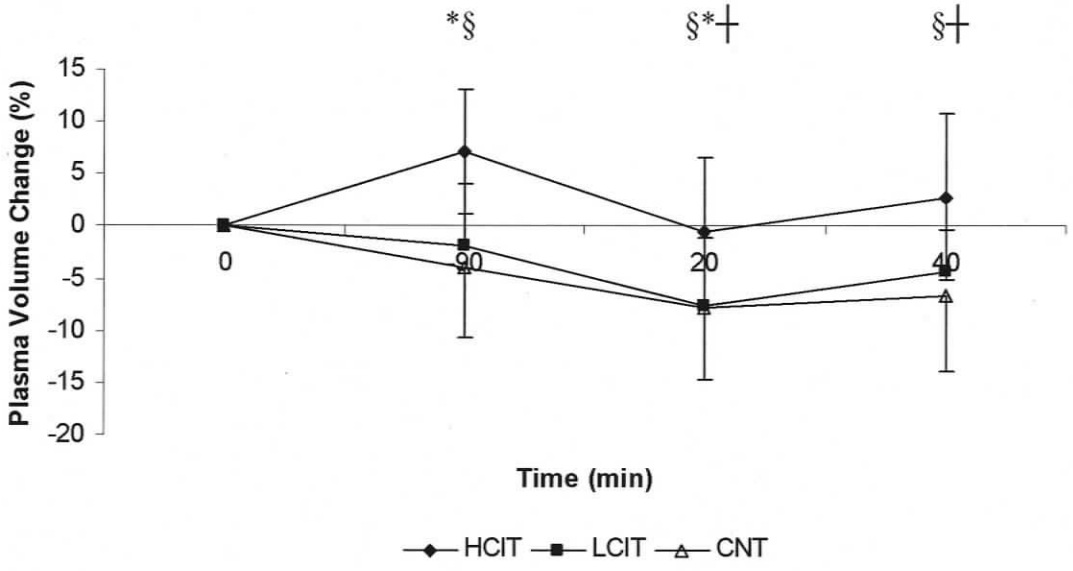

Figure 3 Plasma volume changes (%) after the ingestion of one of the three treatment solutions from baseline (time 0-min). * Significantly different than previous value (HCIT 0-90 min and HCIT – 90-20 min). ┼ Significantly different than baseline (LCIT 20- and 40-min and CNT 20- and 40-min). § Significantly different than each other (HCIT-90, -20, -40-min). P < 0.05.

4.2.3 Electrolyte analysis

There was no significant differences between treatments across time for plasma sodium. Both the HCIT and LCIT trials decreased in potassium from baseline to 90-min with significant interaction from 0-90 minutes in the CNT trial (Figure 4). All three trials showed an increase in potassium between resting (-90-min) and 20-min. Potassium was reduced in all three trials from 20-min to 40-min. Figure 4 illustrates the sodium/potassium trends for the whole blood samples.

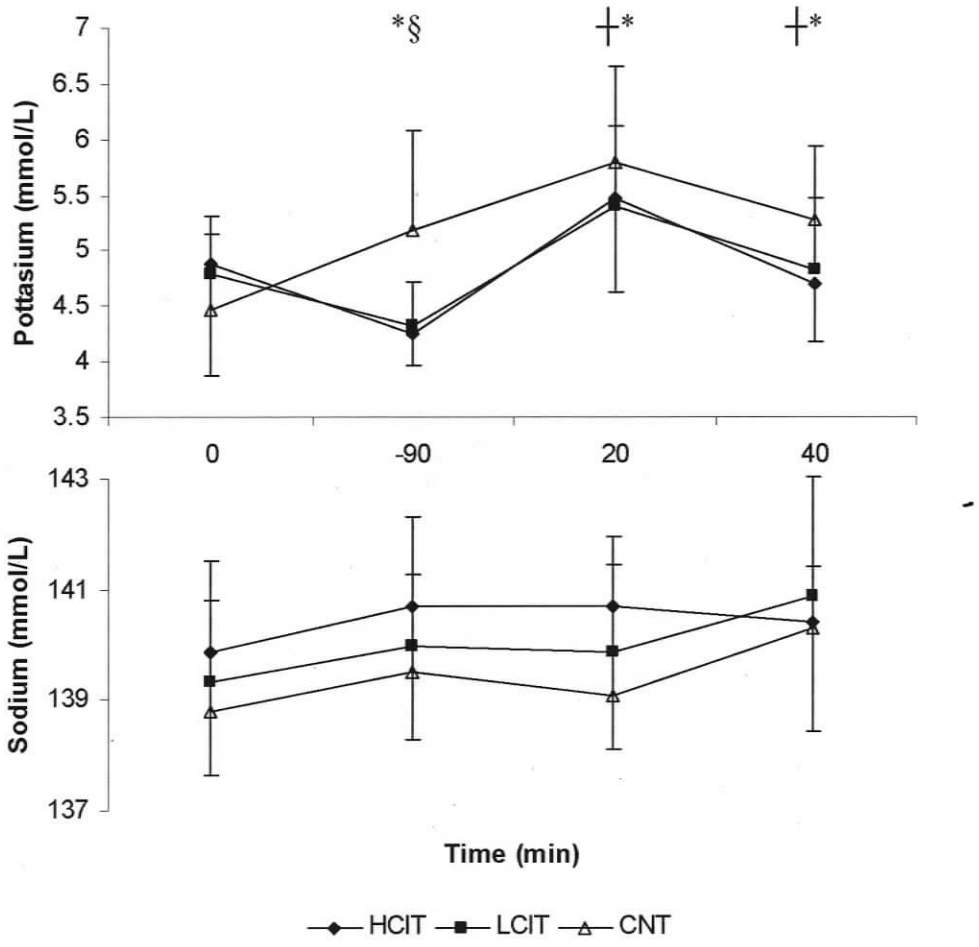

Figure 4 Sodium and Potassium capillary blood concentrations starting with baseline (time 0) and throughout each time trial (time -90 through 40). * Significantly different than previous value. + Significantly different than baseline. § Significant difference between treatments. P < 0.05.

Capillary blood glucose concentrations decreased from resting baseline levels (-1.2 ± 0.8 mmol/L) following the ingestion of all three treatment solutions. Blood glucose was further reduced by 20-min (-0.3 ± 0.8 mmol/L) of exercise. Blood glucose increased between times 20- and 40-min, on average, by 0.3 ± 1.0 mmol/L. This is illustrated in Figure 5 below.

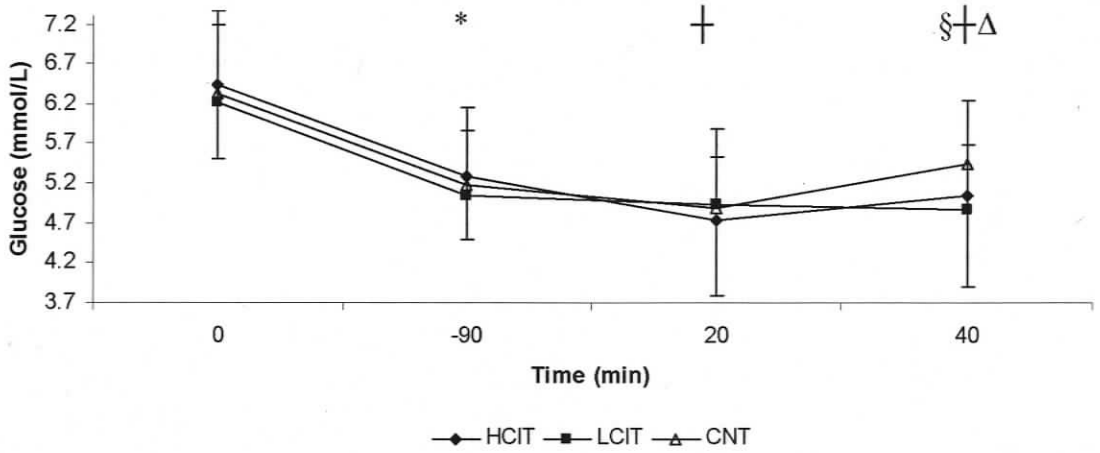

Figure 5 Mean (SD) blood glucose concentrations before- and during a self-paced TT. * Significantly different than previous value (CNT and LCIT). + Significantly different than baseline (20-min = HCIT and CNT; 40-min = HCIT and LCIT). § Significantly different than each other (LCIT-CNT). Δ Significantly different than 90-min (CNT). P < 0.05.

4.2.4 Buffering capacity

Blood bicarbonate (HCO3-) was significantly elevated from baseline to 90-min in the HCIT compared to the LCIT and CNT trials. This was also replicated in blood base excess (BE) concentrations (Figure 6). HCO3- and BE were also reduced between pre-exercise and 20-min. Between time 20-min and 40-min HCO3- and BE expanded to values similar to baseline (Figure 6). Blood pH increased in all treatment groups from baseline to the end of exercise, increasing by an average 0.1 $\pm$ 0.03 pH by minute 40 (Figure 7).

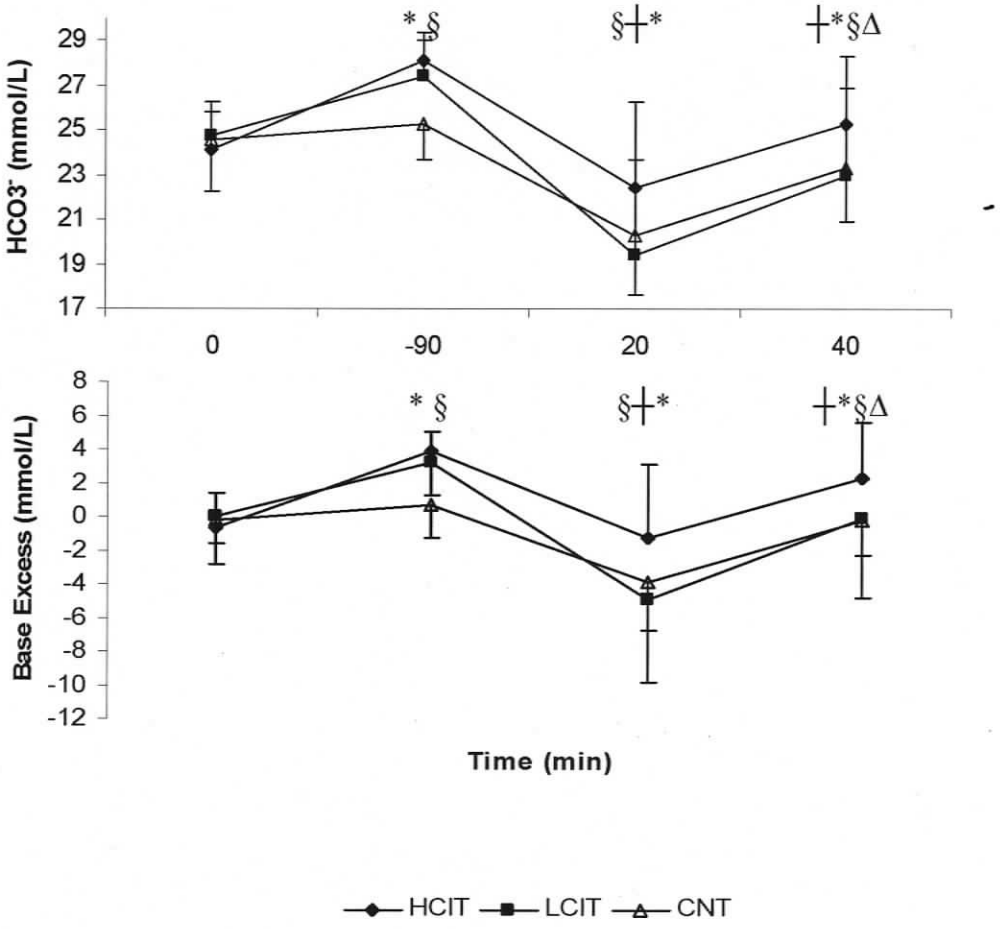

Figure 6 Average bicarbonate and base excess capillary blood concentrations (+SD). * Significantly different than previous value. ┼ Significantly different than baseline. § Significantly different than each other (LCIT-CNT). Δ Significantly different than 90-minutes (CNT). $P < 0.05$.

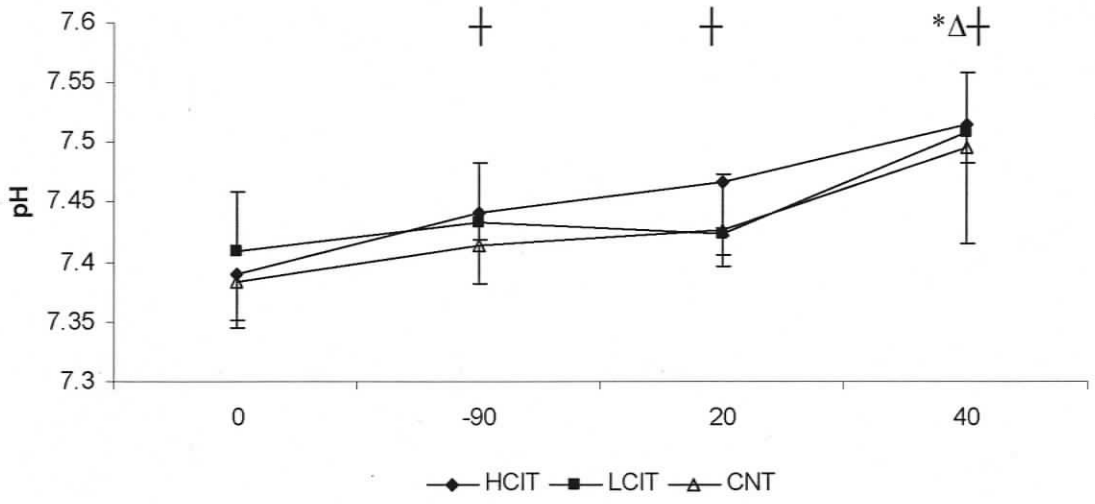

Figure 7 Mean (SD) blood pH starting from resting baseline levels to 40-minutes of a self-paced TT.). * Significantly different than previous value (LCIT and CNT). + Significantly different than baseline. Δ Significantly different than 90-minutes. P < 0.05.

4.3 Physiological Strain

4.3.1 Body temperature

Rectal temperature (T_r) was similar before the start of the TT in all three trials (37.1°C ± 0.3). A significant (p = 0.000) core temperature time effect was found with T_r steadily increasing in all three trials similarly, reaching significance after the warm-up (Figure 8). There was no time x group effect found between treatments. Skin temperature changes mirrored rectal temperature; increasing steadily throughout all three trials. A significant time effect (p = 0.005) was found in all three trials without any time x group interaction. Skin temperature during the warm-up (33.6 ± 0.7°C) was significantly elevated (35.6 ± 0.7°C) 5-minutes after the commencement of the 1-hr TT (p = 0.000). Skin temperature was also found to be significantly different between times 5 and 10, 20 and 25, and 30 and 35 (Figure 8).

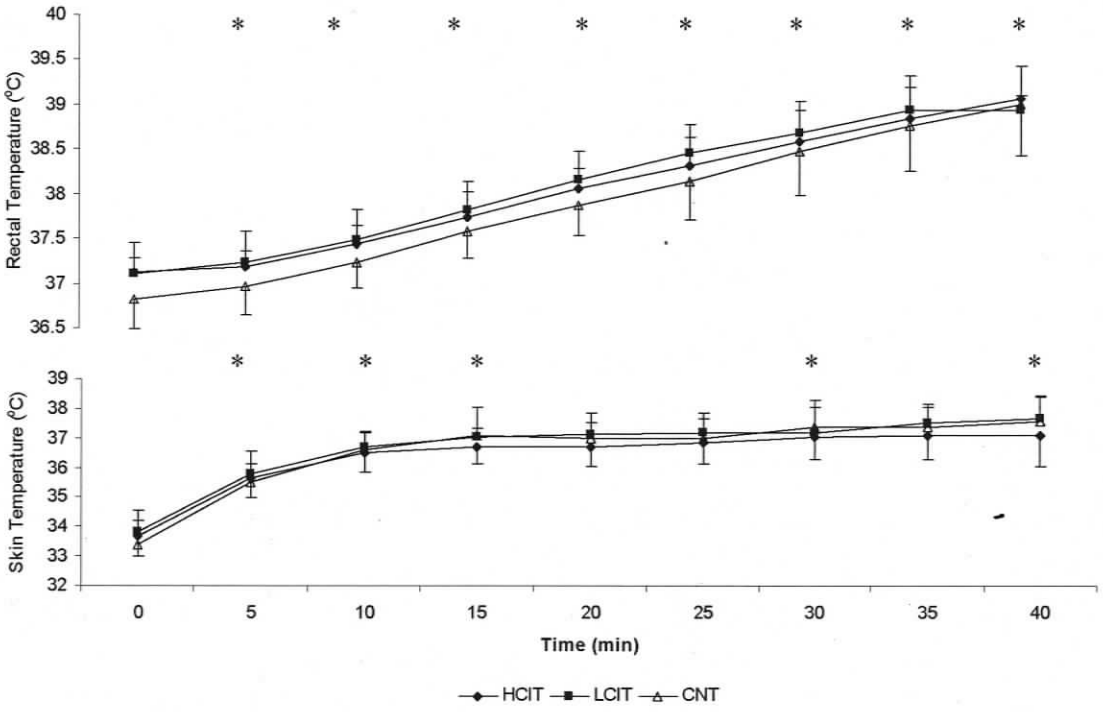

Figure 8 Average (SD) rectal and skin temperatures taken every five minutes starting during the warm-up (100 W, time 0-5) and continuing throughout the time trial. Rectal temperature was significantly different (*) after the warm-up (p = 0.000). Skin temperature was also found to be significantly elevated (*) compared to its previous value throughout the time trial (P < 0.05).

Core temperature was found to be similar in the CNT trial compared to both the HCIT and LCIT trials despite showing a lower raw value. Core temperature tended to increase the least amount per unit of time (5 minutes) in the HCIT compared to the LCIT and CNT group (Figure 9).

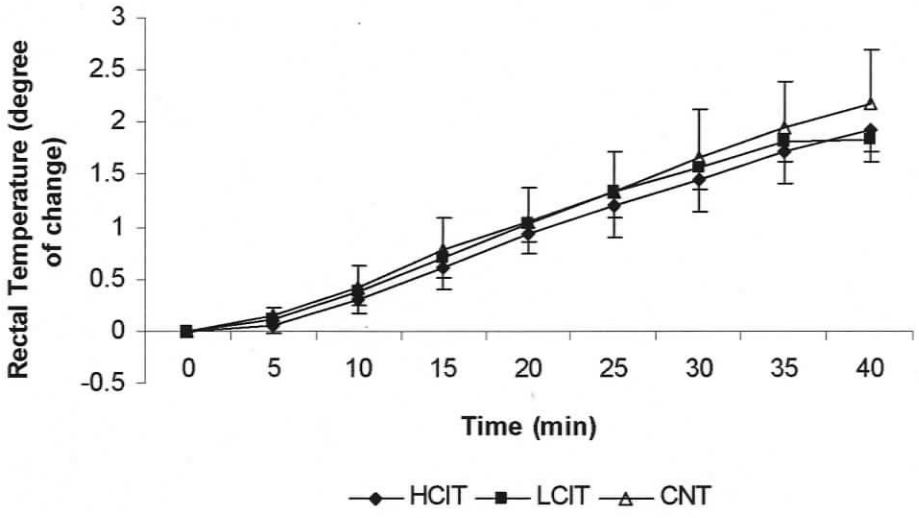

Figure 9 Mean (SD) rectal temperature change from baseline values for each of the three treatments per unit of time (5 minutes).

4.3.2 Cardiovascular Strain

Heart rates (HR) were similar (105.0 ± 19.3 bpm) during the five minute warm-up at 100 W for all three trials. HR was significantly increased (155 ± 13.3 bpm) during the first 5-min ($p = 0.002$) of all three TT without a significant difference between trials. HR was similar across all three trials and steadily increased at a similar rate as illustrated in Figure 10. There was a significant increase found at times10-min, 15-min and 30-min compared to the previous time increment (Figure 10).

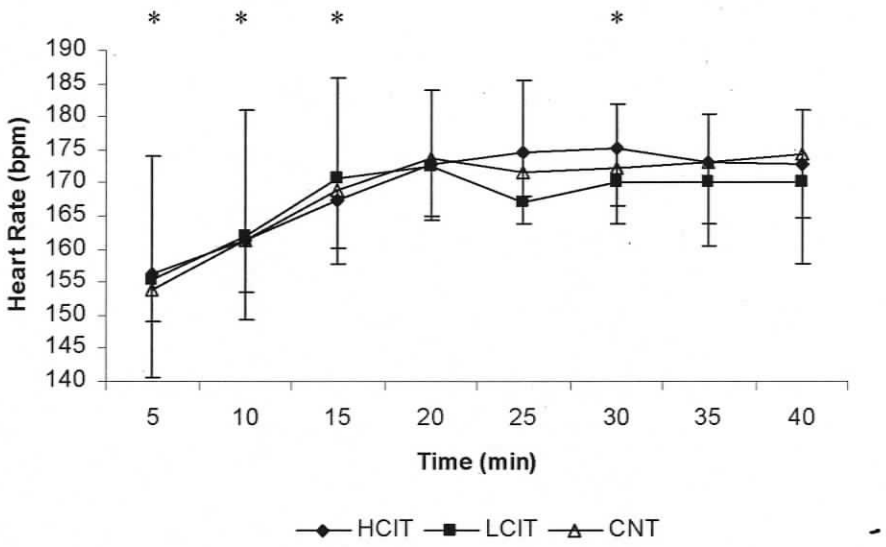

Figure 10 Mean (SD) five minute heart rate (bpm) during 40-min of a self-paced time trial. * Significantly different than all other values.

Physiological strain (PSI) significantly changed across time ($P < 0.01$), beginning after the 5-minute warm-up and continuing until the termination of exercise. The greatest increase in strain occurred after the first 10 minutes of heat exposure (5-min warm-up plus first 5-min of TT) with a mean core temperature increase of 0.11 ± 0.1°C and an average increase in heart rate of 49.1 ± 12.9 bpm (Figure 11)

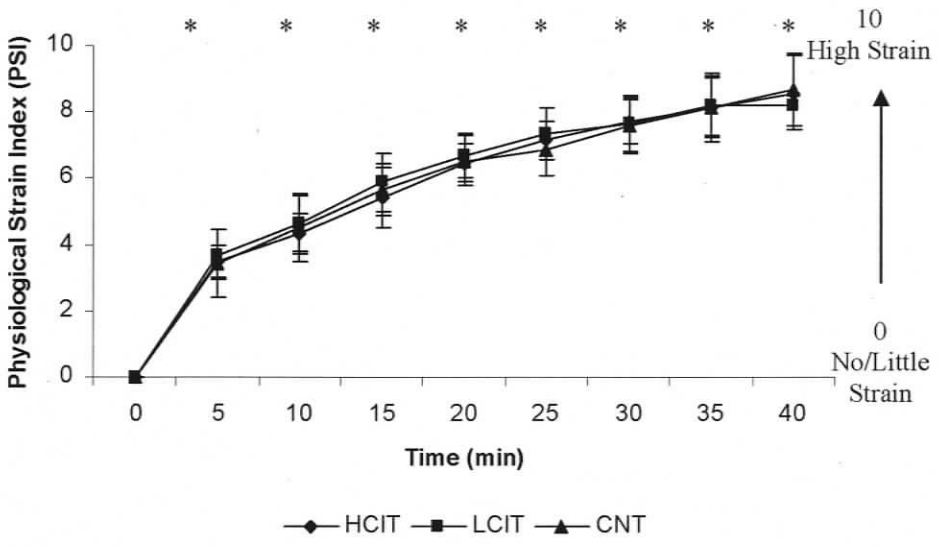

Figure 11 Physiological strain during 40-min of a maximal time trial effort. n = 8 except at times 30 and 35 (n = 7 and n = 6). PSI reached significance (P = 0.000) at time 0 and was maintained throughout the TT.

4.4 Psychophysical Strain

Subjects reported feeling significantly less exerted and more comfortable during the five minute warm-up (100 W) under the same heat stress as the TT compared to their following reports during the TT. Rating of perceived exertion (RPE), Thermal Comfort (TC), and Thermal Sensation (TS) were all significantly higher at 5-min compared to the warm-up ($P < 0.01$). RPE, TC, and TS were all reported similarly throughout all three trials and all increased at a similar rate. No significant differences were found between treatments; however, RPE, TC, and TS reached statistical significance at 5-min and continued to increase significantly throughout the TT's. Psychophysical measures are illustrated in Figures 12-14.

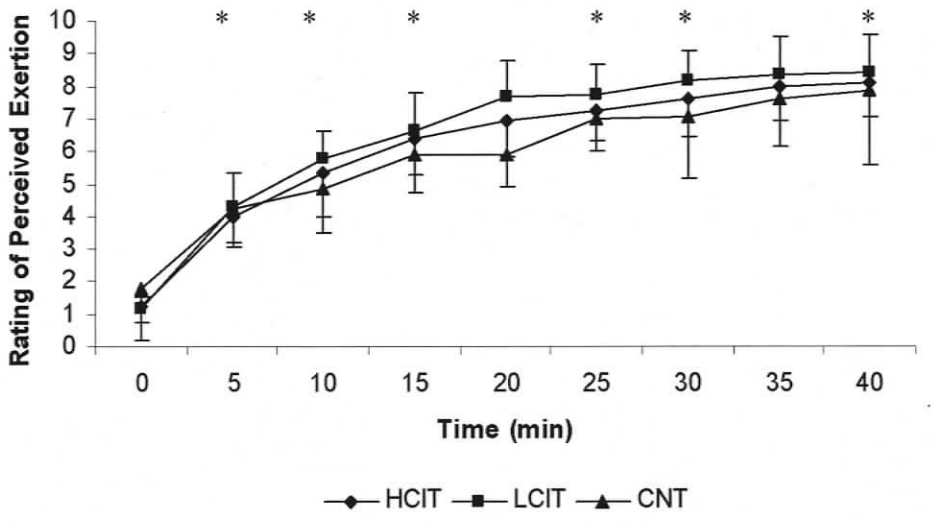

Figure 12 Mean (SD) ratings of perceived exertion taken using a modified Borge scale. * Significant difference from previous value.

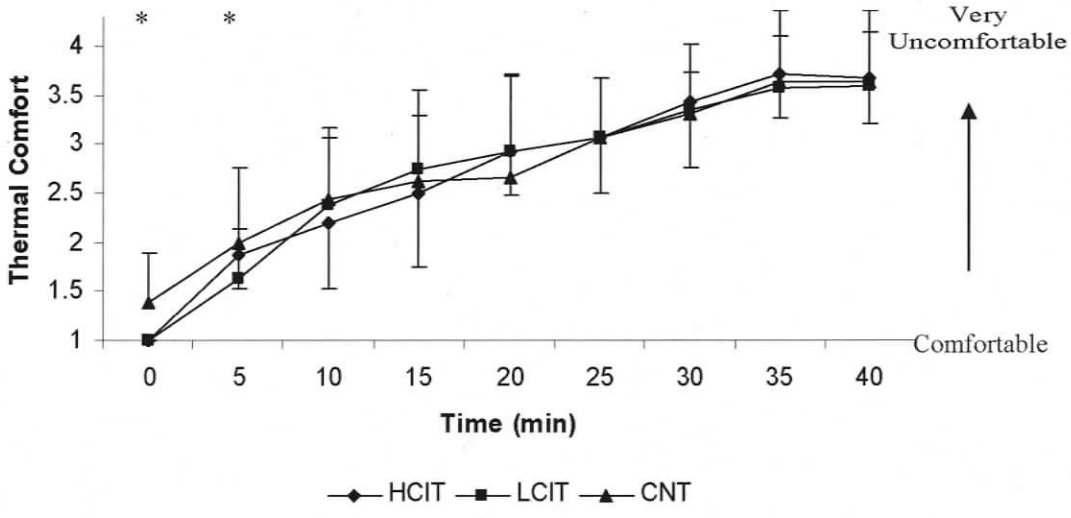

Figure 13 Mean (SD) thermal comfort during a 5-minute warm-up (100 W) and 40-minutes of self paced TT. * Significantly different than all other values.

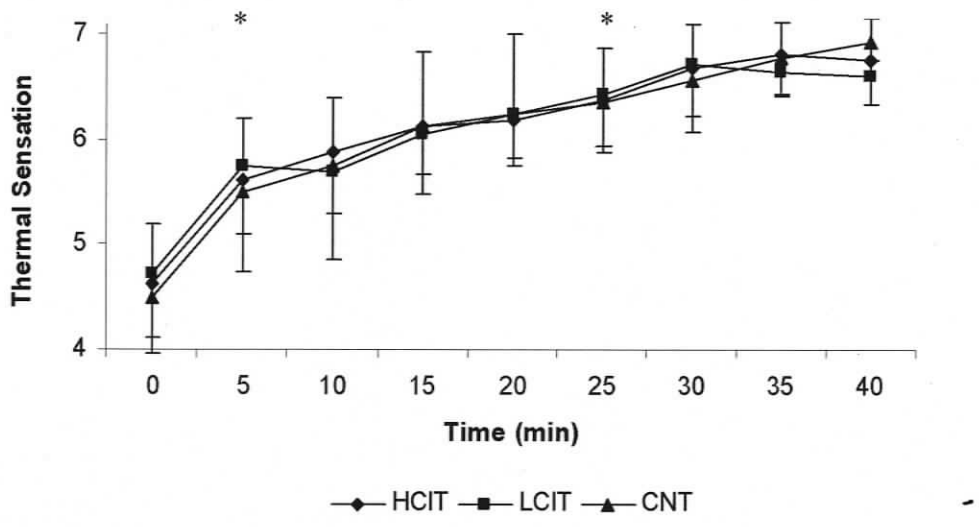

Figure 14 Mean (SD) thermal sensation during a 5-minute warm-up (100 W) and 40-minutes of a self paced TT. * Significantly different than the previous value.

4.5 Self paced performance

4.5.1 Power Output

Power output started at a similar wattage for all three trials (267.3 $\pm$ 32.8 W) and was similarly reduced in all three trials. All three treatments resulted in a significant difference in power output across all times compared to the previous 10-minute average in all cases except one (LCIT watts at 30-minutes compared to watts at 40-min). Linear regression ($R^2 = 0.99$) using all three treatment values indicates a 17.7 watt decrease for every 10-minutes spent in the heat chamber.

$$y = -17.723(x) + 284.49$$

Let x represent one single 10-minute period. For example at time 30-minutes, x = 3.

The HCIT trial was not found to maintain a higher power output at times 30-min and 40-min compared to the LCIT and CNT trial. There was a small difference reported at

20-minutes and 40-minutes between the HCIT and LCIT trial ($P < 0.10$). A significant difference was found between the LCIT and CNT trial at 20-minutes ($P < 0.05$).

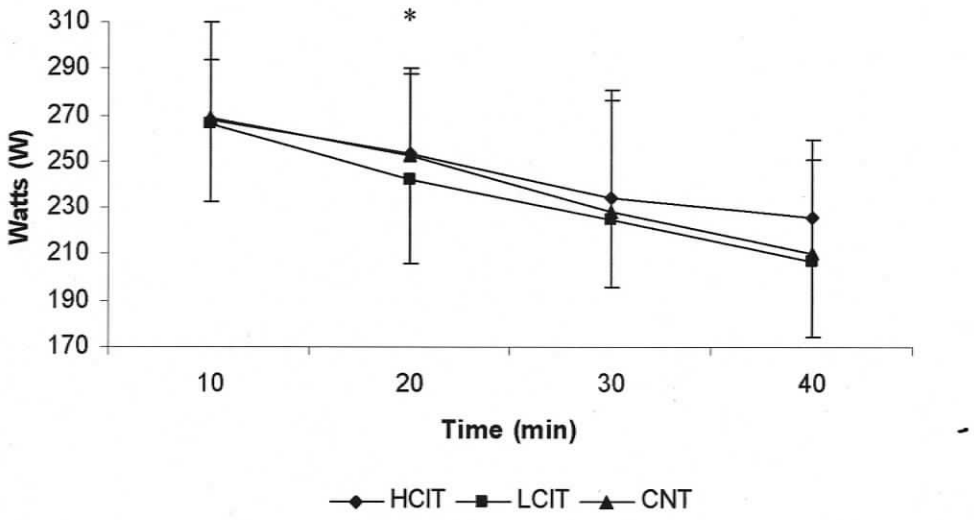

Figure 15 Mean (SD) power output for all three treatments during 40-minutes of a self-paced TT. * Significant difference between LCIT and CNT treatments.

4.5.2 Split times and pacing

No statistical difference was found between treatments for individual split times (25% and 50%). The HCIT trial completed the first 25% of the TT 3.0 seconds faster than the LCIT trial and 0.5 seconds faster than the CNT trial. The HCIT trial also completed the second quarter of the TT 19.4 seconds and 29.1 seconds faster than the LCIT and CNT trials, respectively (Figure 16).

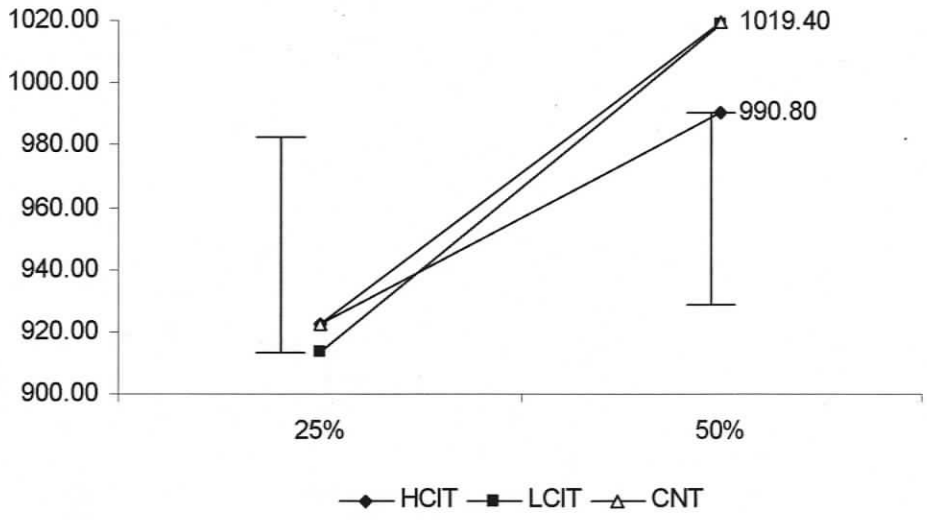

Figure 16 Mean split times for each treatment for the completion of the first and second quarter of a TT. Error bars on either side of the graph are meant to represent the average SD (+ 78 sec) of the first quarter split time (left) and the average SD (+ 69 sec) of the second quarter split time (right).

Table 3 Mean (SD) TT split times: Time (sec) taken to complete 25% and 50% of the predetermined target amount of work (KJ).

Treatment	25% of Target KJ (Seconds)	50% of Target KJ (Seconds)
HCIT	922.2 ± 67.4	1913.0 ± 51.5
LCIT	913.6 ± 61.1	1932.4 ± 52.6
CNT	922.7 ± 107.1	1942.1 ± 104.9

4.5.3 Total Test Time and Work Achieved

None of the eight subjects completed the required amount of work and therefore no data is available on total test time. The HCIT trial did cycle for a greater amount of time than the LCIT and CNT trial before voluntarily withdrawing or reaching a critical core temperature (2852 sec HCIT, 2623 sec LCIT, and 2743 sec CNT). The CNT trial, on

average, cycled for a greater amount of time (+120 sec) than the LCIT trial. The above data confirms the greater amount of work achieved by the HCIT and CNT trial, compared to the LCIT trial, respectively. Table 4 shows this trend.

Table 4 Mean (SD) total test time (sec) and total amount of work (KJ) completed during each of the three treatments.

Treatment	Total Test Time (seconds)	Total Work Complete (KJ)
HCIT	2852.6 ± 442.5	697.2 ± 148.6
LCIT	2623.8 ± 555.4	636.7 ± 131.8
CNT	2705.3 ± 277.4	664.1 ± 96.7

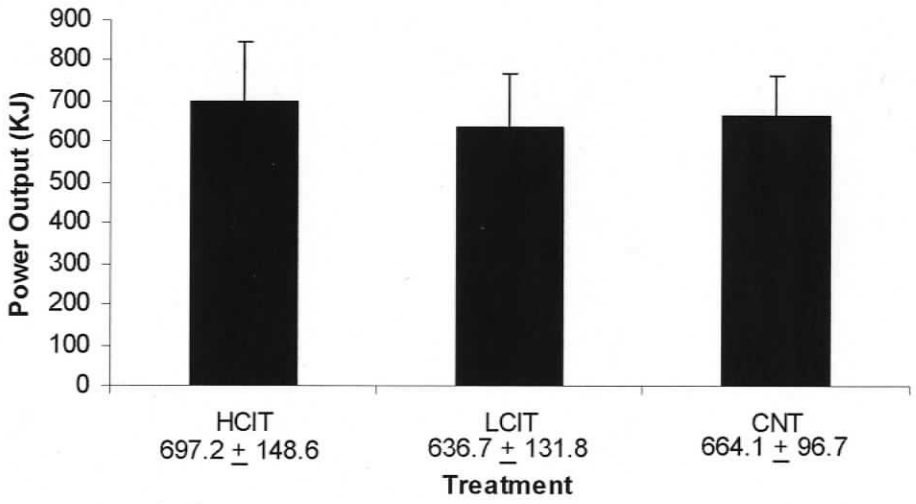

Figure 3 Total average (SD) amount of work complete (KJ) by each treatment.

4.6 Hydration Status

Mean subject body mass significantly decreased in all three trials, from 74.0 ± 4.2 kg prior to starting the warm-up, to 72.7 ± 4.3 kg upon leaving the heat chamber. On average, subjects lost 1.8 ± 0.5% of their total body mass during the three trials. This

was a result of an average sweat loss equal to 1606.7 $\pm$ 395.3 ml and an average fluid intake of 316.7 $\pm$ 81.6 ml of tap water (Figure 17).

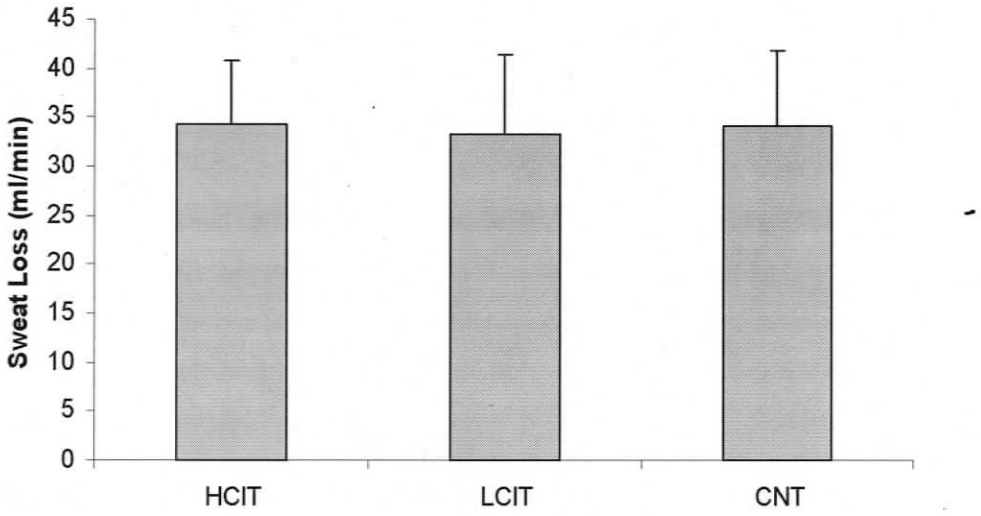

Figure 4 Mean (SD) sweat loss (ml/min) during a TT following the ingestion of one of three solutions.

Chapter 5

Discussion

The purpose of this study was to examine whether hypervolemia, via acute sodium citrate ingestion, improved cycling time trial performance under severe environmental conditions. Sodium Citrates ability to artificially increase the buffering capacity of blood was also assessed. Acute oral ingestion of sodium with the intention of expanding plasma volume has already been studied (Greenleaf et al. 1998, Coles and Luetkemeier, 2005). To the best of our knowledge only one other study (Sims et al. 2007) has examined the effects of hypervolemia on exercise performance in athletes in a hot environment after oral ingestion of sodium citrate. They found that following acute sodium-fluid load, plasma volume was expanded by 4.5% and physiological strain was improved. Exercise capacity, greater time to exercise termination, was also significantly improved following plasma volume expansion.

There has been a great deal of research examining the effects of acute PV expansion via intravenous infusion (Sawka et al. 1983, Hopper et al. 1988, Watt et al. 2000, Roy et al. 2000, Berger et al. 2006, and Grant et al. 1997). Research using clinical plasma expanders (i.e. Dextran, Hemaccel or Gelofusine) is currently inconclusive. Sawka et al. (1983) found heart rate to be lower during submaximal exercise in the heat after a 13% increase in PV following human albumin infusion; however, no thermoregulatory benefit was found. These findings are in accordance with Roy et al. (2000) who showed that following a 15.8% expansion in PV, heart rate was lower and SV and cardiac output was higher but no difference in mean arterial pressure, total peripheral resistance or core temperature was found. Watt et al. (2000) also examined submaximal exercise in the

heat following Hemaccel infusion but found no difference in core temperature, heart rate response, and skin blood flow between the treatment and control trial.

The present study found that sodium ingestion did not change body temperature or cardiovascular response to exercise under severe heat stress. It is possible that these changes were made unclear due to the type of work trial chosen for this experiment. It was found that the HCIT trial completed both the first and second quarter of the time trial faster than the two other treatment groups. The HCIT trial tended to work at a higher power output throughout the trial while still exercising for a longer total time than either the LCIT or CNT. Although rectal temperature did not suggest an improved thermoregulatory it is important to note the similarity between the core temperatures for each treatment. The time trials were not a fixed power output, meaning any differences in thermal strain between treatments may have been masked by differences in power output. There was a trend for a higher power output in the HCIT group, and although not significant, the slightly higher power output was achieved with no added physiological strain.

Treatment Effect

The highest concentration of CIT in the present study significantly expanded resting PV by 7.1%, and this increase was significant when compared against the other two treatments.. This is in accordance with the findings of Coles and Luekemeier (2004) who showed that after ingesting a high concentration of sodium (~164 mEq), PV expanded by 3.1% but decreased by 4.7% from baseline values in the placebo group. $HCO3^-$ and base excess were also significantly elevated from baseline to post-ingestion in the HCIT trial compared to both the LCIT and control group. Other research examining the effect of

CIT on athletic performance has also shown similar results (Hausswirth et al. 1995, Faff et al. 1996, Potteiger et al. 1996, Kowalchuk et al. 1989, Potteiger et al. 1996b, Schabort et al. 2000, Van Someren et al. 1998, Van Montfoort et al. 2004). Our findings suggest that the HCIT treatment had both a hypervolemic and an alkaline effect prior to and throughout the TT compared to the other two treatments..

None of the eight subjects who volunteered for the study completed any of the three TT. Of the 24 TT started, two trials were voluntarily aborted, five trials were stopped due to the inability of the subject to maintain a power output $\geq$ 150 W, and the remaining TT were stopped by the primary investigator due to a core body temperature $\geq$ 39.5 $^{\circ}$C. It is important to examine the effects of potentially performance enhancing tools in an applied setting. The use of a TT was chosen for this reason; as a mechanism for studying the effects of sodium citrate and hypervolemia on athletic performance. In the future, both intensity, and the central research question, should be examined more closely. Steady-state exercise could potentially highlight some of the major physiological differences not supported in the present study.

Rectal temperature and heart rate steadily increased throughout exercise showing no differences throughout the time trial between any of three groups. Power output was, however, higher and better maintained in the HCIT trial, and with greater statistical power ($\gamma = 0.4$), could possibly be significantly different ($n = 10$). This suggests that the subjects self-selected a higher wattage throughout the HCIT trial compared to the control trial and therefore were working harder. Thus, reflecting similar cardiovascular strain while producing a similar core temperature under differing amounts of stress. It is possible that thermoregulation was improved in the HCIT trial but was not reflected in

the data due to the nature of the performance measure chosen. Moreover, it is possible that heart rate in the HCIT trial is actually depressed compared to a control trial at a similar intensity. More work is needed to distinguish if submaximal, steady-state exercise would yield a difference in rectal temperature and heart rate. Sims et al. (2007) identified a similar trend, showing a slightly ($P = 0.08$) lower heart rate and cardiovascular drift in the high sodium trial compared the low sodium. In that study, subjects ran at the same intensity in both trials.

Acute CIT loading did improve total test time in the HCIT vs CNT trials and better maintained power output compared to the LCIT and CNT trial. As witnessed in the present study, when PV is expanded both hemoglobin and hematocrit are reduced, negatively affecting oxygen carrying capacity. Despite this reduction in oxygen carrying capacity several studies have shown a reduced cardiovascular strain following PV expansion (Sawka et al. 1983, Hopper et al. 1988, Roy et al. 2000, Berger et al. 2006, Grant et al. 1997). It is possible that the increased exercise time and increased total amount of work achieved, as seen in the HCIT trial, is a reflection of this improved cardiovascular function and not dependent on heat dissipation. Berger et al. (2006) showed that PV expansion increased VO_{2peak} despite a reduced oxygen carrying capacity, as well as improved time to exhaustion and oxygen pulse. Heart rate was also found to be depressed, while SV and cardiac output were improved.

Two theories have been put forth to account for this increase in cardiovascular function following a decrease in hemoglobin and hematocrit (O_2 carry capacity): (1) muscle oxygen delivery does not limit oxygen uptake, and (2) adjustments in skeletal blood flow, or in muscle oxygen extraction, "pick up the slack" (Berger et al. 2006). The first

possibility suggests that under controlled conditions there was an excess amount of oxygen available to the working muscles and that following a reduction in oxygen content, VO_2 is not impeded. The second suggestion infers that the additional blood available following PV expansion does not increase/maintain skin blood flow through an elevated central venous pressure, as the original hypothesis suggests; rather, that the additional blood volume is redirected to the working muscles in order to supply the necessary oxygen to the already deprived system as a compensatory method of maintaining arterial oxygen content (Berger et al. 2006, Roy et al. 2000). It is therefore possible that in the present study, a fraction of the elevated central blood volume was directed toward the working muscles (legs) and not to the cutaneous vasculature supporting heat dissipation (Roy et al. 2000). This assumption is supported with the present results, as skin temperature was not different between trials and the environment was uncompensible. It is not likely that the additional blood volume aided with heat dissipation in the present study, since no amount of thermoregulation would counter-act the uncompensible heat stressed witnessed in the present study. Sweat rate (ml/min) was also found to be similar in all three trials and therefore may not have been the primary contributor to heat loss.

Combinations of these two suggestions likely work together to improve cardiovascular function following PV expansion. While SV, and subsequently cardiac output, can be accounted for by an increase in left ventricular end-diastolic volume, a depression of working heart rate is not as easily explained (Roy et al. 2000). If heat storage and oxygen carry capacity are jeopardize/stressed, as suggested above, heart rate would be expected to increase or be maintained compared to a controlled condition (i.e. no PV expansion).

Most of the previous work done in this area have based their assumptions on the larger assumption that (a-v)O_2 difference can be measured with a constant. This seems like a rather presumptuous assumption and one which needs further attention in the future. Given the present results, both heart rate and core temperature have responded positively to acute PV expansion and potentially worked to improve both total test time and total work achieved.

Recent research has suggested that subjects exercising in the heat seem to reach a point of voluntary fatigue at a similar core temperature, rightfully called the *critical internal temperature theory* (Cheung and Sleivert, 2004). This hypothesis suggests that subjects exercising in hot environments will eventually reach a internal temperature at which will force them to volitionally stop exercising despite fitness level (Cheung and McLellan,1998; Gonzalez-Alonso et al. 1999), adiposity (Selkirk and McLellan, 2001) and/or hydration or acclimation (Cheung and McLellan, 1998). Despite differences between groups (above) it is clear that a specific temperature set-point exists in which human beings cannot continue exercising. In accordance with this hypothesis, there exists a theory known as the teleoanticipatory theory (Lambert et al. 2005) which suggests that both feed forward planning and feedback control from afferent changes, as well as previous knowledge acquired from prior exercise bouts contributes to the subtle changes affecting physiological responses to exercise and volitional exhaustion.

Based on the above two theories, it is possible that the inclusion of high sodium-fluid prior to exercise, and the subsequent hypervolemia which follows, contributes to an increased time to critical core temperature; as witnessed in the present study. The teleoanticipatory effect is therefore reduced of afferent input, which "should" otherwise

provide resistance to exercise in uncompensible conditions, reducing the reserve-like mechanism attempting to avoid the critical core temperature by altering pacing (metabolic heat production).

Time Trial Performance

None of the eight subjects completed the 1-hr TT. This is likely a result of the uncompensible heat stress endured and the intensity of work chosen. The TT was set-up in such a way that a pedaling cadence of 100 RPM would correspond to a wattage equal to 75% W_{max}. Likewise, a pedaling rate of 90 RPM would achieve a wattage equal to 70% W_{max} ($W = L * (RPM)^2$); the target amount of work was based on the assumption that each subject could maintain 75% W_{max} for 3600 seconds (Work (J) = 0.75 x W_{max} x 3600) (Jeukendrup et al. 1996). Future studies should recognize the metabolic shift found to occur during heat-stressed exercise, and choose a work load (~60% W_{max}) which would respect the temperature induced metabolic changes found in the present study. This may achieve a more preferential TT performance while still maintaining the competitive nature of the study. The results of the present study still provide an accurate reflection of a 30 minute TT under the same conditions and therefore allow for performance speculation. As mentioned above, the HCIT trial performed more work (KJ), a reflection of the greater test time achieved, and completed both the first and second quarter faster than the two other trials (although not significant).

Linear regression models were used to predict power output at 60-min (the anticipated time to completion).

HCIT $y = -14.423x + 281.21$

LCIT $y = -19.464x + 283.69$

CNT $y = -19.813x + 289.38$

Let x equal each individual 10-minute time increment (i.e. 20-minutes = 2). Assuming that the subjects were allowed and willing to ride for 60-minutes, it is anticipated that the HCIT trial would finish the TT with an average power output equal to 194.7 W. The LCIT and CNT trials would finish nearly 30 watts lower with an average power output of 166.9 W and 170.5 W, respectively. The above models do not allow us to predict core temperature, and are therefore only extrapolations of a pre-critical core temperature self-paced TT. It is therefore unlikely that any subject would have reached this time and/or completed the time trial if allowed to ignore the ethical core temperature cut-off value (39.5°C).

HCO_3^-, Base Excess and Cycling Performance

The increased performance could also be attributed to an enhanced buffering capacity following CIT loading. $[H^+]$ accumulation is believed to affect the interaction of actin and myosin through the inhibition of calcium release from the sarcoplasmic reticulum (Requena et al. 2005; Jain et al. 2003). Decreases in pH are therefore believed to reduce the contractile ability of muscle and impair muscle tension via impaired cross-bridge cycling (McNaughton, 1990; Jain et al. 2003, McNaughton and Cedaro, 1992; Ibanez et al. 1995). Muscular contraction relies on the formation of an actin-myosin complex; the inhibition of such a complex limits muscular contraction (Schabort et al. 2000). Increased $[H^+]$ concentrations are therefore assumed to be one of the primary contributors of fatigue during exercise (Cho et al. 1992).

Approximately 15 to 18% of the $[H^+]$ ions generated during intense exercise are buffered by blood HCO_3^- (Faff, 1993). HCO_3^- is, therefore, one of the main buffers in

the body and acts by neutralizing protons to form carbons dioxide (CO_2) and water (H_2O) (Crowe, 2001). Extra-cellular pH also increases in the presence of $HCO3^-$ which in turn raises the H^+ and lactate [Lac^-] efflux from active muscles via the Lac^-/ H^+ cotransporter (Requena et al. 2005; Fernandez-Castanys et al. 2002; Hausswirth et al. 1995; Ibanez et al. 1995; Jain et al. 2003; Van Montfoort et al. 2004). Lac^- and H^+ diffuse from working muscles through a monocarboxylate transporter; one which is pH sensitive. The maintenance of a higher pH during exercise, through HCO_3^-, may allow this transport mechanism to facilitate greater lactate flux from the muscle (Jain et al. 2003; Potteiger et al. 1995; Potteiger et al. 1996; Teryaki and Atterbom, 1995). A greater efflux of Lac^- through the monocarboxylate transporter could potentially enhance the contractile performance of muscle and delay the onset of fatigue (Requena et al. 2005; Hausswirth et al. 1995; Ibanez et al. 1995).

Extra-cellular alkalosis may therefore promote lactate transport through muscle cell membrane and delay the onset of critical intra-cellular acidosis (Hausswirth et al. 1995). The rate of proton and lactate release from the muscle to the blood in therefore increased, which potentially delays the fall in muscle pH and thus its inhibitory effect on phosphofructokinase, a rate limiting enzyme in glycolysis. Acidity is believed to contribute to decreased athletic performance through the inhibition of key glycolytic enzymes, the inhibition of calcium release from the sarcoplasmic reticulum and its binding to troponin C, and the reduced contractility of muscle fibres (Oopik et al. 2003; Shave et al. 2001; Parry-Billings and MacLaren, 1986; Ibanez et al. 1995). Elevated concentrations of [H^+] ions limit glycolysis, glycogenolysis, and the re-phosphorylation of adenosine diphosphate (ADP) (Faff et al. 1996). PFK, the primary enzyme of

anaerobic glycolysis, is believed to be inhibited when functioning in an environment with a decreased pH (Cho et al. 1992; Faff, 1993; Ibanez et al. 1995). Phosphorylase is also inhibited by elevated [H^+] concentrations; thereby, further inhibiting glycolysis (Schabort et al. 2000; van Someren et al;. 1998).

By reducing the accumulation of H^+ ions in active muscles, and decreasing muscle pH, HCO_3^- delays the inhibition of glycolysis and promotes the formation of lactate (Faff et al. 1996; Linossier et al. 1997). Enhanced glycogenolysis will then contribute to improved muscle contraction and athletic performance during intense exercise (Oopik et al. 2004). The maintenance of an optimal intramuscular pH will therefore improve glycolysis and promote higher blood lactate formation (Potteiger et al. 1995). Through this buffering mechanism, the inhibition of key glycolytic enzymes would be less prominent, which would allow greater energy turnover through anaerobic glycolysis and would promote greater force production over a greater period of time (Shave et al. 2001; van Someren et al. 1998).

HCO3- and BE were both significantly increased during the present experiment. pH was measured from capillary blood samples in the upper extremities and is not a likely reflection of the muscle pH during each of the time trials. Subjectively, all subjects reported "feeling a lot stronger" during the HCIT trial and many were surprised when asked to stop exercising (critical core temperature). Although this was not reflected in the psychophysical measures, it is an important factor which future studies should account for and quantify. Muscle biopsies and venous blood samples should also be taken in order to understand if muscle pH and venous pH are similar/different and

explore the above mechanisms as a potential explanation to improved exercise performance.

Plasma Volume trends throughout TT

PV was expanded by 7.1% in the HCIT trial, where PV was depressed in both the LCIT and CNT trials by 1.8% and 3.9%, respectively. Coles and Luekemeier (2004) previously showed a similar trend, reporting a 3.1% expansion of PV in the high sodium trial compared to the placebo who suffered a 4.7% reduction in resting baseline plasma volume. PV was also found to show a small restoration following its initial drop in all trials, but more in the LCIT and CNT trial. One explanation for this restoration is water release into the extracellular fluid from the liver and skeletal muscle. Prolonged exercise will result in glycogen oxidation, as 2-4 g of water is stored with every gram of glycogen (Laursen et al. 2006, Olsson and Saltin, 1970).

As glycogen is depleted, the stored water is then released from the liver and/or muscles and enters the extracellular fluid. Pastene et al. (1996) showed that marathon runners oxidized approximately 557 g of their own energy stores during a marathon. Muscle glycogen was estimated to be 375 g and liver glycogen was assumed to contain 100 g of glycogen. Therefore, the depletion of glycogen stores would have decomplexed 1280 [(375 + 100) x 2.7] g of water (Olsen and Saltin (1970) showed that 2.7 g of water is complexed to each gram of stored glycogen). The 2.0% reduction in body mass suffered by the runners could therefore be considered negligible in light of these findings.

One other possible mechanism for plasma volume restoration, although it is unclear how significant the effect would be, is the water contribution following proton neutralization during bicarbonate buffering; bicarbonate neutralizes protons to form CO_2

and H_2O (Crow, 2001). The two treatment trials did not show any evidence of improved PV maintenance throughout the TT and therefore it is unlikely that this significantly contributed to the performance changes.

Blood glucose was measured during the three time trials and showed a very interesting trend. Despite a similar decrease in all three trials during the first 20-minutes of exercise, blood glucose levels similarly increased between times 20-min and 40-min. This increase in blood glucose mimicked a similar rise in blood PV and could represent some initial evidence supporting the above phenomenon. Two possible explanations exist, and are likely linked. A systemic rise in blood glucose would suggest that muscular glycogen levels have been reduced and liver glycogen is necessary to continue metabolic function. The rise in glucose could suggest the release of excess water from the skeletal muscles (and liver) into the extracellular spaces, and this increasing PV. However, it is also possible that the increased concentration of blood glucose is creating an osmotic shift of water into the extracellular spaces; resulting in a similar trend. The present study has only a limited amount of evidence to support this theory and more work is needed to understand this phenomenon.

Greenleaf and Brock (1980) showed that following the ingestion of hypertonic and isotonic beverages under different environmental conditions, total extracellular fluid volume (ECV) expansion was lower or less than the fluid intake volume when a hypertonic solution was ingested. They also showed that when an isotonic solution was ingested, total ECV expansion was greater than the fluid intake volume. This was the primary premise of this study, which assumed that PV expansion would come about through the uptake of fluid from the gut as it followed both sodium and glucose across

the intestinal wall (Guyton and Hall, 2006). It is possible that a hypertonic solution would either be dragged back into the intestine or shifted to the interstitial compartment after absorption and therefore reduce PV expansion capacity (Sims et al. 2007). We are confident that our drink concentration and absorption time was sufficient enough to allow complete, if not hyper, absorption and expansion of the ECF as the approximate 7% expansion of PV corresponds to 1050-1120 ml of an estimated 15-16 L pre-drink ECV (Sims et al. 2007, Greenleaf and Brock, 1980); a value considerably higher than the average sodium-fluid volume ingested before exercise (880 ml). The authors therefore do not believe that tonicity of the high-sodium beverage negatively influenced the absorption and subsequent expansion of PV or had a negative impact on exercise performance due to a hyper/hypo-osmotic plasma volume.

Practical Application

The current study investigated the application of acute sodium citrate loading on athletic performance. The 1-hour time trial protocol employed in the present study was likely too rigorous and demanding and did not provide a clear understanding of the effects of CIT ingestion on TT performance. Due to the environmental stress involved in the study, the trials became a test of "Time to Critical Core Temperature" rather than a test of volitional exhaustion or performance. In the future, intensities below 75% W_{max} should be employed to ensure subject compliance and to be able to compare TT performance.

The results from this study show promise, since PV was significantly expanded, and HCO_3^- was increased above the other two treatment groups. Despite showing no difference in thermoregulatory and/or cardiovascular strain, likely because of the TT

protocol chosen and statistical power, HCIT loading was shown to increase total exercise time, and tended to better maintain a higher power output throughout the TT. Split times for 25% and 50% of total work were also faster in the HCIT trial compared to the CNT and LCIT groups suggesting a faster overall TT in optimal conditions. More work is needed into the investigation of sodium citrate loading and its potential use in future sporting events. Future work should examine the steady state response to exercise after sodium citrate loading in severe heat stress.

Bibliography

Armstrong, L. E., C. M. Maresh, J. W. Castellani, M. F. Bergeron, R. W. Kenefick, K. E. LaGasse and D. Riebe. (1994). Urinary indices of hydration status. *International Journal of Sport Nutrition*. 4: 265-79.

Armstrong, L. E., Carl Maresh, Catherine Gabaree, Jay Hoffman, Stavros Kavouras, Robert Kenefick, John Castellani and Lynn Ahlquist. (1997). Thermal and circulatory responses during exercise: Effects of hypohydration, dehydration, and water intake. *Journal of Applied Physiology*. 82(6): 2028-35.

Armstrong, L. E., J. A. Herrera Soto, F. T. Hacker, Jr., D. J. Casa, S. A. Kavouras and C. M. Maresh. (1998). Urinary indices during dehydration, exercise, and rehydration. *International Journal of Sport Nutrition*. 8:345-55.

Ball, Derek and Ronald Maughan. (1997). The effect of sodium citrate ingestion on the metabolic response to intense exercise following diet manipulation in man. *Experimental Physiology*. 82: 1041-1056.

Bartok, Cynthia, Dale Schoeller, Jude Sullivan, R. Clark, and Gregory Landry. (2004). Hydration testing in collegiate wrestlers undergoing hypertonic dehydration. *Medicine and Science in Sports and Exercise*. 36(3): 510-517.

Barr, Susan. (1999). Effects of dehydration on exercise performance. *Canadian Journal of Applied Physiology*. 24(2): 164-172.

Berger, N. J. A., I. Campbell, D. Wilkerson, and A. Jones. (2006). Influence of acute plasma volume expansion on VO2 kinetics, VO2 peak, and performance during high-intensity cycle exercise. *Journal of Applied Physiology. 101*: 707-714.

Burdon, JGW, EF Juniper, KJ Killian, FE Hargrave and EJM Campbell. (1982). The perception of breathlessness in asthma. *American Review of Respiratory Disease*. 126: 825-828.

Casa, Douglas. (2004). Proper hydration for distance running – Identifying individual fluid needs. *Track Coach*. 167: 5321.

Charkoudian, Nisha and John Johnson. (2000). Female reproductive hormones and thermoregulatory control of skin blood flow. *Exercise and Sport Science Reviews*. 28(3):108-116.

Cheung, S.S., and T. M. McLellan. (1998). Influence of heat acclimation, aerobic fitness, and hydration effects on tolerance during uncompensible heat stress. *Journal of Applied Physiology*. 84: 1731-1739.

Cheung, S.S., and Gordon G. Sleivert. (2004). Multiple triggers of hyperthermic fatigue and exhaustion. *Exercise and Sport Sciences Reviews*. 32(3): 100-106.

Cheuvront, Samuel and Michael Sawka. (2005). Hydration assessment of athletes. *Sports Science Exchange*. 18(2): 1-9

Chevront, Samuel and Emily Haymes. (2001). Thermoregulation and marathon running. *Sports Medicine*. 31(10): 743-762.

Cho, Sung-Gye, Young-Su Kim, Tae-Hyung Kim, and Dong-Ju Yoon. (1992). Responses of urine pH, blood pH, blood lactate concentration and anaerobic endurance to sodium bicarbonate, sodium citrate and glutamate administration. *Korean Journal of Sport Science*. 4: 22-34.

Coles M. G. and M. J. Luetkemeier. (2005). Sodium-facilitated hypervolemia, endurance performance, and thermoregulation. *International Journal of Sports Medicine*. 26: 182-187.

Coyle, Edward. (2005). Fluid and fuel intake during exercise. *Journal of Sport Science*. 22: 39-55.

Coyle, E., M. K. Hopper, and A. R. Coggan. (1990). Maximal oxygen uptake relative to plasma volume expansion. *International Journal of Sports Medicine. 11*(2): 116-119.

Crowe, M. J. (2001). The effects of sodium citrate on repeated maximal cycling bouts. In sports medicine Australia, 2001: a sports medicine odyssey: Challenges, controversies and change: Australian Conference of Sports Science and Medicine in Sport. Perth, A.C.T., Sports Medicine Australia, p. 63.

Deschamps, A., R. D. Levy, M. G. Cosio, E.B. Marliss, and S. Magder. (1989). Effect of saline infusion on body temperature and endurance during heavy exercise. *Journal of Applied Physiology*. 66(6): 2799-2804.

Dill, D. B. and D. L. Costill. (1974). Calculation of percent changes in volumes of blood, plasma, and red cells in dehydration. Journal of Applied Physiology. 37(2): 247-248.

England, Penelope, Scott Powers, Stephen Dodd, Thomas Callender and Ellen Brooks. (1984). The effect of acute thermal dehydration on blood lactate accumulation during incremental exercise. *Journal of Sports Sciences*. 2: 105-111.

Enoka, Roger and Douglas Stuart. (1992). Neurobiology of muscle fatigue. *Journal of Applied Physiology*. 72(5): 1631-1648.

Erickson, Katherine and Peter Wilding. (1993). Evaluation of a noval point-of-care system, the i-STAT portable clinical analyzer. *Clinical Chemistry*. 39(2): 283-287.

Faff, J., M. Ladyga, K. Burkhard-Jagodzinska, and D. Sitkowski. (1996). Effects of alkalization on the changes in circulatory, respiratory and biochemical indices induced by a short, intense exercise. *Biology of Sport*. 13(4): 247-257.

Faff, J. (1993). Can the work capacity be improved by inducing pre-exercise alkalosis? *Biology of Sport*. 10(3): 127-143.

Fernandez-Castanys, Feriche, M. Fernandez and J. Garcia. (2002). The effect of sodium citrate intake on anaerobic performance in normoxia and after sudden ascent to a moderate altitude. *Journal of Sports Medicine and Physical Fitness*. 42: 179-185.

Fortney, S. M., N. B. Vroman, W. S. Beckett, S. Permutt, and N. D. LaFrance. (1988). Effect of exercise hemoconcentration and hyperosmolality on exercise responses. *Journal of Applied Physiology*. 65(2): 519-524.

Gagge AP, JAJ Stolwijk and JD Hardy. (1967). Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environmental Health Perspectives*. 1: 1-20.

Gault, M. and Cheryl Harding. (1996). Evaluation of i-STAT portable clinical analyzer in a hemodialysis unit. *Clinical Chemistry*. 29(2): 117-124

Gisolfi, C. V., R. W. Summers, G. P. Lambert, and T. Xia. (1998). Effect of beverage osmolality on intestinal fluid absorption during exercise. *Journal of Applied Physiology*. 85(5): 1941-1948.

Gisolfi, Carl and Stanley Duchman. (1991). Guidelines for optimal replacement beverages for different athletic events. *Medicine and Science in Sports and Exercise*. 24(6): 679-687.

Greenleaf, J. E. and P. J. Brock. (1980). Na+ and Ca2+ ingestion: plasma volume-electrolyte distribution at rest and exercise. *Journal of Applied Physiology*. 48:838-847.

Greenleaf, J. E., R. Looft-Wilson, J. L. Wisherd, C. G. R. Jackson, P. R. Fung, A. C. Ertl, P. R. Barnes, C. D. Jensen and J. H. Whittam. (1998). Hypervolemia in man from fluid ingestion at rest and during exercise. *Aviation, Space, and Environmental Medicine*. 69(4): 374-86.

Greenleaf, J. E., R. Looft-Wilson, J. L. Wisherd, M. A. McKenzie, C. D. Jensen, and J. H. Whittam. (1997). Pre-exercise hypervolemia and cycle ergometer endurance in men. *Biology of Sport*. 14(2): 103-114.

Greenleaf, John. (1992). Problem: thirst, drinking behavior, and involuntary dehydration. *Medicine and Science in Sports and Exercise*. 24(6): 645-656.

Grant S. M., H. J. Green, S.M. Phillips, and J. R. Sutton. (1997). Effects of acute expansion of plasma volume on cardiovascular and thermal function during prolonged exercise. *European Journal of Applied Physiology*. 76: 356-362.

Gonzalez-Alonso, Jose, C. Teller, S. Andersen, F. Jensen, T. Hyldig and B. Nielsen. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of Applied Physiology*. 86(3): 1032-1039.

Gonzalez-Alonso, Jose, Ricardo Mora-Rodrigues, Paul Below and Edward Coyle. (1997). Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *Journal of Applied Physiology*. 82(4): 1229-1236.

Gonzalez-Alonso, Jose, C. L. Heaps and E. F. Coyle. (1992). Rehydration after exercise with common beverages and water. *International Journal of Sports Medicine*. 13(5): 399-406.

Hausswirth, C., A-X. Bigard, R. Lepers, M. Berthelot, and C. – Y. Guezennec. (1995). Sodium citrate ingestion and muscle performance in acute hypobaric hypoxia. *European Journal of Applied Physiology*. 71: 362-368.

Heaps, C. l., J. Gonzalez_Alonso, E. F. Coyle. (1994). Hypohydration causes cardiovascular drift without reducing blood volume. *International Journal of Sports Medicine*. 15(2): 74-79.

Helyar, R., H. Green, D. Zappe and J. Sutton. (1996). Comparative effects of acute plasma volume expansion and short-term training on thermal and cardiovascular responses to prolonged exercise. *Canadian Journal of Physiology and Pharmacology*. 74: 1087-1094.

Hessemer, V. and K. Bruck. (1985a). Influence of menstrual cycle on thermoregulatory, metabolic, and heart rate responses to exercise at night. *Journal of Applied Pysiology*. 59(6): 1911-1917.

Hessemer, V. and K. Bruck. (1985b). Influence of menstrual cycle on shivering, skin blood flow, and sweating responses measured at night. *Journal of Applied Physiology*. 59(6): 1902-1910.

Hopper, M., A. Coggan and E. Coyle. (1988). Exercise stroke volume relative to plasma-volume expansion. *Journal of Applied Physiology. 64*(1): 404-408.

Ibanez, J., T. Pullinen, E. Gorostiaga, A. Postigo, and A. Mero. (1995). Blood lactate and ammonia in short-term anaerobic work following induced alkalosis. *Journal of Sports Medicine and Physical Fitness.* 35: 187-193.

Jacobs, Ellis, Elizabeth Vadasdi, Laszio Sarkozi and Neville Colman. (1993). Analytical evaluation of i-STAT portable clinical analyzer and use by nonlaboratory health-care professionals. *Clinical Chemistry.* 39(6): 1069-1074.

Jain, Preeti, Peeyush Jain, Harish Tandon, and Rashmi Babbar. (2003). Effect of sodium citrate ingestion on oxygen debt and exercise endurance during supramaximal exercise. *Indian Journal of Medicine and Res____.* 118: 42-46.

Janse de Jonge, Xanne. (2003). Effects of the menstrual cycle on exercise performance. *Sports Medicine.* 33(11): 833-851.

Jentjens, R. L. P. G., C. Cale, C. Gutch and A. E. Jeukendrup. (2003). Effects of pre-exercise ingestion of differing amounts of carbohydrate on subsequent - metabolism and cycling performance. *European Journal of Applied Physiology.* 88: 444-452.

Jentjens, Roy and Asker Jeukendrup. (2002). Prevalence of hypoglycaemia following pre-exercise carbohydrate ingestion in not accompanied by higher insulin sensitivity. *International journal of Sport Nutrition and Exercise Metabolism.* 12: 398-413.

Jeukendrup, Asker; Saris, Wim H. M.; Brouns, Fred, and Kester, Arnold D. M. (1996). A new validated endurance performance test. *Medicine and Science in Sports and exercise.* 28(2): 266-270

Kargotich, Stephan, C. Goodman, D. Keast and A. Morton. (1998). The influence of exercise-induced plasma volume changes on the interpretation of biochemical parameters used for monitoring exercise, training and sport. *Sports Medicine.* 26(2): 101-117.

Kovacs, E. M. R., J. M. G. Senden, and F. Brouns. (1999). Urine color, osmolality and specific electrical conductance are not accurate measures of hydration status during postexercise rehydration. *Journal of Sports Medicine and Physical Fitness.* 39: 47-53.

Kowalchuk, John, Stephan Maltais, Keiji Yamaji, and Richard Hughson. (1989). The effect of citrate loading on exercise performance, acid-base balance and metabolism. *European Journal of Applied Physiology.* 58: 858-864.

Lambert, E.V., St. Clair Gibson, and T. D. Noakes. (2005). Complex systems model of fatigue: intergrative homoestatic control of peripheral physiological systems during exercise in humans. *British Journal of Sports Medicine.* 39: 52-62.

Lauks, Imants. (1998). Microfabricated biosensors and microanalytical systems for blood analysis. *American Chemical Society*. 31(5): 317-324.

Laursen, P. B., R. Suriano, M. J. Quod, H. Lee, C. R. Abbiss, K. Nosaka, D. T. Martin, D. Bishop. (2006). Core temperature and hydration status during an ironman triathlon. *British Journal of Sports Medicine*. 00: 1-6.

Laursen, P. B., C. M. Shing and D. G. Jenkins. (2003). Reproducibility of a laboratory-based 40-km cycle time-trial on a stationary wind-trainer in highly trained cyclists. *International Journal of Sports Medicine*. 24: 481-485.

Lindinger, Michael. (1999). Exercise in the heat: Thermoregulatory limitations to performance in humans and horses. *Canadian Journal of Applied Physiology*. 24(2): 152-163.

Linossier, M., D. Dormois, P. Bregere, and A. Geyssant. (1997). Effect of sodium citrate on performance and metabolism on human skeletal during supramaximal cycling exercise. *European Journal of Applied Physiology*. 76: 48-54.

Luetkemeier, Maurie, Michael Coles, and Eldon Askew. (1997). Dietary sodium and plasma volume levels with exercise. *Sports Medicine*. 23(5): 279-286.

Luetkemeier M. J. and E. L. Thomas. (1994). Hypervolemia and cycling time trial performance. *Medicine and Science in Sports and Exercise*. 26(4): 503-09.

Marsh, Susan and David Jenkins. (2002). Physiological responses to the menstrual cycle. *Sports Medicine*. 32(10): 601-614.

Maughan, Ron and John Leiper. (1999). Limitations to fluid replacement during exercise. *Canadian Journal of Applied Physiology*. 24(2): 173-187.

Maughan, R. J. and S. M. Shirreffs. (1999). Rehydration and recovery after exercise. *Acta Kinesiologiae Universitatis Tartuensis*. 4: 27-45.

Maughan, Ronald, Marion McArthur and Susan Shirreffs. (1996). Influence of menstrual status on fluid replacement after exercise induced dehydration in healthy young women. *British Journal of Sports Medicine*. 30: 41-47.

Murray, Robert, William Bartoli, John Stofan, Mary Horn, and Denis Eddy. (1999). A comparison of the gastric emptying characterisitics of selected sport drinks. *International Journal of Sport Nutrition*. 9: 263-274.

McNaughton, Lars, and Rod Cedaro. (1992). Sodium citrate ingestion and its effects on maximal anaerobic exercise of different durations. *European Journal of Applied Physiology*. 64: 36-41.

McNaughton, Lars. (1990). Sodium citrate and anaerobic performance: Implications of dosage. *European Journal of Applied Physiology*. 61: 392-397.

Melinda, Ray, Mark Bryan, Timothy Ruden, Shawn Baier, Rick Sharp, and Douglas King. (1998). Effect of sodium in a rehydration beverage when consumed as a fluid or meal. *Journal of Applied Physiology*. 85(4): 1329-1336.

Mock, Thomas, Douglas Morrison, and Randall Yatscoff. (1995). Evaluation of the i-STAT system: A portable chemistry analyzer for the measurement of sodium, potassium, chloride, urea, glucose, and hematocrit. *Clinical Biochemistry*. 28(2): 187-192.

Montain, Scott and Edward Coyle. (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *Journal of Applied Physiology*. 73(4): 1340-1350.

Morimoto, Taketoshi, T. Itoh, and A. Takamata. (1998). Thermoregulation and body fluid in hot environment. *Progress in Brain Research*. 115:499-508.

Moseley, Luke, Graeme Lancaster and Asker Jeukendrup. (2003). Effects of timing of pre-exercise ingestion of carbohydrate on subsequent metabolism and cycling performance. *European Journal of Applied Physiology*. 88: 453-458.

Mounier, R., V. Pialoux, I. Mischler, J. Coudert, and N. Fellmann. (2003). Effect of hypervolemia on heart rate during 4 days of prolonged exercises. *International Journal of Sports Medicine. 24:* 523-529.

Olsson, K-E. and B. Saltin. (1970). Variation in total body water with muscle glycogen changes in man. *Acta Physiologica Scandinavia*. 80: 11-18

Oopik, Vahur, Ivi Saaremets, Saima Timpmann, Luule Medijainen, and Kalle Karelson. (2004). Effects of acute ingestion of sodium citrate on metabolism and 5-km running performance: A field study. *Canadian Journal of Applied Physiology*. 29(6): 691-703.

Oopik, V., I Saaremets, L. Medijainen, K. Karelson, T. Janson, and S. Timpmann. (2003). Effects of sodium citrate ingestion before exercise on endurance performance in well trained college runners. *British Journal of Sports Medicine*. 37: 485-489.

Oopliger, Robert, Scott Magnes, LeRoy Popowski and Carl Gisolfi. (2005). Accuracy of urine specific gravity and osmolality as indicators of hydration status. *International Journal of Sport Nutrition and Exercise Metabolism*. 15: 236-251.

Oopliger, Robert and Cynthia Bartok. (2002). Hydration Testing of Athletes. *Sports Medicine*. 32(15): 959-971.

Parry-Billings, M. and D. P. M. MacLaren. (1986). The effect of sodium bicarbonate and sodium citrate ingestion on anaerobic power during intermittent exercise. *European Journal of Applied Physiological*. 55: 524-529.

Pastene, J., M. Germain, A. M. Allevard, C. Gharib, and J-R. Lacour. (1996). Water balance during and after marathon running. *European Journal of Applied Physiology*. 73: 49-55.

Popowski, LeRoy, Robert Oppliger, G. Lambert, Ralph Johnson, Alan Johnson and Carl Gisolfi. (2001). Blood and urinary measures of hydration status during progressive acute dehydration. *Medicine and Science in Sport and Exercise.* 33(5): 747-753.

Potteiger, Jeffrey, Michael Webster, Graig Nickel, Mark Haub, and Robert Palmer. (1996). The effects of buffer ingestion on metabolic factors related to distance running performance. *European Journal of Applied Physiology*. 72: 365-371.

Potteiger, J. A., G. L. Nickel, M. J. Webster, M. D. Haub, and R. J. Palmer. (1995). Sodium citrate ingestion enhances 30km cycling performance. *International Journal of Sports Medicine*. 17(1): 7-11.

Powers, Scott, John Lawler, Stephen Dodd, Richard Tulley, Greg Landry, and Keith Wheeler. (1990). Fluid replacement drinks during high intensity exercise: Effects on minimizing exercise-induced disturbances in homeostasis. *European Journal of Applied Physiology*. 60: 54-60.

Prashad, D. N. and P. A. Fletcher. (1987). Exercise-induced changes in urinary water and mineral output during the menstrual cycle. *British Journal of Sports Medicine*. 21(1): 9-12.

Ramanathan, N. C. (1964). A new weighting system for mean surface temperature of the human body. *Journal of Applied physiology*. 19: 531-533.

Rehrer, Nancy. (2001). Fluid and electrolyte balance in ultra-endurance sport. *Sports Medicine*. 31(10): 701-715.

Rehrer, Nancy. (1994). The maintenance of fluid balance during exercise. *International Journal of Sports Medicine*. 15(3): 122-25.

Requena, Bernardo, Mikel Zabala, Paulino Padial, and Belen Feriche. (2005). Sodium bicarbonate and sodium citrate: Ergogenic aids? *Journal of Strength and conditioning Research*. 19(1): 213-224.

Robergs, Robert, Keith Hutchinson, Shonn Hendee, Sean Madden, and Jason Siegler. (2005). Influence of pre-exercise acidosis and alkalosis on the kinetics of acid-base recovery following intense exercise. *International Journal of Sport Nutrition and Exercise Metabolism*. 15: 59-74.

Robergs, Robert. Blood acid-base buffering: Explaination of the effectiveness of bicarbonate and citrate ingestion. *Journal of Exercise Physiology*. 5(3): 1-5.

Roy, B. D., H. J. Green, S. M. Grant, and M. A. Tarnopolsky. (2000). Acute plasma volume expansion alters cardiovascular but not thermal function during moderate intensity prolonged exercise. *Canadian Journal of Physiology and Pharmacology. 78*: 244-250.

Ryan, A. J., G. P. Lambert, X. Shi, R. T. Chang, R. W. Summers, and C. V. Gisolfi. (1998). Effect of hypohydration on gastric emptying and intestinal absorption during exercise. *Journal of Applied Physiology*. 84(5): 1581-1588.

Sanders, B., T. D. Noakes and S. C. Dennis. (2001). Sodium replacement and fluid shifts during prolonged exercise in humans. *European Journal of Applied Physiology*. 84: 419-425.

Sawka, M., R. Hubbord, R. Francesconi, and D. Horstman. (1983). Effects of acute plasma volume expansion on altering exercise-heat performance. *European Journal of Applied Physiology. 51*: 303-312.

Schabort, Elske, G. Wilson, and Timothy Noakes. (2000). Dose-related elevations in venous pH with citrate ingestion do not alter 40-km cycling time-trial performance. *European Journal of Applied Physiology*. 83: 320-327

Selkirk, G. A., and T. M. McLellan. (2001). Influence of aerobic fitness and body fatness on tolerance to uncompensible heat stress. *Journal of Applied Physiology*. 91: 207-214.

Shave, Robert, Greg Whyte, ART Siemann, and Lance Doggart. (2001). The effects of sodium citrate ingestion on 3,000-meter time-trial performance. *Journal of Strength and Conditioning Research*. 15(2): 230-234.

Shirreffs, Susan and Ronald Maughan. (1998). Urine osmolality and conductivity as indices of hydration status in athletes in the heat. *Medicine and Science in Sports and Exercise*. 30(11): 1598-1602.

Shirreffs, S. M. (2000). Markers of Hydration status. *Journal of Sports Medicine and Physical fitness*. 40: 80-84.

Shirreffs, Susan. (2003). The optimal sports drink. *Schweizerische Zeitschrift fuer Sportmedizin und Sporttraumatologie*. 51(1): 25-29.

Shirreffs, Susan, Lawrence Armstrong, and Samuel Cheuvront. (2004). Fluid and electrolyte needs for preparation and recovery from training and competition. *Journal of Sports Science*. 22: 57-63.

Speedy, Dale, John Thompson, Ian Rodgers, Malcolm Collins, and Karen Sharwood. (2002). Oral salt supplementation during ultradistance exercise. *Clinical Journal of Sport Medicine*. 12: 279-284.

Stewart, Ian, Darren Warburton, Alastair Hodges, Donald Lyster and Donald McKenzie. (2003). Cardiovascular and splenic responses to exercise in humans. *Journal of Applied Physiology*. 94:1619-1626.

Street, Darrin, Jens-Jung Nielsen, Jens Bangsbo, and Carsten Juel. (2005). Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. *Journal of Physiology*. 566(2): 481-489

Tenaglia, Sandra, Tom McLellan and Panagiota Klentrou. (1999). Influence of menstrual cycle and oral contraceptives on tolerance to uncompensable heat stress. *European Journal of Applied Physiology*. 80:76-83.

Tiryaki, Gul and Hemming Atterbom. (1995). The effects of sodium bicarbonate and sodium citrate on 600m running time of trained females. *Journal of Sports Medicine and Physical Fitness*. 35: 194-198.

Thomas, Jerry, Jack Nelson,and Stephen Silverman. (2005). Research Methods in Physical Activity (5th ed). Human Kinetics: Champaign.

Van Montfoort, Marije, Lotte Van Dieren, William Hopkins, and Jeremy Shearman. (2004). Effects of ingestion of bicarbonate, citrate, lactate, and chloride on sprint performance. *Medicine and Science in Sports and Exercise*. 36(7): 1239-1243.

Van Someren, Ken, Kathy Fulcher, John McCarthy, Jonathan Moore, Gill Horgan, and Richard Langford. (1998). An investigation into the effects of sodium citrate ingestion on high intensity exercise performance. *Internaltional Journal of Sport Nutrition*. 8: 356-363.

Warburton, D., N. Gledhill and H. A. Quinney. (2000). Blood volume, aerobic power, and endurance performance: Potential ergogenic effect of blood volume loading. *Clinical Journal of Sport Medicine. 10*: 59-66.

Watt, M., A. Garnham, M. Febbraio, and M. Hargreaves. (2000). Effect of acute plasma volume expansion on thermoregulation and exercise performance in the heat. *Medicine and Science in Sport and Exericse. 32*(5): 958-962.

Wilmore, Jack and David Costill (Ed). (1999). Physiology of Sport and Exercise (2nd Ed). Champaign: Human Kinetics.

Table of Appendices

Appendix A – Recruitment Letter for Participants – Letter distributed to the national training centers, athletes, and coaches in order to attract volunteers to participate

Hypervolemia, thermoregulation and exercise performance under severe heat stress

You are being invited to participate in a study entitled *Hypervolemia, thermoregulation and exercise performance under severe heat stress* that is being conducted by Michael Nelson, Dr. Gordon Sleivert and Dr. Lynneth Wolski at the University of Victoria, School of Physical Education.

The purpose of this research project is to determine if the ingestion of different concentrations of sodium citrate will improve athletic performance.

Research of this type is important because the current state of knowledge in this area is limited. Many inconsistencies exist in the literature. Very little work has been conducted looking at the effects of sodium citrate ingestion on athletic performance and the physiological outcomes associated with it. Most research in this area has used either sodium bicarbonate or sodium chloride, and often investigates the potential buffering capacity of the ergogenic aid not its hypervolemic (blood plasma volume expansion) tendency. Therefore, more work is needed involving sodium citrate ingestion as it relates to hypervolemia and athletic performance.

You are being asked to participate in this study because you are a trained, healthy, male triathlete and/or cyclist between the ages of 18 and 30 years of age.

If you agree to voluntarily participate in this research, your participation will include:

A preliminary information session where the primary investigator will explain the experiment protocol to you. During this time you will be encouraged to ask questions and gain familiarity with the test protocol. You will also be asked to complete a graded, stepwise test of maximal oxygen consumption (VO2max) if you have not completed one within the past two months. If you have completed a VO_{2max} test within the past two months you are not required to complete the following: On a cycle ergometer you will be asked to cycle at a given rpm until volitional exhaustion. The intensity of this test will increase in a stepwise manner until you cannot physically continue or until the investigator indicates you have achieved VO_{2max} and does not require you to continue. Expired gases will be collected during this time using a mounted face mask.

Experiment Procedure:

1) You will next be randomly assigned a group number in a random double-blind fashion.

2) Upon arrival to the study you will be asked to provide a urine sample

   a) Urine will be immediately analyzed for specific gravity to ensure hydration

3) You will be weighed immediately after providing a urine sample to establish a baseline weight measurement.

4) You will then be asked to ingest one of three solutions in double-blind fashion, so that you or the investigators do not know which solution you are ingesting. The solution will amount to 12 ml per kg of your body mass (note: all solutions will contain 6% glucose):

- 0.2 g/kg Na-citrate
- 0.1 g/kg Na-citrate

- Gatorade

5) You will have 30 minutes to completely ingest the solution

6) You will be asked to insert a rectal thermometer into your anus so that core temperature can be measured.

7) You will be asked to rest in the laboratory for 90 minutes after completely ingesting the solution.

8) Several drops of blood will be taken from either your finger tip or ear lobe before you ingest the solution and at 90 minutes post-ingestion

9) Ceramic chips will be placed on your chest, arm, calf, and thigh in order to measure skin temperature

10) 90 minutes post-ingestion you will sit on a cycle ergometer after your nude body weight has been recorded:

a) 65 μL (several drops) of blood will be drawn prior to exercise

b) The exercise environment will be held constant at 30-35 degrees Celsius

11) 100 minutes post-ingestion you will be asked to complete a 40-km time-trial on a cycle ergometer as quickly as possible. You will have to rely on subjective pace measurement throughout each trial.

a) 65 μL of blood will be drawn every 15 minutes until exercise is complete

b) Heart rate will be recorded every five minutes using a polar heart rate monitor

c) Rectal temperature and skin temperature will be recorded every five minutes

12) Immediately after exercise you will be weighed using a digital scale

You will be asked to complete the above experiment three times in randomized fashion.

The potential benefits of your participation in this research include: (1) the further development of much needed knowledge about sodium citrate ingestion; (2) you will gain valuable knowledge regarding your rate of oxygen consumption and sweat rate (both valuable tools to aid with your training and future competitions). Both of these measures are valuable to you in regards to training and fluid consumption in high ambient temperatures. Each trial is expected to replace a training session and will likely help prepare you for future competitions of similar magnitude, in similar environments.

It is anticipated that the results of this study will serve to help our athletes in 2008 at the Beijing Olympics. Your participation in this study will assist us with this goal.

Thank you for your consideration.

If you are interested in participating, or if you have any further questions, please contact Michael Nelson at (250) 514-9110, mdn@uvic.ca; or Dr. Gordon Sleivert at gsleivert@pacificsport.com or Dr. Lynneth Wolski at lwolski@uvic.ca or (250) 721-7884.

In addition to being able to contact the researcher and his advisor at the above phone numbers, you may verify the ethical approval of this study, or raise any concerns you might have, by contacting the Associate Vice-President, Research at the University of Victoria (250-472-4545).

Appendix B – Informed Consent – This letter was given to each participant prior to starting the study to ensure they understood their rights as a volunteer

University of Victoria | British Columbia Canada

Participant Consent Form

Sodium citrate, hypervolemia and exercise performance under sever heat stress

You are being invited to participate in a study entitled *Sodium citrate, hypervolemia and exercise performance* that is being conducted by Michael Nelson, Dr. Gordon Sleivert and Dr. Lynneth Wolski.

Michael Nelson is a graduate student in the department of Physical Education at the University of Victoria and you may contact him if you have further questions by email (mdn@uvic.ca) or phone (250-514-9110).

As a graduate student, I am required to conduct research as part of the requirements for a degree in M.Sc. It is being conducted under the supervision of Dr. Gordon Sleivert and Dr. Lynneth Wolski. You may contact my supervisor at gsleivert@pacificsport.com (250-744-5536) or lwolski@uvic.ca (250-721-7884).

The purpose of this research project is to determine if the ingestion of sodium citrate has an ergogenic benefit on athletic performance.

Research of this type is important because the current state of knowledge in this area is lacking. Many inconsistencies exist with regard to sodium citrate research. Very little work has been conducted looking at the effects of sodium citrate ingestion of athletic performance and the physiological outcomes associated with it. Therefore, more work is needed to add to the growing body of knowledge currently supporting the use of sodium citrate as a potential tool for inducing plasma volume expansion. Sodium citrate and sodium bicarbonate are often used by athletes to improve buffering capacity and reduce muscle fatigue; more research is needed to monitor sodium citrates ability to expand plasma volume.

You are being asked to participate in this study because you are a trained, healthy, triathletes and/or cyclist between the ages of 18 and 30 years of age.

If you agree to voluntarily participate in this research, your participation will include:

A preliminary information session where the primary investigator will explain the experiment protocol to you. During this time you will be encouraged to ask questions and gain familiarity with the test protocol. You will also be asked to complete a graded, stepwise test of maximal oxygen consumption (VO2max) if you have not completed one within the past two months. If you have completed a VO_{2max} test within the past two months you are not required to complete the following. On a cycle ergometer you will be asked to cycle at a given rpm until volitional exhaustion. The intensity of this test will increase in a stepwise manner until you cannot physically continue or until the investigator indicates you have achieved VO_{2max} and does not require you to continue. Expired gases will be collected during this time using a mounted face mask.

Experiment Procedure:

1) You will next be randomly assigned a group number in a random double-blind fashion.

2) Upon arrival to the study you will be asked to provide a urine sample

a) Urine will be immediately analyzed for specific gravity to ensure hydration

3) You will be weighed immediately after providing a urine sample to establish a baseline weight measurement.

4) You will then be asked to ingest one of three solutions in double-blind fashion, so that you or the investigators do not know which solution you are ingesting. The solution will amount to 12 ml per kg of your body mass (note: all solutions will contain 6% glucose):

- 0.5 g/kg Na-citrate
- 0.1 g/kg Na-citrate
- Gatorade

5) You will have 30 minutes to completely ingest the solution

6) You will be asked to insert a rectal thermometer into your anus so that core temperature can be measured.

7) You will be asked to rest in the laboratory for 90 minutes after completely ingesting the solution.

8) Several drops of blood will be taken from either your finger tip or ear lobe before you ingest the solution and at 90 minutes post-ingestion

9) Ceramic chips will be placed on your chest, arm, calf, and thigh in order to measure skin temperature

10) 90 minutes post-ingestion you will sit on a cycle ergometer after your nude body weight has been recorded:

a) 65 μL (several drops) of blood will be drawn prior to exercise

b) The exercise environment will be held constant at 30-35 degrees Celsius

11) 100 minutes post-ingestion you will be asked to complete a 40-km time-trial on a cycle ergometer as quickly as possible. You will have to rely on subjective pace measurement throughout each trial.

a) 65 μL of blood will be drawn every 15 minutes until exercise is complete

b) Heart rate will be recorded every five minutes using a polar heart rate monitor

c) Rectal temperature and skin temperature will be recorded every five minutes

12) Immediately after exercise you will be weighed using a digital scale

Participation in this study may cause some inconvenience to you. The greatest inconvenience to you if you participate in this study is the time requirement necessary for this research. It is expected that a total of four test days will be required of each participant. One day used as an information/orientation session and to run a VO2max test; three more days will be devoted to the experiment. The orientation is expected to take approximately one hour total. Each test day for the experiment is expected to take 220 minutes (20 minutes to ingest, 100 minutes of rest, 60 minutes of exercise, 20 minutes miscellaneous, 30 minutes to conclude test session). It is

estimated that each collection day will last approximately 250 minutes for a total of 3 test days. Secondly, in order to standardize the treatment protocol you will be asked to refrain from strenuous exercise, social drugs, and known diuretics (soda, coffee, tea, alcohol, etc.) at least 24 hours prior to each test day.

There are some risks to you if you participate in this study. As with any physical activity, there is always a risk of muscle soreness, stiffness, strains, and other unforeseen injuries. It is, however, unlikely that you will experience these risks because you are healthy, well trained, and practice a very similar exercise protocol during your normal training cycle. It is the purpose of this study to fatigue you, therefore, you are expected to experience some acute muscle soreness and be temporarily short-of-breath; as you would expect during any strenuous physical activity. Serious risks to you will be avoided by using standard warm-up and cool-down procedures pre-and-post-exercise. Furthermore, you will be encouraged to stretch prior to exercise to reduce the chance of injury. All the investigators present in the lab during your test are required to possess a current CPR and first aid certification.

The potential benefits of your participation in this research include the further development of much needed knowledge about sodium citrate ingestion. By participating in this research project you are expected to gain valuable knowledge regarding your rate of oxygen consumption and sweat rate. Both of these measures are valuable to you in regards to training and fluid consumption in high ambient temperatures. The experiment trial is also designed to act as a training session, providing you with valuable knowledge about your physical abilities and tendencies in hot environments; that is, environmental conditions which you might expect in competitions around the world.

Your participation in this research must be completely voluntary. If you do decide to participate, you may withdraw at any time without any consequences or any explanation. Written informed consent will be obtained on the first day of data collection. You will then be asked to provide verbal consent on each of the following test days. It is important that you understand that at any time throughout the entire experiment (all four days) you have the right to withdraw your consent and leave the study without any explanation. If you do withdraw from the study your data will only be used with your permission. If it is not your will for your data to be used it will immediately be destroyed.

It is not the intent of this research project to develop and promote a commercial product or service. In the event that this research gains the interest of a commercial manufacturer, the investigator may decide to present the findings and enter into discussion with this company. You personal information and results would be kept completely confidential, as they will always be kept confidential.

In terms of protecting your anonymity your results will be kept confidential and be displayed in future reports as group means. No identifiers will be used which would lead others to believe you participated in this research.

Your confidentiality and the confidentiality of the data will be protected through the use of group means and generalizations. No future report will contain any identifier or marker which would distinguish you from the other volunteers. You cannot be granted complete confidentiality, as other subjects involved in the study will likely come in contact with you or witness your participation. Your results will not be shared with other participants or any other interested party.

It is anticipated that the results of this study will be shared with others in the following ways: (1) This is a thesis project, thus the results will be published accordingly, (2) The primary investigator may wish to publish his findings in a peer reviewed journal as part of an article collection, (3) The results may also be presented at scholarly meetings (i.e.: conferences).

Data from this study will be disposed of two years after the completion of the study. All electronic data will be erased from a password protected computer and all paper copies will be shredded and recycled.

In addition to being able to contact the researcher and his advisor at the above phone numbers, you may verify the ethical approval of this study, or raise any concerns you might have, by contacting the Associate Vice-President, Research at the University of Victoria (250-472-4545).

Your signature below indicates that you understand the above conditions of participation in this study and that you have had the opportunity to have your questions answered by the researchers.

Name of Participant	*Signature*	*Date*

A copy of this consent will be left with you, and a copy will be taken by the researcher.

Appendix C – Physical Activity Readiness Questionnaire – Used to determine if an individual is able to participate in strenuous exercise.

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

→ **DELAY BECOMING MUCH MORE ACTIVE:**

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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Appendix D – Rating of Perceived Exertion Scale – a subjective psychophysical tool used to gain a measure of the subjects perceived exertion

Scale	Severity
1	Nothing At All
0.5	Very Very Slight
1	Very Slight
2	Slight
3	Moderate
4	Somewhat Severe
5	Severe
6	
7	Very Severe
8	
9	Very Very Severe
10	Maximum

Modified Borge Scale, From Burdon JGW, Juniper EF, Killian KJ, Hargrave FE, and Campbell EJM. (1982). The Perception of breathlessness in asthma. *American Review of Respiration Disease*. 126: 825-828.

Appendix E – Thermal Sensation Scale – a subjective psychophysical tool used to gain a measure of the subjects perceived thermal sensation

Scale of Thermal Sensation

1	Cold
2	Cool
3	Slightly Cool
4	Neutral
5	Slightly Warm
6	Warm
7	Hot

from, Gagge et al. 1967, 1969

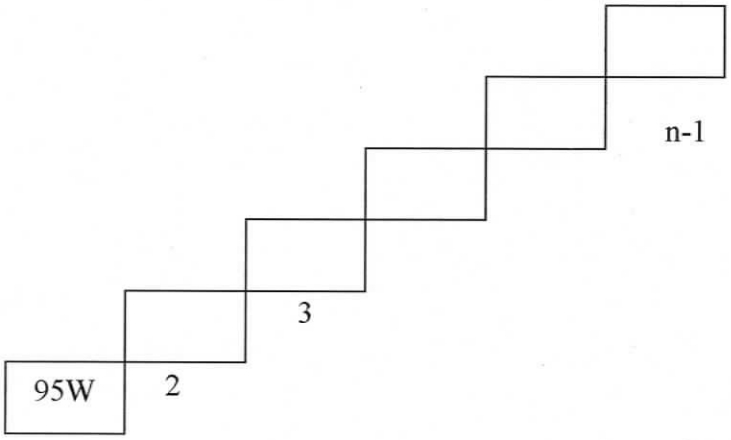
Appendix F – Thermal Comfort Scale - a subjective psychophysical tool used to gain a measure of the subjects perceived thermal comfort

Scale of Comfort Sensation

1	Comfortable
2	Slightly Uncomfortable
3	Uncomfortable
4	Very Uncomfortable

Gagge et al. 1967, 1969

Appendix G – VO_{2max}/W_{max} Protocol – Graded exercise test protocol used to gain a measure of the subject's maximal oxygen consumption and maximum wattage



Let each step represent one stage. Let n = stage number. Because Wattage starts at 95W, and n = stage number; 95 + [(n-1) x 35] = total watts at a given stage.

Appendix I – Time Trial Protocol – A description of the 1-hr TT protocol used during the present study

Endurance Performance Test

Jeukendrup et al. (1996) validated a performance test using a standard Lode ergometer (Lode Excalibur Sport, Lode, Groningen, The Netherlands). It is often difficult to assess athletic performance in a laboratory when work (watts/rpm) is kept constant. The Lode bike is an electromagnetically braked ergometer with the capability of operating in a pedaling rate dependent mode (linear mode). When in the linear mode, an increasing pedelling rate increases the work rate according to the following formula:

$$W = L * (RPM)^2$$

RPM is the pedelling rate and L is a constant.

After a short warm-up (100 W, 5 minutes) the subjects were asked to complete a predetermined amount of work (J) which would be equal to approximately 60 minutes of exercise. The subjects were instructed to achieve this as fast as possible. TT performance is therefore dependent on the target amount of work.

Total amount of work (J) was based on the maximal workload (W_{max}) achieved during the preliminary graded exercise test (Appendix H). The formula used to calculate total work is described below:

$$\text{Target amount of work (J)} = 0.75 * W_{max} * 3600 \text{ (s)}$$

According to this formula, if the subject cycled at an intensity equal to 75% Wmax they would achieve the target work (J) in exactly 60 minutes. Any deviation from this intensity (higher/lower) would result in a respective decrease or increase in completion time.

1-hr time trial, From Jeukendrup, Asker; Saris, Wim H. M.; Brouns, Fred; Kester, Arnold D. M. (1996). A validated endurance performance test. *Medicine and Science in Sports and Exercise*. 28(2): 266-270.

Appendix J – Dill and Costill Equation for determination of Plasma Volume

$$BV_a = BV_b\ (Hb_b/Hb_a)$$

$$CV_a = BV_a\ (Hct_a)$$
$$PV_a = BV_a - CV_a$$

$$BV\% = 100\ (BV_a - BV_b)/\ BV_b$$
$$CV\% = 100\ (CV_a - CV_b)/\ CV_b$$
$$PV\% = 100\ (PV_a - PV_b)/\ PV_b$$

where,

BV = blood volume
CV = red cell volume
PV = plasma volume

and

b = before treatment
a = after treatment

From: Dill, D. B. and Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration. *Journal of Applied Physiology*. 37(2): 247-248.