EFFECT OF FLUID TEMPERATURE AND VOLUME ON THERMOREGULATION IN THE HEAT

Michelle M. Johannsen
University of Montana, Missoula
EFFECT OF FLUID TEMPERATURE AND VOLUME ON THERMOREGULATION
AND PERFORMANCE IN THE HEAT

By

MICHELLE MARIE JOHANNSEN

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Approved by:

Scott Whittenburg, Dean of The Graduate School
Graduate School

Brent Ruby, Ph.D., FACSM, Chair
Health and Human Performance

Charles Dumke, Ph.D., FACSM,
Health and Human Performance

Stephen Lodmell, Ph.D.
Division of Biological Sciences
Effect of Fluid Temperature and Volume on Thermoregulation and Exercise Performance in the Heat

Chairperson: Brent Ruby, Ph.D., FACSM

The link between thermoregulation, hydration status, and exercise performance in hot humid environments is controversial. The purpose of this study was to evaluate the effects of volume and temperature of ingested fluid on hydration status, thermoregulation and exercise performance. Recreationally active males (N=11, 25±1.8 years; VO$_2$max=58.3±1.8 mL/kg/min) completed two 3-hour intermittent exercise trials in the heat (WBGT=35.5 °C with 50% humidity). Participants consumed either 1 mL/kg body weight (BW) of room temperature water (35.5°C; ROOM) or 0.5 mL/kg of an ice slurry mixture (~0°C; COLD) every ten minutes throughout the trials in a randomized crossover design. Subjects walked on a motorized treadmill at 40% VO$_2$max for 25-minutes followed by 5-minutes of standing rest after which a 1.6 km time trial was completed as quickly as possible on a non-motorized treadmill (Woodway Curve). After completion of the time trial, participants remained seated for the rest of the 1-hour time period. This series of steady state (SS) and time trial (TT) segments was repeated three times over each 3-hour trial. Core temperature and heart rate were monitored continuously throughout the 3-hour trials and used to calculate physiological strain index (PSI). Nude BW was measured pre and post to calculate sweat loss. Body weight loss was significantly higher for the COLD trial (2.2±0.7 and 3.0±0.8 % for the ROOM and COLD respectively, p<0.05). In contrast, sweat loss was not different between the trials (1.2±0.2 and 1.2±0.2 L/hr, for the ROOM and COLD, respectively). Time trial performance was not different between treatments (ROOM: 9.7±1.3, 10.8±1.4, 12.8±2.4 min for hours 1, 2, and 3, respectively, and COLD: 10.1±1.6, 11.2±2.0, 12.8±2.6 min for hours 1, 2, and 3, respectively) but was impaired over time. These data suggest that it is not simply the volume, but the temperature of the ingested fluid that aids in thermoregulation.
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Chapter One: Introduction

Introduction
It has been well documented that pre-cooling interventions have been shown to attenuate decrements in performance in hot, humid environments. These interventions have also demonstrated usefulness in preventing hyperthermia and heat related illnesses during prolonged bouts of exercise. As such, cooling methods must be practical for use in the field. Ice-slurries are easily transportable and have been shown to be an effective mid-cooling treatment for preventing hyperthermia and heat related illness. Ice-slurries have also been shown to be an effective pre-cooling technique for attenuating decrements in performance while exercising in the heat.

Problem
Pre-cooling and mid-cooling methods have been shown to increase athletic performance in the heat, whereas research studying the effects of mid-cooling is scarce. Half-volume ice-slurry treatments have been shown to be not significantly different from full-volume ambient temperature water as a mid-cooling technique in reducing thermal stress. It has not been examined whether or not reduced fluid temperatures in a restricted volume is similarly effective at attenuating performance decrements when compared to ambient water treatment of a higher volume.

Null Hypotheses
There will be no difference in time trial performance between the two treatments. There will be no difference in physiological metrics including core temperature, skin temperature, heart rate, physiological strain index (PSI) score, sweat rate, hematocrit, electrolyte concentration, urine specific gravity, and rate of perceived exertion (RPE) between the two trials.

Significance of Study
The purpose of this study is to determine the effectiveness of the half-volume of ice-slurry when given as a mid-cooling intervention on preventing decrements in performance while exercising in a hot, humid environment in comparison to a full-volume of ambient temperature water.

Rationale of Study
This study will provide a greater understanding of the utility of ice-slurry mid-cooling treatments during prolonged bouts of exercise in the heat. This is especially useful for individuals with limited means of accessing cooling interventions in the field such as military and wildland fire personnel. By maintaining performance, workers will be able to complete job specific duties in which failure to do so could result in the risk of injury. Smaller fluid volumes could lead to potentially lighter
pack weights, and thereby decrease the workload and total energy expenditure and therefore heat production of an individual.

Limitations
- Subjects will be asked to maintain a diet and activity log 48 hours prior to the first trial. The subjects should attempt to replicate these conditions in an effort to reproduce the condition in which the subject arrives at the lab between the two trials.
- Subjects will be asked to abstain from heavy lifting and intense exercise at least four days prior to arriving at the lab to avoid influences on performance.
- To limit human error, all research personnel will be similarly trained with all laboratory procedures.
- Subjects must arrive at the lab at similar times of day for both trials to prevent influences on performance.
- Motivation on the part of the participants to complete maximal effort time trials each time may be limited.
- To avoid potential influences on motivation subject testing will be staggered so that the self-selected workload portions of testing do not occur at the same time while two subjects are in the chamber.

Delimitations
- Due to variations in body temperatures throughout the menstrual cycle, female subjects will be excluded from the study.

Definition of Terms
- Ice Slurry – a drink mixture in which 1/3 of the total volume is crushed/shaved ice and the remaining 2/3 is composed of chilled/refrigerated water
- Mid-cooling – a cooling intervention provided during exercise/physical activity
- Physiological Strain Index (PSI) – a measure of heat stress on the body
- Pre-cooling – a cooling intervention provided prior to exercise/physical activity (see methods for equation)
- Sweat Rate – loss of sweat over a period of time (see methods for equation)
- VO₂Max – maximal oxygen consumption capable for an individual; also known as maximal aerobic capacity

Chapter Two: Review of Literature

Thermoregulation and Performance in the Heat
The human body exists in a delicate balance that allows it to perform at an optimal level. If core temperature varies by more than a few degrees catastrophic results, even death, may occur. (Nolte, Hew-Butler, Noakes, & Duvenage, 2015; Kolka M. A., Latzka, Montain, & Sawka, 2003) Despite this narrow margin of optimal existence, humans are resilient when exposed to extreme environmental and physiological stressors. (Jovanovic, Karkalic, Zeba, Pavlovic, & Radakovic, 2014; Trinity, Pahnke, Lee, & Coyle, 2010; Veghte & Webb, 1961) Humans have been able to withstand extremes in temperature of upwards of 120 degrees (F), all while running over one hundred miles. Our nearly hairless bodies and ability to sweat at extremes of up
to four liters per hour, suggest humans are uniquely built to dissipate large amounts of heat. (Veghte & Webb, 1961)

Humans experience heat loading from two major sources, the environment (exogenous heat), and heat due to metabolic process (endogenous heat). (Booth, Marino, & Ward, 1997; Cochrane, Cuddy, Hailes, & Ruby; Veghte & Webb, 1961) Humans are approximately 20% efficient at utilizing fuel sources for energy. This means that for all of the energy produced through the breakdown of foods, only about 20% is converted to locomotion or other energy demanding processes, where the other 80% is lost to heat. This heat must be unloaded in some way to prevent us from leaving our zone of optimal temperature. (Booth, Marino, & Ward, 1997; Cuddy, Hailes, & Ruby; 2014; Gonzalez-Alonso, Teller, Andersen, Jensen, Hylidig, & Nielsen, 1999)

Heat can be unloaded by two major methods in humans, convection-conduction and evaporative cooling (sweating). (Booth, Marino, & Ward, 1997; Cochrane, Cuddy, Hailes, & Ruby; Veghte & Webb, 1961) Convection-conduction cooling works by releasing heat into the environment by following a gradient from the higher temperatures of the body, to the cooler surroundings. (Cochrane, Cuddy, Hailes, & Ruby; Veghte & Webb, 1961) When the ambient temperature rises above skin temperature (~32°C) convection-conduction methods of cooling are impeded and the body becomes almost completely reliant on sweating to cool the body. (Cochrane, Cuddy, Hailes, & Ruby; 2014) If the body is not able to unload this heat, a condition known as uncompensable heat, the individual must then make changes to their activity or their environment. (Booth, Marino, & Ward, 1997; Cochrane, Cuddy, Hailes, & Ruby; Cuddy, Hailes, & Ruby, 2014; Glitz, et al., 2015; Gonzalez-Alonso, Teller, Andersen, Jensen, Hylidig, & Nielsen, 1999)

Changes in activity to reduce metabolic heat means the individual must lower the intensity and therefore energy demands of their activity, or cease the activity all together. (Gonzalez-Alonso, Teller, Andersen, Jensen, Hylidig, & Nielsen, 1999) Environmental changes may include removing the individual from the environment, removing clothing that is impeding evaporative heat loss, or implementing a cooling intervention. (Booth, Marino, & Ward, 1997; Cochrane, Cuddy, Hailes, & Ruby; Glitz, et al., 2015)

Acclimatization training has been shown to benefit those who are expected to perform in hot environments. (Jovanovic, Karkalic, Zeba, Pavlovic, & Radakovic, 2014; Lui, Cuddy, Hailes, & Ruby, 2014) Despite these benefits, it has been shown that adaptations gained through acclimatization are not enough to overcome impedances to evaporative cooling when protective encapsulating clothing is worn. (Glitz, et al., 2015; Jovanovic, Karkalic, Zeba, Pavlovic, & Radakovic, 2014; Kofler, et al., 2014; Yamazaki, 2013)

Military personnel are frequently required to work in hot humid environments where removal to a cooler location is often not available. (Cochrane, Cuddy, Hailes, & Ruby; Jovanovic, Karkalic, Zeba, Pavlovic, & Radakovic, 2014; Welles, Buller, Margolis, Economos, Hoyt, & Richter, 2013) These individuals are also required to wear uniforms intended to provide protection specific to their duties, as such, removal of clothing is also typically not an option when attempting to unload heat. (Cochrane, Cuddy, Hailes, & Ruby; Glitz, et al., 2015; Kofler, et al., 2014; Welles, Buller, Margolis, Economos, Hoyt, & Richter, 2013) This leaves the third method of implementing a cooling intervention as the most likely method to alleviate heat stress on the body for these individuals. (Cochrane, Cuddy, Hailes, & Ruby; Jovanovic, Karkalic, Zeba, Pavlovic, & Radakovic, 2014; Welles, Buller, Margolis, Economos, Hoyt, & Richter, 2013)
Exercising in hot and humid environments has been shown to decrease performance across various modalities and event durations. (Booth, Marino, & Ward, 1997; Gonzalez-Alonso, Teller, Andersen, Jensen, Hylød, & Nielsen, 1999; Jovanovic, Karkalic, Zeba, Pavlovic, & Radakovic, 2014) In situations such as races and other competitions participants may slow their pace in order to reduce metabolic heat production. (Peiffer & Abbiss, 2011) Reductions in performance during a military operation could lead to an inability to perform necessary tasks, which may ultimately result in injury, possibly even death. (Welles, Buller, Margolis, Economos, Hoyt, & Richter, 2013) It thus becomes apparent that decrements in performance must be prevented and that cooling interventions are vital to the health and safety of military personnel. (Glitz, et al., 2015)

**Cooling Methods**

Various cooling methods have been used to prevent hyperthermia and heat related illnesses, such as ice vests, whole body immersion, ice sheets, cold water and ice slurry ingestion. (Booth, Marino, & Ward, 1997; Cochrane, Cuddy, Hailes, & Ruby; Kenny, et al., 2011; Riera, Trong, Sinnapah, & Hue, 2014) Many methods when used as a pre-cooling intervention have also been shown to attenuate decrements in performance in the heat. (Booth, Marino, & Ward, 1997; Kenny, et al., 2011; Riera, Trong, Sinnapah, & Hue, 2014; Stevens, Dascombe, Boyko, Sculley, & Callister, 2013)

Cooling methods such as whole body immersion, though effective at reducing thermal metrics and increasing exercise performance in the heat, are not logistically realistic in situations in which the cooling method must be easily transportable. (Booth, Marino, & Ward, 1997; Kenny, et al., 2011) When offered as a mid-cooling intervention, cold-water treatments have been shown to be similarly effective in reducing hyperthermic symptoms in hot humid environments compared to ambient temperature water that is double the volume. (Cochrane, Cuddy, Hailes, & Ruby) Cold-water beverages are easily transported and have been shown to be similarly effective in attenuating performance decrements in the heat compared to alternative cooling methods, such as whole-body immersion, when given as a pre-cooling intervention. (Cochrane, Cuddy, Hailes, & Ruby; Siegel, Mate, Brearley, Watson, Nosaka, & Laursen, 2010; Stevens, Dascombe, Boyko, Sculley, & Callister, 2013) Ingestion of cold fluids has been shown to extend time to exhaustion in hot humid environments by up to 23% compared to an equal volume of warm fluids (Marino & Noakes, 2009; Stevens, Dascombe, Boyko, Sculley, & Callister, 2013)

**Hydration Recommendations**

Current fluid replacement recommendations for the military are based on studies advocating replacing fluids equal to mass lost during exercise. (Montain, Latzka, & Sawka, 1999; McLellan, 2004; Kolka M., Latzka, Montain, Corr, O’Brien, & Sawka, 2003) Revised in 2003, the current military guidelines suggest personnel consume ¾ of a quart (710 mL) of fluids per hour under Category 4 Red Flag Heat Conditions (WBGT Index 31.1-32.2°C). The recommendations also limit individuals to ingest no more than 1.5 quarts (1.4L) per hour, and no more than 12 quarts (11.4 L) per day. (Kolka M., Latzka, Montain, Corr, O’Brien, & Sawka, 2003; Kolka M. A., Latzka, Montain, & Sawka, 2003; McLellan, 2004) While these values appear aggressive in contrast to calculated 24 hour fluid intake during occupational (Ruby, Schoeller, Sharkey, Burks, & Tysk, Water turnover and changes in body composition during arduous wildfire suppression, 2004; Ruby, Schoeller, Sharkey, Burks, & Tysk, Water turnover and changes in body composition during arduous wildfire suppression, 2003; Ruby, Shriver, Zderic, Sharkey, Burks, & Tysk, 2002; Cuddy, Sol, Hailes, & Ruby, 2015) and military training operations (Ruby, Shriver, Zderic, Sharkey, Burks, & Tysk, 2002;
Ruby, Schoeller, Sharkey, Burks, & Tysk, Water turnover and changes in body composition during arduous wildfire suppression, 2003; Cuddy, Sol, Hailes, & Ruby, 2015), these revised guidelines have successfully prevented the incidence of hyponatremia and water intoxication that had become troublesome under the previous recommendations. (Kolka M., Latzka, Montain, Corr, O’Brien, & Sawka, 2003; Kolka M. A., Latzka, Montain, & Sawka, 2003) The new recommendations also provide suggested work:rest schedules and intensities according to WBGT environmental conditions in an attempt to reduce overall physiological strain and the risk for heat related injury. (Kolka M., Latzka, Montain, Corr, O’Brien, & Sawka, 2003; Kolka M. A., Latzka, Montain, & Sawka, 2003) However, the present guidelines do not consider the possible implications regarding fluid temperatures.

While it has been demonstrated that cold water ingestion improves an individual’s capacity to maintain performance in hot humid environments, and that even slight incidences of hypohydration may result in significant decrements in performance, the combined effects of these factors has not been adequately addressed. (Siegel, Mate, Brearley, Watson, Nosaka, & Laursen, 2010; Riera, Trong, Sinnapah, & Hue, 2014; Hailes, Cuddy, Cochrane, & Ruby, 2016) It is not well understood if body heat can be adequately regulated with the aid of cold-water ingestion in order to maintain exercise performance in the heat despite reductions in fluid replacement volumes.

**Chapter Three: Methodology**

**Subjects**

Twelve recreationally active males will be recruited from the University of Montana and surrounding community, ranging from 18 to 40 years of age. Subjects must pass a preliminary testing process, including a Physical Activity Readiness-Questionnaire (PAR-Q), obtain a VO$_2$Max of at least 40 ml·kg$^{-1}$·min$^{-1}$, and sign an informed consent form approved by the Institutional Review Board of the University of Montana. Subjects will be given a detailed explanation of the experimental procedures and expectations of subjects participating in this study, as well as any risks that they may incur as a result of participating in the study.

**Data Collection**

**Preliminary Testing**

**Physical Activity Readiness- Questionnaire (PAR-Q)**

A PAR-Q will be used to identify whether or not subjects will be physically capable of performing the exercise tasks that will be asked of the subjects in the study. Subjects that do not meet the PAR-Q standards will be excluded from the study.

**Maximal Aerobic Capacity (VO$_2$Max)**

Maximal aerobic capacity (VO$_2$Max) will be determined for each subject using the Bruce protocol (Bruce, Kusumi, & Hosmer, 1973) Subjects will be at least 3 hours fasted prior to arriving at the lab for testing. Testing will be done on a motorized treadmill (Fullvision, Inc., Newton, KS). Expired gases will be captured and analyzed using a metabolic cart every 15 seconds (Parvomedics, Inc., Sandy, UT). Heart rate will be recorded using a heart rate strap and watch (Polar Electro, Kemple, FL). Stages of the Bruce protocol are as follows:

1. 1.7 mph – 10% grade
2. 2.5 mph – 12% grade
3. 3.4 mph – 14% grade
4. 4.2 mph – 16% grade
5. 5 mph – 18% grade
6. 5.5 mph – 20% grade
7. 6.0 mph – 22% grade

Each phase of the protocol will last three minutes. Upon successfully completing a stage of the protocol the subject will be moved on to the subsequent stage. A subject must meet one of the following criteria to qualify as meeting their VO$_2$Max:

1. There is a plateau in oxygen consumption despite an increase in workload.
2. The subject’s RER is greater than 1.10.
3. The subject’s HR is within 10 beats of the participant’s predicted max HR
4. The subject experiences volitional fatigue and reports an RPE of greater than 17.

Body Composition

Body composition will be determined for each subject through the use of a hydrodensiometry and estimates of residual lung volume based on height and weight. Subjects must be at least three hours fasted prior to testing. Height will be measured and dry weights will be collected prior to entering the water tank using a scale (Befour Inc, Cedarburg, WI). Subjects will be instructed to expel as much air as possible while fully submerged under water and seated on the weighing platform. Net underwater weights will be recorded using load cells (Exertech, Dresbach, MN). Subjects will be weighed multiple times until a measure within 0.1kg is obtained to ensure a reliable measure is recorded. The Siri equation will be used to calculate body density as well as percent body fat. (Siri, 1993)

Siri equation:

\[
\% \text{ Body Fat} = \frac{495}{\text{Body Density}} - 450
\]

Experimental Testing

Exercise Protocol

Subjects will be asked to maintain a food and activity log 48 hours prior to arriving at the lab. Subjects will arrive at the lab at least 8 hours fasted prior to testing. Subjects are permitted to drink only water during the 8 hour fast. Subjects will be required to visit the lab twice for testing. Testing interventions will be assigned in a randomized cross-over manner. Visits to the lab will be separated by a period of at least two weeks to prevent heat acclimatization effects that may influence performance results. Subjects will be asked to replicate diet and activity as best as possible to that which they reported in their food and activity log submitted prior to the first trial. (Cochrane, Cuddy, Hailes, & Ruby)

Subjects will be dressed in standard issue Air Force pants and shirt. Subjects will be given a Mon-a-therm general purpose temperature probe (Mon-a-therm, Mallinckrodt, Inc, St. Louis, MO) and instructed on how to properly insert the device rectally. Subjects will also be outfitted with skin temperature sensors, placed on the left pectoralis muscle approximately 5 cm above the nipple and the scapula at a similar location (Mini Mitter, A Respironics Company, Bend, OR), and heart rate
monitor strap prior to entering the climate chamber. \textsuperscript{(Cochrane, Cuddy, Hailes, & Ruby)} Temperatures will be recorded using DASYLab Software (Measurement Computing CO., Norton, MA). Heart rate will be monitored using a Polar Heart Rate watch (Polar Electro, Kempele, FL), values will be recorded to a computer.

Upon entering the climate chamber, set to approximately 31 degrees wet bulb globe temperature (WBGT) (35.5\degree C and 50\% relative humidity), subjects will walk on a treadmill ergometer at a speed that is equivalent to 40\% of their VO\textsubscript{2}Max for 25 minutes followed by 5 minutes of rest. \textsuperscript{(Cochrane, Cuddy, Hailes, & Ruby)} The subjects will then run a 1-mile time trial on a non-motorized treadmill (Woodway Curve, Waukesha, WI). Subjects will be blinded to the intensity at which they are working during the time trial portion of testing. Subjects will be instructed to complete the mile as quickly as possible during the time trial. The time required to complete the one-mile time trial will be recorded. After completing the time trial, subjects will be seated for the remainder of the hour. This intermittent exercise protocol will be repeated three times for a total of three hours in the climate chamber.

Temperatures of the chamber will be monitored using a Kestrel 4400 Heat Stress Tracker (Weather Republic LLC., Downingtown, PA) and recorded to a computer.

**Drink and Food Administration**

Subjects will be given either 1ml $\cdot$ kg of body weight\textsuperscript{-1} of ambient temperature water (~27\degree C) or 0.5mL $\cdot$ kg of body weight\textsuperscript{-1} of ice slurry (~0\degree C) every ten minutes throughout the trial. Subjects will be randomly assigned to one treatment for the first trial and will receive the alternative for the second trial.

Subjects will be given a commercially available sports bar comprised of 250 total kcals and approximately 45\% of carbohydrate. Half will be given at 55 minutes and the other half at 115 minutes within the chamber during the resting phases following the first and second time trials.

**Physiological Strain Index**

Physiological strain index (PSI) scores will be calculated as a safety precaution to determine if subjects are at risk of incurring a heat related illness while participating in the study and to determine differences in response to the two fluid treatments. PSI will be calculated using measures of core temperature (T\textsubscript{c}) and heart rate (HR) to determine whether an individual is experiencing heat stress. \textsuperscript{(Buller, Latzka, Tharion, & Moran, 2008)}

$$\text{PSI} = 5 \left( T\textsubscript{c(t)} - T\textsubscript{c(0)} \right) \cdot \left( 39.5 - T\textsubscript{c(0)} \right) \cdot 5 \left( \text{HR}\textsubscript{t} - \text{HR}\textsubscript{0} \right) \cdot \left( 180 - \text{HR}\textsubscript{0} \right)$$

**Fluid Loss Rate**

Sweat loss during each trial will be calculated utilizing pre and post exercise nude body weight values, urine output, fluid intake, as well as respiratory loss.

$$\text{Fluid Loss (L)} = (\text{BW}\textsubscript{pre}(kg) + \text{Ingested Liquid (kg)}) - (\text{BW}\textsubscript{post}(kg) + \text{Urine Weight (kg)})$$
**Blood Sampling**

Blood samples will be obtained before and after each trial in the heat chamber. Approximately 5mL of venous blood samples will be obtained using a needle and vacutainer containing heparin to prevent clotting.

Blood samples will be analyzed for hematocrit as well as electrolyte concentrations using an iSTAT1 Analyzer (Abbott Point of Care Inc., East Windsor, NJ).

Plasma volume shift will be calculated based on pre and post blood volume measurements:

\[
PV = BV - CV \\
CV = BV \times Hct \\
\Delta PV\% = 100 \left( \frac{PV_A - PV_B}{PV_B} \right)
\]

where PV = plasma volume (mL), BV = blood volume (mL), CV = cell volume, Hct = hematocrit (%), and subscripts A = after and B = before exercise. (Dill & Costill, 1974)

**Urine Specific Gravity**

Urine samples will be collected from each subject before and after each trial. Specific gravity will be measured using a pocket refractometer (ATAGO USA Inc., Bellevue, WA) for each sample to determine relative hydration status of the individual.

**Statistical Procedures**

A 2x3 repeated measures analysis of variance (ANOVA) will be used to analyze steady state and ending time trial core temperature, skin temperature, heart rate, RPE, and PSI. A 2x3 repeated measures ANOVA will be used to analyze the one-mile time trial completion times. A 2x2 repeated measures ANOVA will be used to determine if there is a significant difference in pre and post exercise measurements for sweat rate, electrolyte concentration, and urine specific gravity between the two trials. A paired sample t-test will be used to determine if there is a significant difference in plasma volume shift between the two trials. Data will be considered significant with a 95% confidence interval (p<0.05). Analyses will be performed using StatPlus and Microsoft Excel software.
Chapter 4: Manuscript

EFFECT OF FLUID TEMPERATURE AND VOLUME ON THERMOREGULATION AND EXERCISE PERFORMANCE IN THE HEAT
Michelle M. Johannsen, John S. Cuddy, Walter S. Hailes, Brent C. Ruby
Montana Center for Work Physiology and Exercise Metabolism, The University of Montana, 32 Campus Drive, Missoula, MT 59812-1825, United States

michelle.johannsen@umontana.edu, john.cuddy@umontana.edu, walter.hailes@umontana.edu, brent.ruby@umontana.edu

Corresponding Author Contact Information:
Brent C. Ruby, PhD
Director, Montana Center for Work Physiology Exercise Metabolism
Department of Health and Human Performance
The University of Montana
McGill Hall – HHP
Missoula, MT 59812-1825
Tel: (406) 243-2117
Fax: (406) 243-6252
E-mail: brent.ruby@umontana.edu
Abstract
The link between thermoregulation, hydration status, and exercise performance in hot humid environments is controversial. The purpose of this study was to evaluate the effects of volume and temperature of ingested fluid on hydration status, thermoregulation and exercise performance. Recreationally active males (N=11, 24.7±5.9 years; VO$_2$max=58.2±6.0 mL/kg/min) completed two 3-hour intermittent exercise trials in the heat (31° WBGT=35.5°C with 50% humidity). Participants consumed either 1 mL/kg body weight (BW) of room temperature water (35.5°C; ROOM) or 0.5 mL/kg of an ice slurry mixture (~0°C; COLD) every ten minutes throughout the trials in a randomized crossover design. Subjects walked on a motorized treadmill at 40% VO$_2$max for 25-minutes followed by 5-minutes of standing rest after which a 1.6 km time trial was completed as quickly as possible on a non-motorized treadmill (Woodway Curve). After completion of the time trial, participants remained seated for the rest of the 1-hour time period. This series of steady state (SS) and time trial (TT) segments was repeated three times over each 3-hour trial. Core temperature and heart rate were monitored continuously throughout the 3-hour trials and used to calculate physiological strain index (PSI). Nude BW was measured pre and post to calculate sweat loss. Body weight loss was significantly higher for the COLD trial (2.2±0.7 and 3.0±0.8 % for the ROOM and COLD respectively, p<0.05). Total water loss was not different between the trials (1.2±0.2 and 1.2±0.2 L/hr, for the ROOM and COLD, respectively). Time trial performance was not different between treatments (ROOM: 9.7±1.3, 10.8±1.4, 12.8±2.4 min for hours 1, 2, and 3, respectively, and COLD: 10.1±1.6, 11.2±2.0, 12.8±2.6 min for hours 1, 2, and 3, respectively) but was impaired over time. These data suggest that it is not simply the volume, but the temperature of the ingested fluid that aids in thermoregulation.

Keywords
Thermoregulation; Hydration; Performance; Heat stress; Physiological strain index; Core Temperature; Sweat Rate

Highlights
- No difference in subject performance in the heat between treatments.
- Difference in percent dehydration between treatments
- No difference in sweat rate between treatments.
- No difference in physiological strain or rate of perceived exertion between treatments.

**Abbreviations:** BW, body weight; V̇O$_2$, volume of oxygen; SS, steady state; TT, time trial; RP, recovery period; PSI, physiological strain index; SR, sweat rate; USG, urine specific gravity; HR, heart rate; Tc, core temperature; Ts, skin temperature; ANOVA, analysis of variance; RPE, rating of perceived exertion; COLD, ice-slurry water treatment; ROOM, room temperature water treatment; SD, standard deviation.
Introduction

Military personnel are frequently subject to working in hot, humid environments for extended periods of time. Prior research has suggested that to maintain thermoregulation and safety in these environments, work rate, work:rest ratio, and adequate fluid intake must be carefully considered. (Montain, Latzka, & Sawka, 1999; McLellan, 2004) Human thermoregulation in hot environments is dependent on adequate evaporative cooling through sweating and the redirection of blood flow to the skin. (Smith & Johnson, 2016) Previous studies have suggested that even minimal changes in body mass loss (1%) can reduce the effectiveness of evaporative cooling and an individual’s ability to maintain exercise performance in the heat. (Bardis, Kavouras, Kosti, Markousi, & Sidossis, 2013; Bardis, Kavouras, Arnaoutis, Panagiotakos, & Sidossis, 2013) However, other data demonstrate minimal differences in exercise performance and common metrics of thermoregulation when body mass loss approaches 2%. (Cheung, et al., 2015; Yamashita, Nakano, & Matsumoto, 2015) Controversies persist in the literature regarding fluid replacement needs so as to minimize body mass loss (Bardis, Kavouras, Arnaoutis, Panagiotakos, & Sidossis, 2013; Bardis, Kavouras, Kosti, Markousi, & Sidossis, 2013), maintain body mass to within no more than 2% loss, or to drink to thirst. (Noakes, 2010)

Current fluid replacement recommendations are based on studies advocating replacing fluids equal to mass lost during exercise. (Montain, Latzka, & Sawka, 1999; McLellan, 2004; Kolka M. , Latzka, Montain, Corr, O’Brien, & Sawka, 2003) Revised in 2003, the current military guidelines suggest personnel consume ¾ of a quart (710 mL) of fluids per hour under Category 4 Red Flag Heat Conditions (WBGT Index 31.1-32.2°C). The recommendations also limit individuals to ingest no more than 1.5 quarts (1.4 L) per hour, and no more than 12 quarts (11.4 L) per day. (Kolka M. , Latzka, Montain, Corr, O’Brien, & Sawka, 2003; Kolka M. A., Latzka, Montain, & Sawka, 2003; McLellan, 2004) While these values appear aggressive in contrast to calculated 24 hour fluid intake during occupational and military training operations (Ruby, Schoeller, Sharkey, Burks, & Tysk, Water turnover and changes in body composition during arduous wildfire suppression, 2003; Ruby, Shriver, Zderic, Sharkey, Burks, & Tysk, 2002; Cuddy, Sol, Hailes, & Ruby, 2015) and military training operations (Ruby, Shriver, Zderic, Sharkey, Burks, & Tysk, Water turnover and changes in body composition during arduous wildfire suppression, 2003; Cuddy, Sol, Hailes, & Ruby, 2015), these revised guidelines have successfully prevented the incidence of hyponatremia and water intoxication that had become troublesome under the previous recommendations. (Kolka M. , Latzka, Montain, Corr, O’Brien, & Sawka, 2003; Kolka M. A., Latzka, Montain, & Sawka, 2003) The new recommendations also provide suggested work:rest schedules and intensities according to WBGT environmental conditions in an attempt to reduce overall physiological strain and the risk for heat related injury (Kolka M. , Latzka, Montain, Corr, O’Brien, & Sawka, 2003; Kolka M. A., Latzka, Montain, & Sawka, 2003) However, the present guidelines do not consider the possible implications regarding varied ingested fluid temperatures.

Offloading heat can be especially difficult in special populations like military personnel because uniforms and protective equipment inhibit evaporative and convective cooling by creating a microclimate and reducing the temperature gradient from the core to skin to ambient environments. Moreover, the core to skin gradient has implications on work tolerance in the heat. (Cuddy, Hailes, & Ruby, A reduced core to skin temperature gradient, not a critical core temperature affects aerobic capacity in the heat, 2014) To combat the limitations created by microclimates, cooling interventions are often implemented to aid the body in its natural thermoregulatory processes. Various cooling interventions initiated prior to exercise or during exercise (Marino & Noakes, 2009) have been shown to increase exercise tolerance in the heat by reducing the amount of heat accumulated by the body. Ingestion of cold fluids has also been shown...
to extend time to exhaustion in hot humid environments by up to 23% compared to an equal volume of warm fluids (Marino & Noakes, 2009)

While it has been demonstrated that cold water ingestion improves an individual’s capacity to maintain performance in hot humid environments, and that even slight incidences of hypohydration may result in significant decrements in performance, the combined effects of these factors has not been adequately addressed. (Siegel, Mate, Brearley, Watson, Nosaka, & Laursen, 2010; Hailes, Cuddy, Cochrane, & Ruby, 2016) It is not well understood if body heat can be adequately regulated with the aid of cold-water ingestion in order to maintain exercise performance in the heat despite reductions in fluid replacement volumes.

The purpose of this study was to evaluate the combined effects of compromised volume and decreased temperature of ingested fluid on hydration status, thermoregulation and exercise performance in the heat for an extended period of time.

Methods

PARTICIPANTS
Recreationally active, non-heat acclimated males were recruited from the university and surrounding community. Subjects were required to pass a preliminary testing process, including a Physical Activity Readiness-Questionnaire (PAR-Q), achieve a VO2max of at least 40 mL·kg⁻¹·min⁻¹, and sign an informed consent form approved by the university Institutional Review Board. Anthropometric data was collected for each study participant prior to the experimental trials. Eleven recreationally active, non-heat acclimated males (age 24.7±5.9 years, height 179.0±7.3 cm, weight 78.4±6.4 kg, peak VO2 58.2±6.0 mL·kg⁻¹·min⁻¹, body fat 14.3±5.9%) completed all trials. All reported data includes all 11 participants.

EXPERIMENTAL TESTING

Preliminary Testing

Hydrodensitometry
Body composition was measured using an underwater weighing tank (Exertech, Dresbach, MN) at estimated residual lung volume based on height and weight. Subjects were ≥3 hours fasted prior to testing. Dry weight was measured using a digital scale (Befour Inc., Cedarburg, WI), and height was measured. Body density and fat percentage were estimated using the Siri equation. (Siri, 1993)

Peak Aerobic Capacity
Maximal aerobic capacity (VO2Max) was determined for each subject using the Bruce protocol treadmill test. (Bruce, Kusumi, & Hosmer, 1973) Subjects were ≥3 hours fasted prior to arriving at the lab for testing. Testing was completed on a motorized treadmill (Fullvision, Inc., Newton, KS). Expired gases were analyzed using a calibrated metabolic cart every 15 seconds (Parvomedics, Inc., Sandy, UT). (Glass, Gregory, & eds., 2007) Heart rate was recorded using a heart rate chest strap and watch (Polar Electro, Kemple, FL).
Experimental Trials

Exercise Protocol

Testing consisted of two visits to the laboratory receiving one of two fluid treatments (ROOM or COLD) in a randomized crossover design with at least two weeks between each visit to prevent acclimation. Participants arrived at the laboratory 8-hours fasted, and recorded their food and activity 24-hours and 48-hours, respectively, prior to the exercise protocol, and replicated their log prior to the second visit. Subjects completed a familiarization protocol on the non-motorized treadmill (Woodway Curve) outside the climate chamber prior to each testing session walking and running at various speeds (0.3km at 1.3m/s, 0.3km at 1.8m/s, 0.2km at an easy walk, 0.3km at 2.2 m/s, and 0.2km at an easy walk). Following the familiarization protocol participants were given 100 mL of cool water to drink (1.6°C). A 5 mL venous blood sample, urine sample, and nude body weight was collected prior to each exercise protocol and immediately afterwards. Blood samples were analyzed using an iSTAT CHEM8+ handheld analyzer (Abbott Point of Care Inc., Princeton, NJ) for electrolytes, hemoglobin, and hematocrit. Urine samples were analyzed using a refractometer (PAL-10S, Atago, Cohasset, MA) to determine urine specific gravity (USG). Nude weights were measured using a digital scale (CW-11, Ohaus, Pine Brook, NJ) and used to calculate amount of fluid to administer to each subject, changes in body weight, and sweat loss. Heart rate was monitored continuously throughout the exercise protocol using a Polar Heart Rate chest strap and watch (Polar Electro, Kempele, FL). Core (T_c) and skin (T_s) temperature was monitored continuously throughout the protocol using a rectal temperature probe (Mon-a-therm, Mallinckrodt, Inc., St. Louis, MO), and a skin temperature sensor (T200, PhysiTemps, Clifton, NJ) placed approximately 5 cm above the nipple on the left pectoral region. Temperature values were recorded using a computer data logger (DASYLab Software, Measurement Computing CO., Norton, MA). Core temperature and heart rate were used to calculate physiological strain index (PSI) using the equation developed by Moran et al. (Moran, Shitzer, & Pandolf, 1998) Subjects were dressed in standard issue battle dress uniforms (BDU).

All experimental exercise testing was conducted within a Tescor Climate Chamber adjusted to 31°C WBGT (35.5°C and 50% humidity). Temperatures within the chamber were monitored using a Kestrel 4400 Heat Stress Tracker (Weather Republic LLC., Dowingtontown, PA). The exercise protocol required subjects to walk on the motorized treadmill for 25-minutes at 40% of their VO2max (steady state (SS) segment), followed by 5-minutes of standing rest, followed by a 1.6 km maximal effort time-trial (TT segment) on a non-motorized treadmill (Woodway Curve, Woodway Inc., Waukesha, WI), with the remainder of the hour spent as seated rest (resting segment (R)). This intermittent exercise protocol was repeated three times for a total of 3-hours in the climate chamber.

Fluid and Food Delivery

Subjects were administered either 1 mL kg⁻¹ body weight of the ROOM (35.5°C) fluid treatment or 0.5 mL kg⁻¹ body weight of the COLD (0°C) fluid treatment to drink every ten minutes throughout the exercise protocol. The ROOM treatment fluid was brought to temperature by remaining in a Brita Water Filter Dispenser (Ultramax Filtered Water Dispenser, Britta, L.P., Taunusstein, Germany) within the climate chamber where subjects were tested. The COLD treatment fluid was created by mixing crushed/shaved ice and refrigerated water kept in a separate Brita Water Filter Dispenser in a ratio of 1:2, ice and water respectively. Because of the duration of the trial, subjects were given a commercially available sports bar (250kcal) (ClifBar, Emery,
CA) to eat, half during the first resting phase following the first, and the other half following the second time trials.

Physiological Strain Index
Physiological strain index (PSI) was calculated using heart rate and core temperature utilizing the equation developed by Moran et al. (Moran, Shitzer, & Pandolf, 1998).

$$\text{PSI} = 5(T_c-T_0) \cdot (39.5-T_0)^{-1} + 5(HR_t-HR_o) \cdot (180-HR_o)^{-1}$$

Where $T_c$ is the core temperature at the time point of interest, $T_0$ is the initial core temperature, $HR_t$ is the heart rate at the time point of interest, and $HR_o$ is the initial heart rate. The standardized $HR_o$ that was used was 70 bpm.

Sweat Loss
Pre and post nude body weight values, and ingested fluids were used to calculate sweat loss during the exercise protocol using the equation below.

$$\text{Sweat Loss (L)} = (\text{BW}_{\text{pre}}(kg) + \text{fluid ingested (kg)}) - (\text{BW}_{\text{post}}(kg) + \text{Urine Weight (kg)})$$

STATISTICAL ANALYSIS
Core temperature, skin temperature, heart rate, and PSI (average 30-minute steady state and peak values at the completion of the time trial) were analyzed using a 2x6 repeated measures analysis of variance (ANOVA). Time trial performances and rate of perceived exertion were analyzed using a 2x3 repeated measures ANOVA. Blood chemistry characteristics, body mass values, and USG were analyzed using a 2x2 repeated measures ANOVA. Volume of fluid ingested, and sweat loss were analyzed using a paired sample two-tailed T-test. Statistical analyses were performed using StatPlus (AnalystSoft Inc., Walnut, CA). Statistical significance was evaluated at the $p<0.05$ level.

Results

FLUID INTAKE
Fluid consumption between the trials was 78.4±6.4 mL/10min and 39.2±3.2 mL/10min, ROOM and COLD, respectively. Total fluid ingested was significantly lower in the COLD treatment compared to the ROOM treatment (1.4±0.1 L and 0.7±0.1 L, ROOM and COLD, respectively).

BODY MASS/SWEAT LOSS
Body weight prior to exercise was not different ($p>0.05$) between the two treatments ($n=11$), and decreased significantly ($p<0.05$) following the exercise protocol as a main effect for time. The percent body weight lost in the COLD treatment was significantly greater ($p<0.05$) than the ROOM treatment (ROOM=2.2±0.2%; COLD=3.0±0.2%; $p<0.05$).
CORE TEMPERATURE
The main effect of time was significant for core temperature (n=11) during the intermittent exercise protocol (Figure 1). Mean core temperature was significantly increased during each steady state segment. This was also observed in peak core temperature during the time trial segments. There was no difference between the ROOM and COLD treatments.

SKIN TEMPERATURE
The main effect of time was significant (p<0.05) for skin temperature (n=11) during the intermittent exercise protocol (Figure 2). Mean skin temperature was significantly higher during the second and third steady state segments compared to the initial steady state segment (p<0.05). There was no difference in peak skin temperature during the time trials. There was no interaction, and no difference between the ROOM and COLD treatments.

HEART RATE
The main effect of time was significant for HR (n=11) during the intermittent exercise protocol (Figure 3). Mean HR was significantly higher during the second and third steady state segments compared to the initial steady state segment. There were no differences in peak heart rates across the three time trials. There was no difference between the two fluid treatments.

PHYSIOLOGICAL STRAIN INDEX
The main effect for time was significant for PSI (n=11) during intermittent exercise protocol (Figure 4). Mean PSI was significantly increased during each of the SS and TT portions of the exercise protocol. There was no difference between the two fluid treatments.

TIME TRIAL PERFORMANCES
The main effect for time to completion was significant (n=11) for the time trial portions of the exercise protocol. Time trials 2 and 3 were significantly slower than time trial 1. Similarly, time trial 3 was significantly slower than time trial 2 (Figure 5). However, there was no difference between the two fluid treatments.

USG AND PLASMA ELECTROLYTES
There was no difference in pre and post USG values, and no difference between the two fluid treatments (ROOM: 1.017±0.002 and 1.019±0.002, pre and post, respectively; COLD: 1.018±0.002 and 1.019±0.002, pre and post, respectively).

The main effect for time was significant for change in potassium for both treatment groups. There was a significant interaction for chloride concentrations (Table 1). There was no difference between pre and post values and no difference between treatments for sodium and calcium concentrations. Post-exercise hematocrit and hemoglobin concentrations were different from pre-exercise values for both fluid treatment trials.

RPE
The main effect for time was significant for rating of perceived exertion (RPE) throughout the intermittent exercise protocol with each hour perceived as being harder than the last, but was not
different between the two fluid treatments (ROOM=11±0.3, 13±0.7, 14±0.6, hours 1, 2, and 3, respectively; COLD=11±0.6, 12±0.8, 14±0.5, hours 1, 2, and 3, respectively).

Discussion

The most novel finding of this study was no difference in time trial performance between ROOM vs. COLD trials despite substantial differences of ingested fluid volume. This is consistent with previous work by Cheung et al. where cyclists received fluid replacement to elicit various states of hydration while exercising in a hot dry environment. (Cheung, et al., 2015) In both Cheung (Cheung, et al., 2015) and the present study, participants were dehydrated by approximately 3% from their initial body weight, challenging the current thinking that even mild dehydration can cause negative effects on performance. While both research designs cannot isolate if performance decrements are due to exercise duration, accumulation of heat stress, or hydration status, neither demonstrated significant differences in performance between treatment groups despite differences in percent body weight loss between trials. The present study is unique from Cheung et al. with its incorporation of the microclimate factor (created by personal protective equipment), which reduces evaporative cooling. (Desruelle, Bothorel, Hoefl, & Candas, 1996) When coupled with progressive dehydration, this typically contributes to diminished exercise performance in the heat. Though RPE rose with each subsequent hour, there was no difference in RPE between the two treatment groups (COLD vs. ROOM). Whereas previous studies demonstrated a higher rating of perceived exertion when completing a set exercise task in a dehydrated state compared to a euhydrated state. (Bardis, Kavouras, Arnaoutis, Panagiotakos, & Sidossis, 2013; Lopez, et al., 2011) Several subjects reported the COLD treatment to be more palatable which is consistent with previous studies in which subjects were allowed to drink ad libitum either cold or ambient temperature water. (Riera, Trong, Sinnapah, & Hue, 2014)

Markers of relative hydration status (USG, electrolyte concentration, and hematocrit) were within the acceptable range of normal values for healthy adults in both treatment groups in the present study, despite significant differences in the volume of fluid ingested and significant amount of body weight lost. The only difference in blood chemistry between the two treatments was in chloride concentrations, in which the COLD treatment post-trial blood samples decreased, whereas ROOM treatment blood chloride levels rose. Despite these differences, both values fall within the acceptable range of a normal healthy adult. (Duh & Cook, 2005; Dill & Costill, 1974; Bruce, Kusumi, & Hosmer, 1973; Hamouti, Del Coso, & Mora-Rodriguez, 2013) In contrast, body mass was significantly reduced as indicated above (2.3 and 3.0% initial body weight for ROOM and COLD, respectively), indicating significant dehydration compared to pre-exercise status. This demonstrates that commonly selected biomarkers could not distinguish trial specific changes in whole body hydration status as a result of the exercise protocol or the treatment interventions. These data conflict with past research (Popowski, Oppinger, Lambert, Johnson, Johnson, & Gisolf, 2001; Hamouti, Del Coso, & Mora-Rodriguez, 2013) that demonstrated significant changes in USG during progressive dehydration. Similarly, Jimenez et al. and Myhre et al. have shown marked reduction in plasma volume with progressive dehydration. (Jimenez, Melin, Koulmann, Alleward, Launay, & Savourey, 1999; Myhre, Hartung, & Tucker, 1982) Interestingly, the most common change in serum electrolyte concentration is noted when attempting to minimize fluid loss and maintain body weight with aggressive fluid replacement, which often results in the dilution of essential electrolytes. (Nolte, Hew-Butler, Noakes, & Duvenage, 2015)
A notable finding of this study was that there was no difference in thermoregulatory metrics. Core temperature, heart rate, and PSI were not different between the two treatments. In previous studies in which participants incurred a 2-3% body weight loss, significant increases in core temperature were observed compared to their euhydrated counterparts. Many of the previously conducted studies lack external validity in that the methods for establishing dehydration in participants do not reflect progressive dehydration due to extended exercise. Previous studies have induced pre-exercise hyphodhydration by using passive dehydration, drugs, or prior exercise. In contrast, during exercise and work in the heat, dehydration is gradual and typically progresses over time, commensurate with increases in core temperature. Providing pre-experimental alteration in hydration status creates a less realistic whole body challenge.

As previously mentioned, heart rate was not different between the two fluid treatments. However, steady state heart rate was increased by approximately 24% for both treatments during the second segment compared to segment 1, but did not increase further during the third steady state segment. Interestingly, core temperature increased across each of the steady state segments, but was not different between the two treatments. In contrast, in a study conducted by Cheung et al., subjects were dehydrated by 2.2% body mass and experienced significantly higher heart rates and changes in core temperature compared to when they were euhydrated performing the same intensity and duration of exercise in the heat while wearing protective clothing, despite receiving the same volume of fluid replacement throughout the exercise protocol. This suggests that the temperature of the fluid intervention in the present study may be the determining factor, rather than fluid volume, in aiding the regulation of body temperature while exercising in the heat.

Although skin temperature throughout the time trials was not different, the increase in core temperature generated a diminished core to skin gradient compared to those seen under less extreme environmental conditions (1.4±0.8, 2.3±1.5, and 2.4±0.9; 1.1±0.7, 1.7±0.9, and 1.9±0.9 °C, ROOM and COLD, respectively, p<0.05 for time). Decreasing the core-skin temperature gradient below 2.1°C has been shown to impair exercise tolerance in the heat. Moreover, this has been proposed as more influential to fatigue compared to a critical core temperature or changes in percent body weight loss. The present data show similar core to skin gradient values to the study conducted by Cuddy et al. in 2014 examining effects of exercise in hot environments and thermoregulatory metrics. Exercise tolerance was significantly reduced when the core to skin gradient was diminished below 2.1°C, which may be a function of the microclimate created by the additional thermal burden of the PPE in the present study.

PSI was also not different between the two treatments throughout the exercise protocol demonstrating that although subjects were 3% dehydrated, they were at no greater risk of a heat related irregularities compared to when they were less dehydrated. These results are consistent with a previously conducted study in our lab comparing varying volumes and temperatures of fluids on thermoregulation under steady state exercise in a hot humid environment for an extended period of time.
Conclusions
The results of this study demonstrate that thermoregulation and performance in the heat is not exclusively reliant on the hydration status of the individual and may be significantly impacted by the temperature of the ingested fluid. This study showed that despite a significant loss of body weight, the COLD fluid replacement treatment was no different than ROOM temperature fluid treatment on thermoregulatory and performance metrics. We conclude that hypohydration alone is not a threat to thermoregulation and decreased performance, and that fluid temperature can by itself aid in thermoregulation while exercising in the heat for extended periods of time.

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Bibliography


Appendix

Table 1. Blood chemistry characteristics for both treatments (ROOM and COLD) before and after the exercise protocol (mean±sd, N=11).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pre-trial</th>
<th>Post-trial</th>
<th>Pre-trial</th>
<th>Post-trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mmol/L)</td>
<td>140.9±1.8</td>
<td>141.9±1.9</td>
<td>140.6±1.7</td>
<td>140.4±3.7</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.2±0.3</td>
<td>4.4±0.2*</td>
<td>4.2±0.4</td>
<td>4.5±0.4*</td>
</tr>
<tr>
<td>Cl (mmol/L)</td>
<td>103.8±2.2</td>
<td>105.5±2.2</td>
<td>104.1±2.1</td>
<td>103.1±2.0*†</td>
</tr>
<tr>
<td>iCa (mmol/L)</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>1.2±0.0</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>45.4±2.2</td>
<td>48.0±2.5*</td>
<td>45.5±1.9</td>
<td>48.3±2.8*</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15.4±0.8</td>
<td>16.3±0.8*</td>
<td>15.5±0.6</td>
<td>16.4±1.0*</td>
</tr>
</tbody>
</table>

* - p<0.05 vs. Pre-trial
† - p<0.05 vs. ROOM Post-trial
Figure 1. Changes in rectal temperature (°C) during the incremental exercise protocol in the heat for both treatments (mean±SEM N=11). * p<0.05 vs. steady state walk 1 (average 30 minute value); † p<0.05 vs. steady state walk 2 (average 30 minute value); a p<0.05 vs. time trial run 1 (TT 1); b p<0.05 vs. time trial 2 (TT 2).
Figure 2. Changes in chest skin temperature (°C) during the incremental exercise protocol in the heat for both treatments (mean±SEM N=11). * p<0.05 vs. steady state walk 1 (average 30 minute value)
Figure 3. Changes in heat rate (bpm) during the incremental exercise protocol in the heat for both treatments (mean±SEM N=11). * p<0.05 vs. steady state walk 1 (average 30 minute value)
Figure 4. Changes in physiological strain index (PSI) during the incremental exercise protocol in the heat for both treatments (mean±SEM N=11). * p<0.05 vs. steady state walk 1 (average 30 minute value); † p<0.05 vs. steady state walk 2 (average 30 minute value); a p<0.05 vs. time trial run 1 (TT 1); b p<0.05 vs. time trial 2 (TT 2).
Figure 5. Time trial performance during the incremental exercise protocol in the heat for both treatments (mean±SEM, N=11). a - p<0.05 vs. time trial 1 (main effect for time); b – p<0.05 vs. time trial 2 (main effect for time).