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Effect of Sodium Chloride Supplementation on Serum and Sweat Sodium Concentration, Cardiovascular Function, and Physical and Cognitive Performance

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Effect of Sodium Chloride Supplementation on Serum Sodium Concentration, Cardiovascular Function, and Physical and Cognitive Performance

by

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Dedication

I dedicate this dissertation to my family. For my fiancée, Lauren, who has given me encouragement, support, diversion, and patience throughout this endeavor. And to my parents for always encouraging me to do my best and for instilling the work ethic learned from my late grandparents who also inspired me.

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Effect of Sodium Chloride Supplementation on Serum and Sweat Sodium Concentration, Cardiovascular Function, and Physical and Cognitive Performance

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These studies determined the effects of sodium chloride supplementation on serum and sweat sodium concentration, cardiovascular function, and physical and cognitive performance. Sweat sodium losses, alone, caused a significant decline in serum sodium concentration (-6.4±1.6 mEq/L, p=0.001) during 3h cycling in the heat in endurance-trained athletes with high sweat sodium losses. However, sodium chloride supplementation matching sweat sodium losses (NA; 5.9±1.5g NaCl/h) maintained serum sodium concentration. Post-exercise maximal cycling power declined and was significantly lower than pre-exercise in placebo (PL; p=0.012), but power was not significantly different in NA (p=0.057). Pre- to post-exercise response time during a Stroop Test improved in NA (p=0.009), while there was no change in PL (p=0.597). Post-exercise postural sway was less in NA *vs.* PL (p=0.044).

Three days of sodium chloride supplementation (~15 g NaCl/d) resulted in a significant increase in plasma volume in healthy untrained males at rest (5.9±7.6 %) and

during exercise at 60%VO_2peak (8.6±5.2 %) compared to PL. During NA, stroke volume was 10% higher during exercise *vs.* PL (139±27 *vs.* 126±24 ml/beat, respectively, p=0.004). Cardiac output was 8% higher in NA during exercise *vs.* PL (21.0±3.1 *vs.* 19.4±2.6 L/min, respectively, p=0.013). Mean arterial pressure during exercise was not different in NA *vs.* PL (p=0.548) as total peripheral resistance decreased (p=0.027) with the increased cardiac output. Sweat sodium concentration was 9% higher in NA *vs.* PL during exercise in the heat (70.4±19.5 *vs.* 64.5±21.7 mEq/L, p=0.044).

In summary, serum sodium concentration declines when high sweat sodium losses are not replaced while hydration status is maintained. Acute sodium chloride supplementation during exercise which matches sodium losses maintains serum sodium concentration. This maintenance of serum sodium concentration results in both physical and cognitive benefits compared to when serum sodium concentration declines. Chronic intake of sodium chloride for 3 days increases plasma volume in healthy untrained men and improves cardiovascular function, as both stroke volume and cardiac output are increased, while oxygen consumption and blood pressure are unchanged. Therefore, acute and chronic sodium supplementation positively alters fluid and sodium balance which results in beneficial effects on physical and cognitive performance and cardiovascular function during exercise.

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Chapter I: General Introduction

Approximately 50-60 % of human body mass is composed of water. Total body water is divided between the intracellular and extracellular compartments. The extracellular space is further divided between interstitial and intravascular compartments. Fluid balance between compartments is largely determined by the non-penetrating solutes, intracellular potassium and extracellular sodium. Fluid or solute additions or losses may result in alterations in the content or volume of the compartments. Nutritional intake or metabolic processes may add to the content or volume while sweating, urination, and respiration may decrease it. One potentially severe body fluid and electrolyte imbalance is hyponatremia. Hyponatremia is considered to be mild to moderate with serum sodium concentration of < 135 mEq/L and severe when < 125 mEq/L. Mild cases of decreased serum sodium concentration result in nausea, confusion, or headaches, while severe cases may result in seizures or death. Cases of hyponatremia have been reported in military personnel, laborers, or ultra-endurance athletes during exercise (5, 8, 13, 31, 44, 51, 55, 59, 73, 74, 76, 94). While the effects of large reductions in serum sodium concentration can be quite severe, the effects of small declines (~5-10 mEq/L) in serum sodium concentration on physical and cognitive performance are not well understood.

One of the many adaptations to endurance training is increased stroke volume during exercise (97, 112). The higher stroke volume in trained vs. untrained individuals is partially due to expanded plasma and blood volume (57, 63). The extracellular body water compartment is expanded in endurance trained athletes (57). The increase in blood

volume results in improvements in cardiovascular function, specifically increased stroke volume, which may result in increased cardiac output and/or a lower heart rate at rest and/or during exercise. As extracellular sodium content is partially responsible for the fluid volume of the intravascular compartment (90), an increase in sodium chloride ingestion results in an increased blood volume (28, 29, 50, 107). This increased blood volume may afford some of the same vascular benefits of endurance training to untrained people. One such benefit would be an increase in stroke volume at rest and during exercise. While stroke volume has been shown to increase at rest with sodium chloride supplementation, no data exists on the effects of sodium chloride supplementation on increasing exercising stroke volume in young (20-35 y) healthy males (28, 29, 50, 107). These studies will investigate the effect of sweat sodium losses on serum sodium concentration and performance, as well as the potential benefits of sodium chloride supplementation on cardiovascular function and physical and cognitive performance.

Chapter II: Statement of the Problem

The overall purpose of these studies was to determine the effects of alterations in body sodium stores on serum sodium concentration, blood volume, cardiovascular function, and physical and cognitive performance. The purpose of Study 1 was to determine if sweat sodium losses, alone, in individuals with high sweat sodium losses would result in significant reductions in serum sodium concentration during exercise in the heat. Additionally, we investigated the effects of sodium supplementation on maintaining serum sodium concentration and physical and cognitive performance. The specific research questions for Study 1 were:

1. Will 3h of exercise in the heat, while maintaining body mass through fluid intake, decrease serum sodium concentration in individuals with high sweat sodium losses?
2. Will sodium chloride supplementation matching sweat sodium losses maintain serum sodium concentration while exercising with large sweat sodium losses?
3. Will a decline in serum sodium concentration result in a decreased physical and cognitive performance?

The purpose of Study 2 was to determine the effects of increased sodium chloride intake over 3 days on blood and stroke volume during rest and upright exercise. Additionally, we investigated the effects of increased sodium chloride intake on sweat sodium concentration in the heat. The specific research questions were:

1. Will an increase in daily sodium chloride intake (3.5 mEq Na/kg body mass/d) for 3 days increase blood volume in untrained males at rest and during exercise?
2. Will an increase in daily sodium chloride intake for 3 days result in an increase in stroke volume during exercise in untrained, healthy males?
3. Will an increase in daily sodium intake result in an increase in sweat sodium concentration during exercise in the heat in non-heat acclimatized untrained males?

Chapter III: Experimental Design

Study 1. This first experiment determined the effects of prolonged sweating, during exercise in the heat, in athletes with high sweat sodium losses on serum sodium concentration and physical and cognitive performance. Furthermore, it determined the resulting effects of sodium chloride supplementation matching sweat sodium losses. To accomplish this, 36 male endurance-trained athletes performed sweating analysis testing in order to identify a subject pool with sweat sodium losses greater than 90 mEq/L. In order to familiarize the participants with the physical and cognitive tasks and to ensure heat acclimatization, the participants performed 3 preliminary trials, separated by 48-96 hours. In addition to the familiarization tasks, participants cycled for 1 hour in the heat at 60% VO_2peak in order to determine whole body sweating rate and regional sweat sodium concentration. Approximately 1 week following the preliminary sessions, participants completed 2 experimental trials, separated by approximately 7 days, consisting of 3 hours of cycling at 60% VO_2peak in a warm environment and several performance tasks in a thermoneutral environment. During these trials, the participants received either sodium chloride capsules matching their individual sweat sodium losses or a placebo. A fluid replacement drink with carbohydrate was also provided to maintain body mass, thus preventing dehydration.

Study 2. This experiment determined the effects of increased sodium chloride intake on blood volume and stroke volume during rest and exercise in untrained males. Nine untrained male subjects consumed pills containing sodium chloride (3.5 mEq Na/kg

body mass/day) or placebo, in addition to their normal diet. Following 3 days of sodium chloride supplementation they performed 15 min of upright cycling at 60 % VO_2peak in a temperate environment (22 °C). During and after exericse, cardiac output, blood pressure, and heart rate were measured and blood samples were taken for the determination of hematocrit and hemoglobin, in order to calculate changes in blood volume.

The second part of Study 2 was completed to determine the effects of increased sodium chloride intake on sweat sodium concentration. Immediately following the cardiovascular function task, participants cycled for 30 minutes at 50 % VO_2peak in a warm environment (~34 °C, 50 % RH), following a 15 min warm-up. Sweat samples were collected from 4 regional sites and whole body sweating rate was measured**.** Whole body sweat sodium concentration was calculated and compared between treatments.

Chapter IV: Study 1

Effects of Oral Sodium Chloride Supplementation on Serum Sodium Concentration and Physical and Cognitive Performance

ABSTRACT

Large declines in serum sodium concentration can result in seizures and death, but the effects of modest declines of serum sodium concentration on physical and cognitive performance are not well understood. It is also not clear if sweat sodium losses alone can result in significant declines in serum sodium concentration and whether sodium chloride supplementation can prevent potential declines. The purpose of this study was to determine the effects of prolonged sweating during exercise in the heat in individuals with high sweat sodium losses on serum sodium concentration and physical and cognitive performance. Eleven endurance-trained athletes cycled for 3h at 60% VO_2peak while ingesting sodium chloride matching sweat sodium losses (NA) or a placebo (PL), while maintaining hydration status. Serum sodium concentration significantly declined in PL (-6.4±1.6mEq/L, p=0.001) and was maintained in NA (-1.0±2.4mEq/L) with sodium chloride supplements (5.89±1.48g/h) matching sweat sodium losses. The 4% decline in maximal cycling power during PL was significant (p=0.012) while the 3% decline in NA was not significant (p=0.057). Twenty-minute time trial performance was 4% higher in NA *vs.* PL, yet this difference was not significant (p=0.307). Response time during a Stroop test improved in NA (p=0.009) and was unchanged in PL (p=0.597). Post-test balance was better in NA *vs.* PL (p=0.044). In conclusion, serum sodium concentration declines in athletes with high sweat sodium losses while exercising in the heat for 3h. When serum sodium concentration is maintained by matching losses with sodium chloride supplementation; balance and Stroop Test response time are significantly improved.

INTRODUCTION

Declines in serum sodium concentration have occurred during prolonged exercise in military and athletic personnel (5, 8, 31, 44, 55, 59, 94). While severe hyponatremia, a serum sodium concentration $\leq$ 125 mEq/L, can result in seizures and death (44), little data exists on the effects of a moderate lowering of serum sodium concentration on physical and cognitive performance (5, 81, 104, 108). Overhydration has been implicated as the main cause of lowering serum sodium concentration (75), but models of factors contributing to a decrease in serum sodium concentration (hyponatremia) have also included sweat sodium losses as a potential factor (69). In a recent field investigation, we found a relationship between declines in serum sodium concentration and rates of sweat sodium loss in males competing in the Hawaii Ironman® Triathlon (77). As sweat sodium losses are extremely variable between subjects (77, 89, 106), those who lose large amounts of sodium in their sweat would be at a greater risk of decreasing serum sodium concentration and potentially developing hyponatremia during prolonged sweating.

During exercise, researchers have investigated the effects of sodium supplementation on serum sodium concentration, as it has been recommended for the maintenance of serum sodium concentration during prolonged exercise (54). While some researchers have reported that serum sodium concentration can be maintained by ingesting sodium (104, 108), others have found no benefit (52, 96). One shortcoming of prior investigations is that sodium supplementation was not tailored to the individual's sodium losses.

The purpose of this study was to determine if sweat sodium losses, alone, in individuals with high losses, would result in significant reductions in serum sodium

concentration during exercise in the heat. Additionally, we investigated the effects of sodium chloride supplementation on maintaining serum sodium concentration as well as the effects on physical and cognitive performance. We hypothesized that serum sodium concentration would significantly decline during exercise in the heat and it would be maintained with sodium chloride supplementation matching sweat sodium losses. Furthermore, we hypothesized that physical and cognitive performance would decline without sodium chloride supplementation and would be maintained or improved with sodium chloride supplementation matching individual sweat sodium losses.

METHODS

Subjects

Eleven heat-acclimatized endurance-trained males with high sweat sodium losses (sweating rate: 1.72 ± 0.25 L/h, sweat sodium loss: 101 ± 28 mEq/h) participated in this experiment. Their age (mean ± SD), body mass, height, and VO_2 peak were 33.5 ± 6.2 y 81.1 ± 7.4 kg, 182 ± 7 cm, 4.67 ± 0.55 L/m, respectively. Participants signed a consent form approved by the Institutional Review Board at The University of Texas at Austin.

Preliminary Testing

Thirty-six male endurance athletes were tested from the local triathlon and cycling community in order to identify those with high sweat sodium losses (> 90 mEq/h). Qualifying athletes with high sweat sodium losses were invited to participate in the investigation. Sweating characteristics for these athletes are displayed in Table 1-1.

All pre-experimental testing sessions for those participating in the investigation were completed 3-10 days prior to the first experimental trial in order to determine VO_2peak, lactate threshold, sweat sodium losses, verify heat acclimation status, and to familiarize with testing protocols. Each of the 3 preliminary trials was separated by 48-96 hours. During the first visit, VO_2peak and lactate threshold was determined while subjects cycled a laboratory ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Subjects first performed 5-6 five-minute sub-maximal stages to identify their blood lactate threshold. After resting, subjects then performed an incremental exercise protocol lasting 7-12 minutes to determine VO_2peak, defined as a stabilization of VO_2 with increasing work rate and respiratory exchange ratios of >1.10.

During each preliminary testing session, subjects completed a sweat measurement exercise test in a temperature and humidity controlled environmental chamber (32 °C, 50 % RH) to ensure heat acclimation, and to measure sweating rate and sweat sodium concentration. The test consisted of a 10-minute warm-up and then participants cycled for 60 min (2 x 30 min) at 60 % of VO_2peak. Body mass was measured following the warm-up (Ohaus Champ, Model CQ250W, Pinebrook, NJ), at 30 min, and after the 60 min exercise bout for the determination of sweating rate. Regional sweat sodium concentration was measured during both 30 min exercise periods by applying a 'sweat patch', composed of 7.6 x 7.6 cm gauze sponge (Johnson & Johnson Medical, Arlington, TX) and 10 x 12 cm Tegaderm® bandage (3M Health Care, St Paul, MN). It was applied to the mid-posterior right forearm, right scapula, right mid-anterior thigh, and calf. These regional sites were chosen as they are highly correlated with whole body sweat sodium concentration (r = 0.82, 0.88, 0.89, and 0.93, respectively) (78). Prior to patch application, the area was cleaned with 70 % isopropyl alcohol, rinsed with de-ionized water, and dried with a contaminant-free cloth. When necessary, these areas were gently shaved to allow for secure placement of the patches. The patches were applied after the initial warm-up and again at 30 minutes of exercise, following towel drying and nude body mass measurements. During the patch application the subjects remained seated on the bicycle ergometer and the patches were removed immediately after 30 min, prior to body mass measurement. This duration was chosen so that the patches would not become saturated, which may alter the sweat sodium concentration (111). A subject's heat acclimatization was confirmed by <0.2 °C change in core temperature at the end of the exercise bouts between preliminary tests. In addition to the sweat testing and heat

acclimation verification protocols, subjects completed neuromuscular maximal cycling power, time trial, and cognitive testing protocols in order to become familiarized with the testing procedures.

Experimental Trials

Approximately one week after the final pre-experimental session, each participant participated in 2 randomized double-blind crossover trials, separated by 7-10 d. During the experimental trials, subjects cycled for 3h on a stationary ergometer (Velotron, Racermate, Inc, Seattle, WA) at 60 % of VO_2peak in a warm environment (33.3 ± 0.2 °C DB, 27.9 ± 1.8 °C WB, 54.0 ± 2.8 % RH, fan speed = 4.1 ± 0.6 m/s). A sucrose placebo (PL) or sodium chloride (NA; 5.89 ± 1.48 g NaCl/h) was ingested via capsule to match individual whole body sweat sodium losses measured during the preliminary testing. Nutritional intake and exercise was recorded and repeated for 3 days prior to both experimental trials. No activity was undertaken the day prior to the trial and only moderate exercise was allowed 2-3 days prior. Sixteen ounces of water and a standardized meal (474 ml, Vanilla Boost, Nestle) were consumed 2 hours prior to each experimental trial. Body mass was maintained during the experimental trials by drinking water (6.9 ± 0.7 L) with carbohydrate (0.8 g sucrose/kg/hr). A standard dose of potassium chloride (0.85 g/h) was given to all subjects. Carbohydrate intake was equal during both trials. Body mass was measured (Ohaus Champ, Model CQ25W, Pinebrook, NJ) at 30-60 minute intervals to determine sweating rate. Four-site regional sweat sodium concentration was measured from 30-60 and 120-150 min of exercise. Blood samples (5 ml each) were taken via venipuncture in an antecubital vein, while subjects

were seated, prior to exercise, and after 60 min, 120 min, and 180 min of exercise. All urine was collected during the trial for volume determination and sodium concentration analysis. Core temperature was measured via a rectal thermistor (YSI 401) inserted 12cm past the anal sphincter and heart rate was measured via telemetry (Polar, Finland) every 15 minutes during the exercise bout.

Immediately prior to and after the 3 h cycling bout, participants completed the Stroop Color-Word Interference Task to assess cognitive function. It was performed in a thermoneutral environment. A subset of subjects (n = 6) also completed a balance assessment task by standing on a force plate for 7 minutes (9 – 30 second task intervals, 15 second rest interval) prior to the cognitive task. Participants also performed a maximal neuromuscular cycling power test (Pmax) on an inertial load ergometer prior to exercise, and after 60 min, 120 min, and 180 min of exercise. A 20 min cycling time trial was also performed at the end of the testing session. Figure 1-1 displays the complete protocol.

Experimental fluid/sodium chloride intake protocol

Fluid was consumed immediately prior to and during the first 2.5 hours of the experimental exercise bout. A tapered drinking and sodium chloride intake protocol was utilized to allow time for absorption by the end of the 3h exercise bout. A large bolus of fluid (11.7 % of total) was consumed 15 min prior to exercise. Fluid was provided at 15 min intervals (8.7 % of total per 15 min) during exercise through 105 min. From 120-150 min of exercise smaller volumes were consumed (7.3, 6.7, and 5 % at 120, 135, and 150 min, respectively). No fluid or sodium was consumed during the final 30 min. The

identical fluid and sodium consumption protocol was followed during both experimental trials.

Performance Testing

Cognitive testing

A modified computerized Stroop Color-Word Interference Test (LSA Stroop, FL) consisted of five 45-second modules. The test difficulty level progressively increased during each module. In short, the subjects were presented with a word (red, green, or blue) on a screen. The color of the word either matched the meaning or was incongruent. Subjects were prompted to select the color or the meaning depending on the module. In the final module, the subjects selected the color or meaning based on if the word was framed by a box. Accuracy and response time were recorded.

Balance Testing

The balance assessment task consisted of 3 trials of 3 stances while standing barefoot on a 6 degree of freedom force plate (Bertec, Columbus, OH) which was interfaced with a computer (Dell, Austin, TX). The stances included 2-leg stance with feet together and eyes open, single leg stance with eyes open, and single leg stance with eyes closed. During stances with eyes open, subjects (n = 6) looked at a spot at head level. Each trial lasted 30 seconds and there was a 15 second break between each repetition. The order of stance conditions was a randomized crossover design between subjects and each subject completed the same order for both of their trials. The center-of-pressure amplitude (ACOP) was measured in the anteroposterior (COPap) and

mediolateral (COPml) planes with custom software (Matlab, The Mathworks, Inc., Natick, MA).

Maximal neuromuscular cycling power (Pmax)

Maximal neuromuscular cycling power per pedal revolution, torque, and revolutions per minute were measured on the PowerCycle prior to the 3 h ride and at 60 min, 120 min, and following the 3 h ride. Participants complete 4 all-out efforts lasting 3-4 seconds each during each testing session. Subjects remained seated on the ergometer for a 1 min rest period between each effort. In short, flywheel angular velocity and acceleration were determined by an optical sensor and micro-controller based computer interface which measured time (± 1 µsec) and allowed power to be calculated instantaneously every 3 degrees of crank revolution or averaged over one complete revolution of the cranks (65). All powers expressed in this paper are average values over one complete pedal cycle. As described in Martin et al. (65), maximal power was calculated as the product of moment of inertia, velocity and angular acceleration of the flywheel.

Cycling Time Trial

Subjects (n = 9) completed a 20 min time trial in a thermoneutral environment. The workrate was fixed for the first 5 min at a power output that would elicit an oxygen consumption that was 10 % above lactate threshold. After the first 5 min of the time trial, subjects were free to alter the workrate at 30 sec intervals for the remaining 15 min. The

same researcher gave verbal encouragement during both time trials. Total work completed during the 20 min time trial was calculated following the trial.

Measurements of gas exchange

During the VO_2peak test, inspired air volume was measured with a pneumotach (model 4813, Hans Rudolph, Shawnee, KS) and expired gasses were continuously sampled from a 4 L mixing chamber (Vacumed, Ventura, CA) and were analyzed for oxygen (S-3A/I, Ametek, Pittsburgh, PA) and carbon dioxide (CD-3A, Ametek, Pittsburgh, PA). The analyzers were interfaced to a computer for calculation of the rate of oxygen consumption and rate of carbon dioxide production (Max II, AEI Technologies, Pittsburgh, PA).

Blood and urine measures

Hematocrit was measured in duplicate following microcentrifugation for 15 minutes. Hemoglobin was measured in duplicate using the cyanmethemoglobin method (35). Plasma volume change was determined via the method of Dill and Costill (33). Whole blood was stored at room temperature until clotting occurred and then was centrifuged for 15 min. Serum and urine sodium concentration and osmolality were measured via electrochemistry (NOVA 5, Waltham, MA) and freezing point depression methods (3MO, Advanced Instruments, Needham Heights, MA), respectively. Urine specific gravity was measured to ensure hydration status pre-exercise and throughout the experimental trials.

Sweating rate and sweat sodium concentration analysis

Sweating rate (L/h) was calculated as the change in body mass, accounting for fluid consumption and urine loss. Upon removal of the sweat patches, the gauze sponge was immediately separated from each Tegaderm® bandage and placed into a plastic syringe. The sweat content of the sponges in the syringes was "squeeze plunged" into four 5 ml plastic test tubes and capped. Sweat electrolyte concentration was measured with a Nova 5 Analyzer (Waltham, MA) with a CV of 2 % for sodium analysis. A modified weighted equation was utilized to calculate whole body sweat sodium concentration (sweat sodium concentration = 0.11([Arm]) + 0.276([Back]) + 0.299([Thigh]) + 0.315([Calf])) (8). Whole body sweat sodium loss was calculated as whole body sweating rate x weighted sweat sodium concentration.

Statistical Analysis

Data are reported as mean and standard deviation. A paired student's t-test and a two way repeated measures analysis of variance was performed to analyze differences in blood and sweating characteristics. The Bonferroni correction was utilized to adjust for comparisons. Pearson product moment of correlation was used for relationships of sweating characteristics. Significance was set at an alpha level of 0.05.

RESULTS

Body mass from pre- to post-exercise was successfully maintained in both trials via fluid consumption (Table 1-2). Core temperature was similar between treatments before (37.4 ± 0.4 *vs.* 37.3 ± 0.3 °C) and following 3 h of exercise in the heat (38.0 ± 0.2 *vs.* 38.1 ± 0.2 °C), PL *vs.* NA, respectively. Heart rate after 3 h of steady state exercise was not significantly different between treatments (134 ± 8.3 *vs.* 137 ± 11.1 bpm, PL *vs.* NA, respectively, p = 0.186).

Serum sodium concentration significantly declined from pre- to post-exercise in PL (-6.4 ± 1.6 mEq/L, p = 0.001), but was maintained in NA when consuming sodium to match sweat sodium losses (-1.0 ± 2.4 mEq/L), (Table 1-3). There were no differences in pre-exercise serum sodium concentrations between treatments (p = 0.833). Serum sodium concentration in PL was significantly lower than NA following 3 h of exercise (137 ± 1.3 *vs.* 142 ± 2.5, PL *vs.* NA, respectively, p = 0.001). As serum sodium is a major contributor to serum osmolality, osmolality also significantly declined in PL (290 ± 3.0 *vs.* 276 ± 2.4, pre- *vs.* post-exercise, respectively, p = 0.001), but was maintained in NA (289 ± 2.7 *vs.* 286 ± 5.2, pre- *vs.* post-exercise, respectively, p = 0.284; Table 1-3).

Furthermore, hematocrit significantly increased in PL, but was maintained in NA. There was a significant difference in hematocrit between treatments at 180 min (p = 0.004; Table 1-3). Hemoglobin concentration was not significantly different between treatments (Table 1-3). There was a significant difference in mean corpuscular hemoglobin concentration (MCHC) between treatments at 180 min (p = 0.007; Table 1-3) which would indicate cell swelling in PL *vs.* NA. Plasma volume change from pre to

post trial was not significantly different between PL and NA trials (Δ -0.4% *vs.* 1.3%, PL *vs.* NA, respectively; Table 1-3).

Sweating characteristics

Sweating characteristics for 30-60 min and 120-150 min of the exercise bout are presented in Table 1-4. There were no significant differences between treatments in sweating rate, sweat sodium concentration, or sweat sodium losses at either time point. There were no differences in sweat sodium concentration between treatments (68 ± 18 *vs*. 68 ± 19 mEq/L, PL *vs.* NA, respectively, p = 0.844). There was an overall time effect, as sweat sodium concentration significantly increased from 30-60 min to 120-150 min (65 ± 17 *vs*. 71 ± 19 mEq/L, respectively, p = 0.013). This time effect was significant for both PL (64 ± 16 *vs*. 71 ± 21 mEq/L, p = 0.018) and NA (66 ± 19 *vs.* 71 ± 19 mEq/L, p = 0.046), 30-60 min *vs.* 120-150 min, respectively. There were no significant differences between treatments in sweat sodium losses (p = 0.979). In PL, sweat sodium loss significantly increased from 30-60 to 120-150 min (p = 0.024), while there was no change in NA (p = 0.729). Sweating rate and sweat sodium concentration were not significantly related in PL or NA at either measurement (p > 0.05 for all comparisons).

The change in serum sodium concentration in PL (-6.2 ± 1.6 mEq/L) was significantly and negatively correlated with measured sweat sodium concentration during the 30-60 min and 120-150 min sweat collection (r = -0.741, p = 0.009 and r = -0.775, p = 0.005, respectively; Figure 1-2). The change in serum sodium concentration of individual subjects in PL was also significantly correlated with estimated total sweat sodium lost during the 3 h trial (332 ± 87.5 mEq; r = -0.729, p = 0.011; Figure 1-3),

which was calculated as actual sweating rate and regional sweat sodium concentrations from the 2 sweating analysis tests (30-60 min and 120-150min). As expected, sweat sodium concentration was not significantly correlated with change in serum sodium concentration when supplementing with sodium to match sweat sodium losses ($r = 0.007$, $p = 0.984$ and $r = 0.277$, $p = 0.410$, 30-60 and 120-150 min tests, respectively) (Figure 1-4).

Urine volume/Urine sodium loss

Total urine volume was not significantly different between treatments (1617 ± 791 *vs.* 2018 ± 697 ml, PL *vs.* NA respectively, $p = 0.092$). There were also no significant differences between treatments in total urinary sodium loss during the 3 h trial (22.3 ± 17.6 *vs.* 38.2 ± 6.5 mEq, PL *vs.* NA respectively, $p = 0.083$). However, at the 180 minute void, urinary sodium loss was significantly lower in PL (0.8 ± 1.58 *vs.* 3.5 ± 3.1 mEq, PL *vs.* NA, $p = 0.016$, $n = 10$).

Performance Tests

Maximal Power

Maximal power (Pmax) significantly declined from pre- to post-exercise. Pmax significantly declined during PL ($p = 0.012$), but the change was not significantly lower during NA ($p = 0.057$; Table 1-5). Torque at Pmax also significantly declined from pre- to post-exercise in PL ($p = 0.045$), but not in NA ($p = 0.166$). There were no significant differences in velocity (revolutions per minute) at Pmax during either treatment.

Time Trial

Total work completed following the initial fixed 5 min effort was ~4 % higher during NA vs. PL (27.17 ± 4.93 *vs.* 26.21 ± 3.48 kJ, respectively). However, this difference in work was not statistically significant (p = 0.307). Average power output during the self-selected 15 min effort was 11 watts higher during NA vs. PL (302 ± 55 *vs.* 291 ± 39 watts, p = 0.307, respectively). The pattern of work output during the performance task is displayed in Figure 1-5.

Cognitive data

Comparing before and after the 3 h exercise bout, subjects significantly improved response time performance on the Stroop Color-Word Interference Test in NA (p = 0.009) but not in PL (p = 0.597) (Table 1-6). Pre- and post-tests between trials were not significantly different between treatments (p = 0.189, p = 0.305, pre and post tests, respectively), nor was error rate between treatments (p = 0.752)

Balance Data

The center of pressure displacement, in the mediolateral direction (ACOPml) post-trial for the 1 foot, eyes open stance was significantly greater in PL *vs.* NA (36.20 ± 14.90 *vs.* 30.94 ± 13.71 mm, p = 0.044) which indicates more postural sway in the mediolateral direction. Post-exercise mean anterior-posterior displacement (ACOPap) was also higher for PL *vs.* NA, but the increased displacement was not stastically different (45.9 ± 18.50 *vs.* 41.5 ± 16.4 mm, respectively, p = 0.092). A representative sample of COP tracings is presented in Figures 1-6A and 1-6B which exhibits the

displacement (sway) of the COP measured in meters traverses further along the x-axis (mediolateral) direction in the PL *vs*. NA.

DISCUSSION

Serum sodium concentration declines during prolonged exercise

While exercising in the heat for 3 h and maintaining body mass via sodium-free fluid consumption, serum sodium concentration significantly decreased by ~6 mEq/L in men that lose high amounts of sodium in their sweat. To our knowledge this is the first experimental reporting of a moderate decline in serum sodium concentration solely due to measured sweat sodium losses. It also provides support to the models proposed by Montain and colleagues (69, 70) which includes excessive sweat sodium losses as a contributing factor to reductions in serum sodium concentration. Our protocol was designed to maintain body mass via fluid replacement without replacing sodium lost in the sweat in order to elicit a decrease in serum sodium concentration. As serum sodium concentration is a balance of sodium content and fluid volume, serum sodium concentration declined as sodium content was lost via sweat while fluid status was maintained. This is also in agreement with previous reports of prolonged exercise in the heat while supplementing with low sodium or sodium-free fluid. In an investigation by Vrijens and Rehrer, males cycling for 3 h in the heat decreased serum sodium concentration by ~4 mEq/L, while drinking water (108). In another investigation of prolonged cycling with very low sodium ingestion (5 mEq/L), serum sodium concentration declined 3 mEq/L (85). Potential reasons for a larger decline in serum sodium concentration is our subjects were pre-selected based on their high sweat sodium losses. While this cannot be confirmed as sweat sodium concentration was not measured in the investigation by Vrijens and Rehrer (108), our subjects had twice as high total sweat sodium losses than in the study by Sanders et al (85). However, comparisons

should be made with caution, as sweat sodium concentration was only measured at 1 site in their study, instead of 4 sites, as in the present investigation. Another potential reason for a larger decline in serum sodium concentration in our study compared to the studies of Vrijens and Sanders may be due to a slightly different hydration status, as our subjects maintained body mass. In the other studies, the subjects slightly decreased body mass by ~ 1%. The decreased fluid volume would result in a higher serum sodium concentration.

Sodium chloride supplementation maintains serum sodium concentration

An important finding is that serum sodium concentration was prevented from falling by oral sodium chloride supplementation in these "high salt sweaters". This finding is significant, as this is the first investigation to our knowledge that matched sodium chloride ingestion to sweat sodium loss, thus preventing the decline in serum sodium concentration. In previous investigations, researchers supplemented subjects with different amounts of sodium with varying degrees of success in altering serum sodium concentration. In a field study by Speedy et al., subjects ingested an additional 6 grams of oral sodium supplementation distributed over 12.5 hours which resulted in a significant increase of 1.5 mEq in serum sodium concentration (96). Non-supplemented athletes with similar changes in body mass did not have any change in serum sodium concentration. In another field study during an ultra-distance triathlon, athletes were provided with sodium chloride or placebo pills (52). The investigators reported a non-significant, 1 mEq/L increase in the supplementation group and no change in the control group. However, only a small amount of additional sodium chloride was ingested by the supplementation group, 3.6 grams sodium over 12.6 hours. Food and fluid consumption

during the race was not controlled in either of these field experiments, thus total sodium consumption cannot be determined. Additionally, no measurements were made of sweat sodium losses. As sweat sodium losses are highly variable (77, 89, 106), fluid and sodium balance cannot be estimated with accuracy. In the previously mentioned study by Sanders et al. (85), serum sodium concentration only slightly declined with supplementation of 5 mEq/L and was slightly better maintained with 100 mEq/L, which may be due to the small sweat losses in the temperate environment (20C) or fluid compartment shifts. In support of our findings, Twerenbold et al. also found a better maintenance of serum sodium concentration with high vs. low sodium intake during exercise (104), as did Vrijens and Rehrer (108). However, there was still a small decline in serum sodium concentration while supplementing with sodium (~2 mEq/L) (104). A potential reason for our protocol successfully maintaining serum sodium concentration is that we provided a tapered fluid and sodium ingestion protocol by providing larger volumes early during exercise, smaller volumes as exercise progressed, and no fluid during the final 30 minutes of exercise to allow for fluid and sodium equilibration.

Sweat sodium concentration and extracellular sodium content

Despite losing ~13 % of estimated ECF sodium content (~300 mEq; ECF sodium content ~2350 mEq for 81kg male) or matching sweat sodium losses, sweat sodium concentration was not different between treatments ($p = 0.844$). The effects of daily sodium intake on sweat sodium concentration in salt depleted versus high intake have been previously investigated during heat acclimation (9, 66), but an acute effect on sweat sodium losses has not been thoroughly investigated. In an investigation by Sanders et al

(85), sweat sodium losses tended to decline (~20 %) during exercise as sodium supplementation increased during exercise. However, sweating rate similarly declined, thus comparisons cannot be made between investigations, as our sweating rate was not different between treatments.

An interesting finding was that sweat sodium concentration increased ~7-10 % from 30-60 minutes to 120-150 minutes with both treatments, which cannot be explained by our investigation. The alterations in sweat sodium are not due to a change in whole body sweating rate, as sweating rate was not significantly different between time points and sweat sodium concentration was not correlated with whole body sweating rate. An unlikely possibility is that the local sweating rate increased while the whole body sweating rate did not, as glandular sweating rate and sweat sodium concentration have a positive correlation (87). Another possibility is that the sweat glands fatigued resulting in a decreased reabsorption capability (103). Lastly, while the process of cleaning and drying the area where sweat was sampled from was the same for both measurements, it is possible a saturated stratum corneum resulted in a decreased glandular reabsorption. However, a wet stratum corneum has been shown to decrease sweating rate (18), which may result in a lower sweat sodium concentration, which was not the case.

While ingesting a placebo, the change in serum sodium concentration was significantly negatively correlated with sweat sodium concentration ($p = 0.005$, Figure 1-2) and total sweat sodium lost among the individual subjects ($p = 0 .011$, Figure 1-3). In a previous field study we conducted during an ultra-endurance triathlon, changes in serum sodium concentration were partially accounted for by rates of sweat sodium losses in males (77). Together both of our studies provide support for sweat sodium losses as a

factor in altering serum sodium concentration during exercise in the heat in males. While this finding has been included in hypothetical models of factors which may decrease serum sodium concentration, and potentially result in hyponatremia (69, 70), our investigations are the first to our knowledge that have directly found this relationship during prolonged exercise. Interestingly, in our previous field investigation, this relationship was not present in the female athletes. We postulated that it was due to the females more closely matching their sweat sodium losses with sodium ingestion. The current investigation also provides support for that hypothesis, as there is no relationship between sweat sodium losses and serum sodium concentration while supplementing with sodium matching sweat sodium losses ($r = 0.007$, $p = 0.984$ and $r = 0.277$, $p = 0.410$, at 30-60 and 120-150 min, respectively; Figure 1-4).

Sodium chloride supplementation and performance

Our findings of improved cognition and postural balance following exercise with sodium chloride supplementation vs. a placebo are similar to previous reports of elderly patients with large declines in serum sodium concentration who were admitted to emergency departments (81). Of 122 chronic hyponatremic elderly patients, 21 % were admitted with falls, indicating decreased balance. When compared to 244 matched controls also admitted to the emergency department, the hyponatremic patients had 4-fold more falls than the controls. Sixteen of the hyponatremic patients participated in cognitive testing and 12 participated in postural examination prior to and after treatment. In a small subset of patients, mean response times in cognitive tests were significantly slower (~9 %) when presented with asymptomatic hyponatremia compared to after

treatment. Balance, measured as center of pressure displacement during 3 steps in tandem, was also significantly worse, as the subjects had a 28 % greater displacement in the center of pressure. While our 10-17 % difference in post-exercise displacement between treatments was not as large as the hospital patients, our subjects only had a difference in post-test serum sodium concentration of ~5 mEq/L compared to the elderly patients who had a change of ~10 mEq/L. Additionally, our balance assessment was of young athletes in a static stance compared to the walking gait assessment in the elderly patients.

In our investigation response time during the Stroop Test was unchanged pre- to post-exercise when serum sodium declined in the PL group. However, response time significantly improved from pre- to post-exercise when sodium was given to maintain serum sodium concentration. An improvement in response time is similar to prior reports of athletes before and after a time trial lasting ~1 h (56). The authors concluded the improvement following strenuous exercise was either due to an increase in activation or possibly a placebo effect of exercise. As our investigation was double-blind, an increased arousal state may be a more likely cause for the 10% improvement in response time. This increased arousal may have been negated by the decline in serum sodium concentration during the placebo treatment.

Sodium supplementation resulted in a non-significant decline in maximal sprinting power and torque while power and torque declined significantly when serum sodium concentration was allowed to fall. However, time trial performance was not statistically significantly different between treatments, although subjects completed 4 % more work during the time trial. To our knowledge this is the only study undertaken that

matched sweat sodium losses with sodium intake, but others researchers have investigated performance with high and low amounts of sodium chloride intake during endurance exercise. Vrijens and Rehrer found a significant relationship between serum sodium concentration and exercise duration while cycling in the heat but the beverages were not isocaloric which could confound the interpretation and time to fatigue was not significantly different (108). Twerenbold et al found no difference in 4 h time trial performance in female runners when consuming 4 L of fluid with no, moderate, or high sodium content. However, the field study occurred in drastically different environmental conditions and ambient temperature had more of an effect on running performance than serum sodium concentration (104).

Our investigation is not without limitations. With only 3 h of exercise in the heat serum sodium concentration declined by ~6 mEq/L and resulted in decrements in balance and cognitive performance and small decrements in maximal sprint cycling power compared to when serum sodium concentration was maintained. It is possible that even further declines in performance would occur during longer duration exercise if prolonged sweat sodium losses are not replaced resulting in a further decrease in serum sodium concentration. As it is not ethical to induce hyponatremia, our experiment could not fully investigate the effects of large declines in serum sodium concentration on performance. While we did find small differences in physical performance between treatments, it is plausible that we would find larger differences with a larger decline in serum sodium concentration or possibly with a different endurance task. As declines in serum sodium concentration altered cognitive function, it is also possible that motivation may be altered, which may be more prevalent over a longer exercise task, instead of the 20 min

time trial that we chose. We chose this high intensity effort to replicate an actual race effort and to minimize overall test duration to limit interference by other factors of fatigue. However, a more appropriate protocol may be an exercise task to fatigue or a longer time trial. Our investigation was designed to maintain body mass with fluid intake to approximate complete hydration status. By providing larger volumes early during exercise, the subjects are over-hydrated until the end of the trial when fluid balance is reached. Therefore, the early decreases in serum sodium concentration are due both to fluid overload and sweat sodium loss. Also, as we maintained body mass via fluid consumption, there would be a slight hyperhydration due to substrate oxidation during 3 h of exercise. Additionally, we utilized a 4 site regional sweat patch technique and a weighted regression equation to estimate whole body sweat sodium losses. While some patch techniques may result in overestimation of whole body losses (111), it is unlikely that our method vastly overestimates losses. If there would have been a larger overestimation, it would have resulted in an increase in serum sodium concentration, a large expansion in plasma volume, large urine sodium losses, or a combination thereof. As this was not the case, our technique appears to be suitable for calculation of whole body sweat sodium losses.

In summary, serum sodium concentration declines ~6 mEq/L when athletes with high sweat sodium losses drink sodium-free fluid to maintain body mass while exercising in the heat for 3 h. When sodium chloride supplementation is provided to match sweat sodium losses, serum sodium concentration is maintained. Post-exercise postural balance is 11-14 % more stable following sodium supplementation compared to a placebo and response time during the Stroop Test improves 11 % pre- to post-exercise with sodium

chloride supplementation while there is no change when serum sodium concentration declines. Additionally, the declines in maximal cycling power are not significant while supplementing with sodium chloride, but maximal power is significantly lower when serum concentration decreases. Furthermore, acute sodium chloride supplementation and rapid changes in serum sodium concentration over 3 h do not alter sweat sodium concentration or sweat sodium losses during exercise. Therefore, serum sodium concentration significantly declines during prolonged exercise in males with high sweat sodium losses, but sodium supplementation matching sweat sodium losses will maintain serum sodium concentration and provide beneficial effects on physical and cognitive performance.

FIGURE LEGENDS

Figure 1-1. Testing protocol including preliminary, 3h exercise at 60% VO_2peak, and post tests. BAL – balance, COG – cognitive, BL – blood sample, Pmax – maximal power test, BM – body mass,  sweat testing

Figure 1-2. Relationship between sweat sodium concentration (mEq/L) and change in serum sodium concentration (mEq/L) at 30-60 min ($y = -0.0706x-1.6478$; $r^2=0.55$, $p = 0.009$) and 120-150 min ($y = -0.0571x-2.1351$; $r^2 = 0.60$, $p = 0.005$) sweat loss measurements while ingesting a placebo ($n = 11$).

Figure 1-3. Relationship ($y = -0.0129x - 1.8879$; $r^2= 0.53$, $p = 0.011$) between total sweat sodium (mEq) lost during 3 h of exercise at 60% VO_2peak and change in serum sodium concentration (mEq/L) while ingesting a placebo ($n = 11$).

Figure 1-4. Relationship between sweat sodium concentration (mEq/L) and change in serum sodium concentration (mEq/L) at 30-60 min ($y = 0.0009x-1.1033$; $r^2=0.00$, $p = 0.984$) and 120-150 ($y = 0.0352x-3.5322$; $r^2 = 0.08$, $p = 0.410$) sweat loss measurements while supplementing with sodium chloride matching sweat sodium losses ($n = 11$).

Figure 1-5. Pattern of power output (watts) during 20 min time trial performance starting at 10% above lactate threshold following 3 h of exercise at 60% VO_2peak in the heat with (NA) and without (PL) sodium chloride supplementation matching sweat

sodium losses (n = 9). Subjects were free to change the workrate following 5 minutes of cycling.

Figures 1-6A and 1-6B. Representative force plate tracings of the center of pressure (COP) for subject 2 following 3h of exercise in the heat at 60% VO_2peak with (NA; 1-6A) and without (PL; 1-6B) sodium chloride supplementation matching sweat sodium losses (n = 6).

Figure 1-1

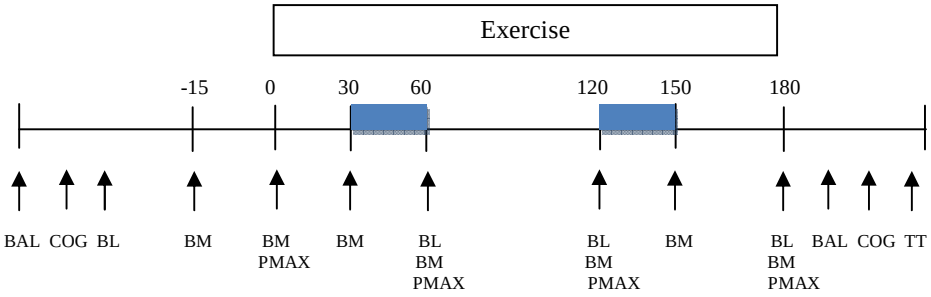
Exercise
-15
0
30
60
120
150
180
BAL COG BL
BM
BM PMAX
BM
BL BM PMAX
BL BM PMAX
BM
BL BM PMAX
BAL COG TT

Figure 1-2

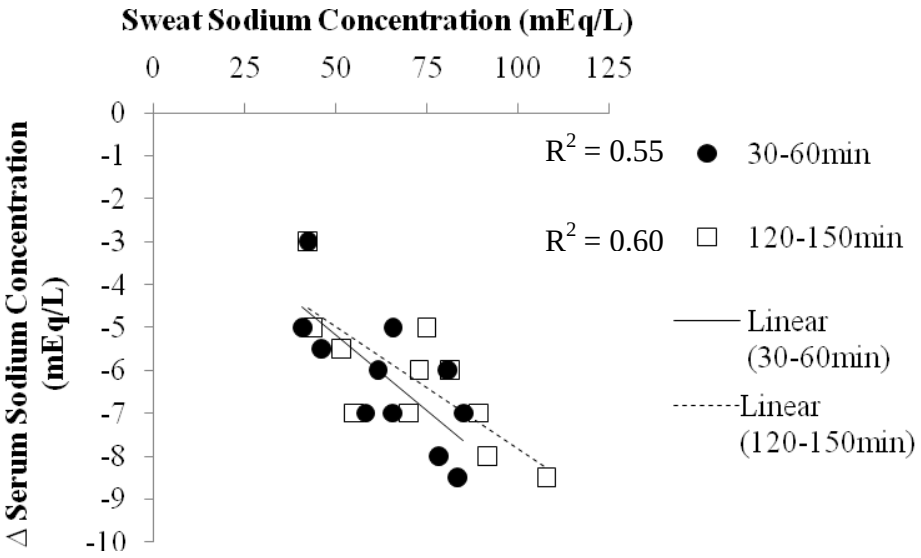
Sweat Sodium Concentration (mEq/L)
0
25
50
75
100
125
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Δ Serum Sodium Concentration (mEq/L)
R2 = 0.55
30-60min
R2 = 0.60
120-150min
Linear (30-60min)
Linear (120-150min)

Figure 1-3

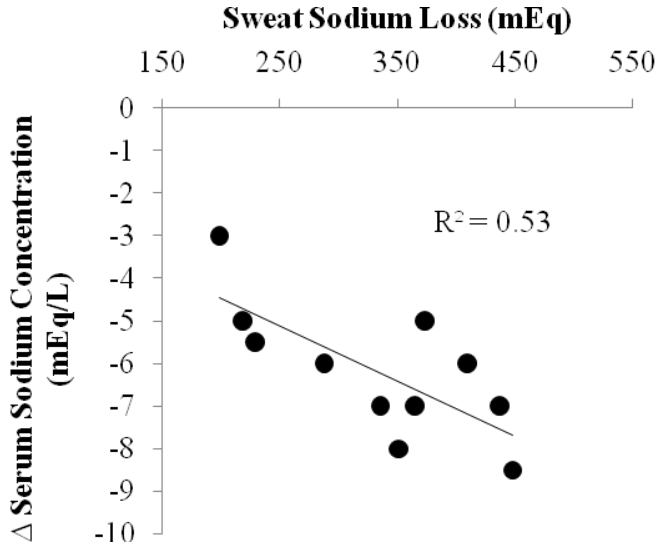
Sweat Sodium Loss (mEq)
150
250
350
450
550
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Δ Serum Sodium Concentration (mEq/L)
R² = 0.53

Figure 1-4

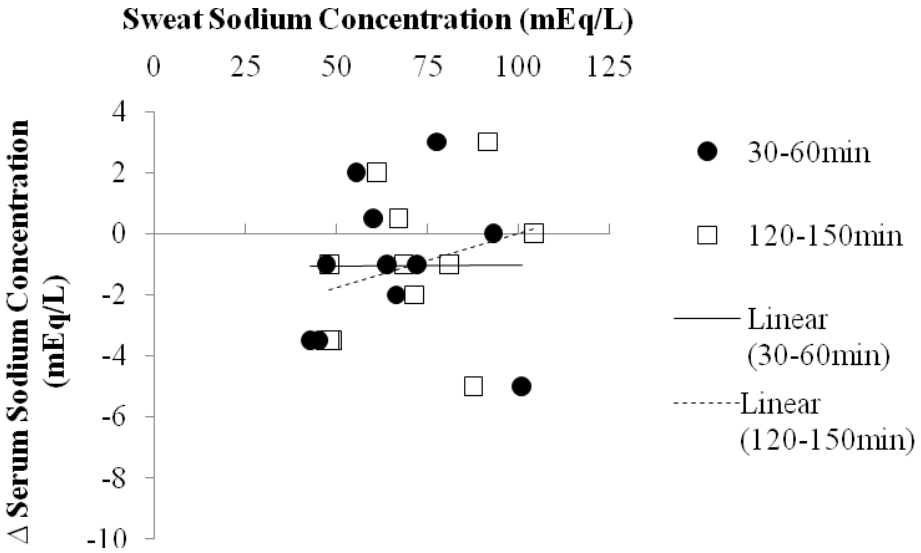
Sweat Sodium Concentration (mEq/L)
0
25
50
75
100
125
4
2
0
-2
-4
-6
-8
-10
Δ Serum Sodium Concentration (mEq/L)
30-60min
120-150min
Linear (30-60min)
Linear (120-150min)

Figure 1-5

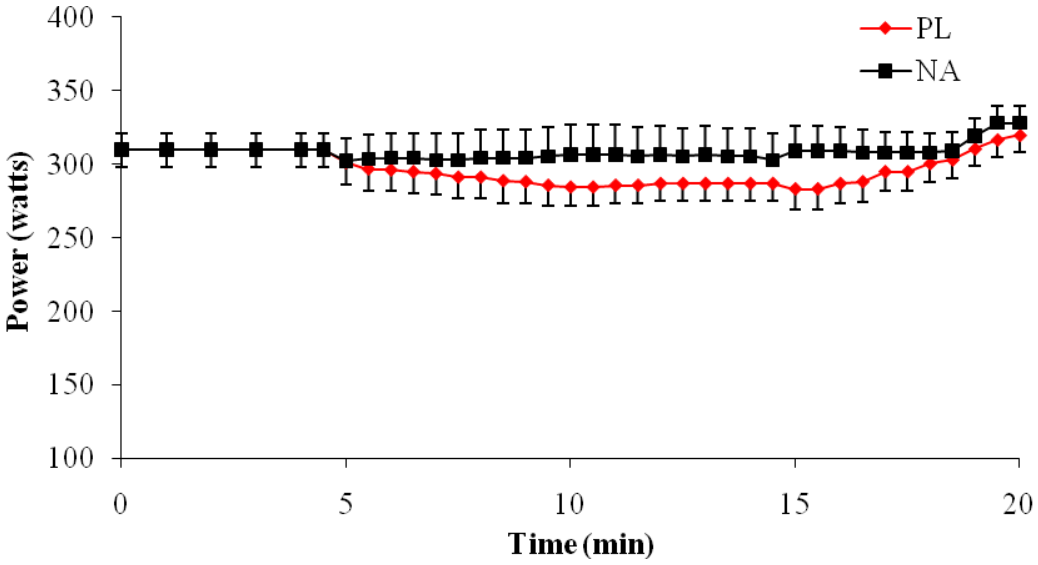
400
350
300
250
200
150
100
Power (watts)
0
5
10
15
20
Time (min)
PL
NA

Figure 1-6A

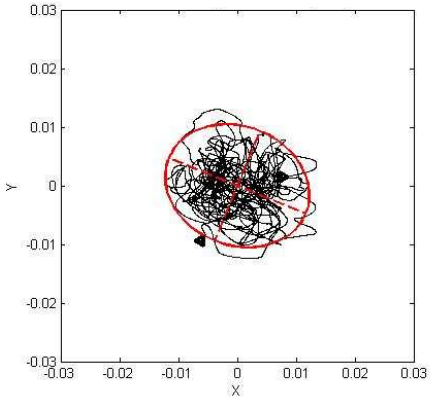

Figure 1-6B

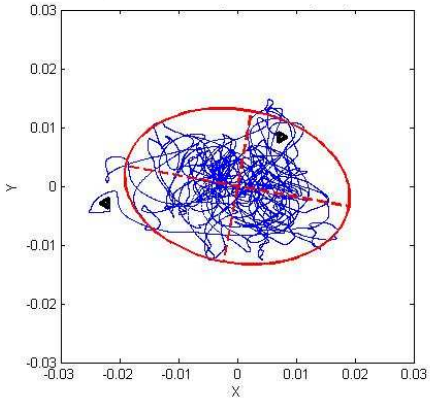

Table 1-1. Sweating characteristics for male endurance athletes (n = 36) cycling in the heat (31.8 ± 2.8 °C DB, 55.3 ± 8.2 % RH) at 70-80% HRmax. Values are mean ± SD.

Sweating Rate (L/h)	1.61 ± 0.55
Sweat Sodium Concentration (mEq/L)	51.4 ± 20.8
Sweat Sodium Loss (mEq/h)	87.4 ± 53.8

Table 1-2. Pre and post body mass during 3h cycling in the heat at 60% VO_2peak with (NA) and without (PL) sodium supplementation matching sweat sodium losses (n = 11). Values are mean ± SD.

	PL	NA
Pre Body Mass (kg)	81.1 ± 7.5	81.3 ± 7.7
Post Body Mass (kg)	81.4 ± 7.5	81.3 ± 7.7

Table 1-3. Blood markers measured before and after 3h cycling at 60% VO_2peak in the heat with (NA) and without (PL) sodium supplementation matching sweat sodium losses (n = 11). Values are mean ± SD.

		Pre-exercise	Post-exercise	p value
Serum Sodium (mEq/L)	PL	143.5 ± 1.79	137.1 ± 1.29*	0.001
	NA	143.4 ± 1.41	142.4 ± 2.54†	1.000
Serum Osmolality	PL	290 ± 3.0	276 ± 2.4*	0.001
(mOsm/L)	NA	289 ± 2.7	286 ± 5.2†	0.284
Hematocrit (%)	PL	45.7 ± 2.2	47.1 ± 2.5*	0.040
	NA	45.7 ± 3.5	44.8 ± 3.0†	0.348
Hemoglobin (mg/dL)	PL	14.9 ± 1.4	15.0 ± 0.8	1.000
	NA	14.8 ± 1.4	14.9 ± 1.2	1.000
MCHC (g Hb/dL)	PL	32.9 ± 2.8	31.9 ± 1.4	1.000
	NA	32.6 ± 2.1	33.2 ± 2.1†	0.561

* Significantly different from 30-60 min, $p < 0.05$. † Significantly different from PL.

Table 1-4. Sweating characteristics from 30-60 min and 120-150 min of the 3h exercise bout at 60% VO_2peak in the heat (n = 11). Values are mean ± SD

		30-60 min	120-150 min
Sweating Rate (L/h)	PL	1.68 ± 0.26	1.74 ± 0.24
	NA	1.76 ± 0.21	1.67 ± 0.11
Sweat Sodium Concentration (mEq/L)	PL	64.2 ± 16.3	70.8 ± 21.1*
	NA	65.8 ± 19.0	70.6 ± 18.9*
Sweat Sodium Loss (mEq/h)	PL	109.0 ± 36.7	123.2 ± 39.8*
	NA	115.5 ± 35.6	117.1 ± 30.2

* Significantly different from 30-60 min, $p < 0.05$.

Table 1-5. Maximal power per revolution, and torque and velocity at maximal power before and after 3h cycling at 60% VO_2peak in the heat with (NA) and without (PL) sodium chloride supplementation matching sweat sodium losses (n = 11). Values are mean ± SD.

		Pre-Exercise	Post-Exercise
Pmax (watts)	PL	1253 ± 142	1201 ± 164*
	NA	1242 ± 142	1207 ± 149
Torque (Nm)	PL	98.4 ± 10.8	94.6 ± 12.4*
	NA	98.8 ± 10.5	95.4 ± 9.7
Velocity (rev/min)	PL	121.1 ± 7.2	120.5 ± 5.1
	NA	121.0 ± 6.2	120.5 ± 5.5

*Significantly different from pre-exercise, $p < 0.05$.

Table 1-6. Mean response time for a correct response during Stroop Color-Word Interference Task before and after 3h cycling at 60% VO_2peak (n = 11). Values are mean ± SD.

	NA	PL
Pre-exercise Response Time (sec)	1.22 ± 0.22	1.14 ± 0.14
Post-exercise Response Time (sec)	1.09 ± 0.21*	1.16 ± 0.25
p value	0.009	0.597

*Significantly different from pre-exercise, $p < 0.05$.

Chapter IV: Study 2

Effects of Oral Sodium Chloride Supplementation on Blood Volume, Stroke Volume, and Sweat Sodium Concentration During Exercise in Untrained Males

ABSTRACT

Plasma volume expansion via intravenous infusion increases stroke volume in untrained men by 10-15%. Oral sodium supplementation is a plausible alternative for increasing plasma volume. The purpose of this investigation was to determine the effects of sodium chloride supplementation on plasma, blood, and stroke volume at rest and during exercise in young healthy untrained/recreationally active males. We also investigated the effects of sodium chloride supplementation on sweat sodium concentration during exercise in the heat. Following 3 d of supplementation with sodium chloride (NA; 3.5 mEq sodium/kg body mass/d) or a placebo (PL) subjects exercised at 60% VO_2peak for 15min. Plasma volume (PV) and blood volume (BV) increased with NA compared to PL at rest (5.9±7.6% and 3.2±4.4%, PV and BV respectively), during exercise at 60% VO_2peak (8.6±5.2% and 4.3±3.1%, PV and BV, respectively), and post-exercise (5.0±4.1% and 2.9±2.8%, PV and BV, respectively) *vs.* PL. Stroke volume increased ~10% during exercise with NA *vs.* PL (139±27.2 ml/beat *vs.* 126±23.7ml/beat, respectively, p=0.004). Cardiac output increased ~8% during exercise with NA (21.0±3.1 *vs.* 19.4±2.6, NA *vs.* PL, respectively, p=0.013). Mean arterial pressure was not different in NA *vs.* PL during exercise (p=0.548). Sweat sodium concentration was significantly higher during exercise in the heat in NA *vs.* PL (70.4±19.5 *vs.* 64.5±21.7 mEq/L, p=0.044, respectively). In conclusion, sodium chloride supplementation increases plasma, blood, and stroke volume during exercise in healthy untrained males. Sweat sodium concentration is higher during exercise in the heat following sodium supplementation compared to a placebo.

INTRODUCTION

One of the many adaptations to endurance training is increased stroke volume during exercise (97, 112). The higher stroke volume in trained versus untrained individuals is partially due to an expanded plasma and blood volume (57, 63). The contribution of plasma volume on stroke volume in trained and untrained subjects has been investigated by intravenous infusion (57, 63). However, intravenous infusion is an invasive technique. Oral sodium chloride ingestion has also been used to increase plasma volume resulting in an increased stroke volume at rest in young healthy adults (28, 29, 50, 107). When comparing a high *vs.* low sodium diet, healthy older males (~57 y) increased plasma and stroke volume during rest and also during a ramp exercise protocol (29). However, to our knowledge, this method of raising blood volume and stroke volume via sodium ingestion has not been investigated during steady state exercise in young healthy individuals.

Sweat sodium concentration and sweat sodium losses in the heat are highly variable between individuals (77, 89). In our prior investigation of 71 male and female subjects, we found a coefficient of variation (standard deviation/mean) of ~31 % in sweat sodium concentration and ~54 % in sweat sodium losses (77). While the reasons for the variability are not entirely clear, daily sodium intake has been implicated as a potential factor (23). The effects of daily sodium chloride ingestion on altering sweat sodium concentration have been investigated during the heat acclimatization process (4, 7, 23, 66). However, as sweat sodium concentration is also reduced with heat acclimatization, the effects of sodium chloride intake on sweat sodium concentration alone cannot be determined from these investigations.

The purpose of this investigation was to determine if 3 days of increased oral sodium chloride ingestion would increase blood volume and stroke volume at rest and during exercise in young healthy untrained/recreationally active males. We also investigated the effects of sodium chloride ingestion on sweat sodium concentration during exercise in non-acclimatized males.

METHODS

Subjects

The nine participants for this investigation were young, untrained or recreationally active males. Their age (mean ± SD), body mass, height, and VO_2peak were 27 ± 6 y, 76.0 ± 7.9 kg, 175.4 ± 9.6 cm, 3.85 ± 0.35 L/m, respectively. Testing was undertaken in early spring so that the participants were not heat acclimatized which is known to increase plasma volume (7, 9, 109). Participants signed a consent form approved by the Institutional Review Board at The University of Texas at Austin.

Preliminary and Familiarization Testing

In order to obtain baseline measures and ensure that participants were adequately prepared and familiarized with the experimental trials, preliminary testing was undertaken 3 to 7 days prior to the first experimental trial. During the preliminary session VO_2peak and the sub-maximal VO_2 to workload relationships were determined. Volume and gas measurements were made during 3 5-minute sub-maximal stages followed by an incremental exercise protocol lasting 7-12 minutes to determine VO_2peak. Peak oxygen consumption was defined as a stabilization of VO_2 with increasing work rate and respiratory exchange ratios of >1.10. Participants were also familiarized with the acetylene wash-in technique for the determination of cardiac output.

Dietary Protocol

Participants maintained a dietary intake record for 3 days prior to the first experimental session and repeated it for the second session. Participants were also

instructed to refrain from eating high sodium containing foods during this time. Participants drank 30 ml of fluid per kilogram body per day for the 3 days leading up to the experimental trials. Subjects also performed an overnight fast ($\geq$ 12 h) and consumed an additional 500 ml of water 2 h prior to each experimental session.

Experimental Protocol

Participants ingested either a sodium chloride supplement (NA, 3.5 mEq sodium/kg body mass per day, ~ 15 g sodium chloride per day) or a placebo (PL, sucrose) via capsule in addition to their normal diet for 3 d prior to experimental sessions. Approximately 7 to 11 days separated the randomized, double-blind treatments to serve as a washout period. Following the supplementation period and the overnight fast, participants performed a bout of upright cycling exercise for 15 min at 60 % of VO_2peak in a thermoneutral environment (22 ˚C, no fan) in order to evaluate cardiovascular function. Core temperature via rectal thermistor (YSI 401) and skin temperature via surface thermistor (YSI 409A) were continuously recorded during the trial and averaged over 1 min at 5 min intervals. Cardiac output via acetylene wash-in (60) was measured at 5, 8, 11, and 14 minutes during exercise, and 5 and 8 minutes post-exercise. Stroke volume was calculated from cardiac output and actual heart rate (Suunto, Finland) during measurement (SV = CO/HR). Blood pressure (Tango+) was measured at 7 and 13 min of exercise, and 6 min post-exercise. Blood samples were taken via an indwelling venous catheter prior to beginning exercise, at 5, 10 and 15 min of exercise and 7 min post-exercise in order to measure hematocrit and hemoglobin, and thus calculate changes in

blood volume. Pre-exercise urine specific gravity was measured to ensure hydration status (USG < 1.020).

Sweating Analysis Protocol

Following the cardiovascular testing in thermoneutral conditions, participants completed a sweating analysis protocol in the heat to measure sweat sodium concentration. Participants exercised for a total of 45 min in warm conditions (35 ˚C dry bulb, 50 % relative humidity). Subjects cycled for 15 min at 50% of VO_2peak without fan cooling to elicit sweating. Following the 15 min warm-up subjects cycled a stationary ergometer for 30 minutes at 50% of VO_2peak with fan cooling from the front and back (1.5 ± 0.2 m/s and 2.1 ± 0.2 m/s, respectively). Whole body sweating rate, calculated via nude body mass changes, and regional sweat sodium concentration of the forearm, upper back, mid-anterior thigh, and calf, utilizing a regional sweat patch technique (77, 89), were measured during the 30 min testing period. Participants consumed 400 mL of water during the exercise bout to prevent excessive dehydration. Core (YSI 401) and skin (YSI 409A) temperature was recorded at 0, 15, and 30 min and blood pressure (Tango+) was measured at 20 min of exercise.

Measurements of gas exchange

Subjects breathed through a pneumotachometer (Hans Rudoloph, Kansas City, MO) and two-way non rebreathing valve (2700 Series, Hans Rudolph, Shawnee, KS). Oxygen and carbon dioxide gases were continuously sampled at the mouthpiece via a 6 ft capillary tube. Gas concentrations were measured by a mass spectrometer (Perkin Elmer

MGA 1100, St. Louis, MO) interfaced with a computer for calculation of breath by breath oxygen consumption and carbon dioxide production (Beck Integrated Physiological Testing System).

Open circuit acetylene wash-in for the determination of cardiac output was performed as described by Johnson et al (60). Briefly, at the end of a full expiration, the participants breathed for a minimum of 8 breaths through a mouthpiece connected to a bag filled with mixed gases, including 0.7% acetylene, 9.0% helium, 21% oxygen, and balance nitrogen. The concentrations of acetylene and helium were monitored by continuous sampling at the mouthpiece using a mass spectrometer (Perkin Elmer MGA 1100, St. Louis, MO) interfaced to a computer for the calculation of cardiac output (Beck Integrated Physiological Testing System).

Blood analysis

Hematocrit was measured in triplicate following microcentrifugation for 15 minutes. Hemoglobin was measured in triplicate using the cyanmethemoglobin method (35). Plasma and blood volume changes were determined via the method of Dill and Costill (33). Whole blood was stored at room temperature until clotting occurred and then was centrifuged for 20 min prior to the removal of serum. Serum sodium concentration was measured via electrochemistry (NOVA 5, Waltham, MA).

Sweating rate and sweat sodium concentration analysis

Sweating rate (L/h) was calculated as the change in nude body mass, accounting for fluid consumption. Upon removal of the sweat patches, the gauze sponge was

immediately separated from each Tegaderm® bandage and placed into a filterless separation tube. The sweat content of the sponges was obtained via centrifugation and transferred into four 5 ml plastic test tubes and capped. Sweat electrolyte concentration was measured with a Nova 5 Analyzer (Waltham, MA) with a manufacturer reported CV of 2% for sodium analysis. A modified weighted equation was utilized to calculate whole body sweat sodium concentration (sweat sodium concentration = 0.11([Arm]) + 0.276([Back]) + 0.299([Thigh]) + 0.315([Calf])) (8).

Statistical Analysis

Data are reported as mean and standard deviation. Multiple measures taken during or post-exercise were averaged and reported as mean values. A paired student's t-test and a two way repeated measures analysis of variance was performed to analyze differences. Significance was set at an alpha level of 0.05.

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RESULTS

Three days of oral sodium chloride ingestion (15.7 ± 1.7 g NaCl/day) in addition to participants' normal diet slightly but significantly increased resting serum sodium concentration above the placebo treatment (146.8 ± 1.0 mEq/L *vs.* 146.1 ± 1.1 mEq/L, NA *vs.* PL, respectively, p = 0.002). Hemoglobin and hematocrit were significantly lower in NA *vs.* PL pre-exercise, during exercise, and post-exercise (16.0 ± 1.1 mg/dL *vs.* 15.4 ± 1.1 mg/dL, PL *vs.* NA, respectively, p = 0.008; 48.6 ± 3.0 % *vs.* 47.0 ± 3.3 %, PL *vs.* NA, respectively, p = 0.006) (Table 2-1). Compared to placebo, plasma and blood volume were increased during exercise at 60 % VO_2peak (8.6 ± 5.2 % and 4.3 ± 3.1 %, PV and BV, respectively). Plasma and blood volume also increased at rest before (5.9 ± 7.6 % and 3.2 ± 4.4 %, PV and BV, respectively), and after exercise (5.0 ± 4.1 % and 2.9 ± 2.8 %, PV and BV, respectively).

There were no significant differences in oxygen consumption between treatments during exercise (2.31 ± 0.23 L/min *vs.* 2.35 ± 0.31 L/min, PL *vs.* NA, respectively, p = 0.625) (Figure 2-1). Stroke volume was significantly higher with sodium chloride supplementation compared to placebo during exercise (126.3 ± 23.7 ml/beat *vs.* 139.0 ± 27.2 ml/beat, PL *vs.* NA, p = 0.004; Figure 2-2) and post-exercise (102.8 ± 23.7 ml/beat *vs.*124.0 ± 27.9 ml/beat, PL *vs.* NA, respectively, p = 0.003).

There was an overall time effect on heart rate as it drifted from 5 to 15 minutes of exercise (145.5 ± 13.4 beats/min *vs.* 161.7 ± 16.1 beats/min, 5 min *vs.* 15 min, respectively, p = 0.001, Figure 2-3). Heart rate was ~2 % lower during exercise and ~4 % lower post-exercise in NA compared to PL, but this difference was not statistically different (p = 0.123 and p = 0.060, exercise and post-exercise, respectively). As heart

rate tended to be lower and stroke volume was significantly higher, there was a left-upward shift in the stroke volume *vs.* heart rate response during exercise in NA compared to PL (Figure 2-4).

Cardiac output was significantly higher during sodium chloride supplementation *vs.* placebo during exercise (21.0 ± 3.1 L/min *vs.* 19.4 ± 2.6 L/min, NA *vs.* PL, respectively, p = 0.013) and post-exercise (12.2 ± 2.4 L/min *vs.* 10.4 ± 2.1 L/min, NA *vs.* PL, respectively, p = 0.004) (Figure 2-5). A summary of the percent changes in cardiovascular measures between treatments is presented in Figure 2-6.

Mean arterial pressure was not significantly different between treatments during exercise (110 ± 8.7 mmHg *vs.* 108 ± 6.9 mmHg, PL *vs.* NA, respectively, p = 0.548) or post-exercise (95 ± 6.1 mmHg *vs.* 96 ± 5.2 mmHg, PL *vs.* NA, respectively, p = 0.817) (Figure 2-7). Systolic blood pressure was also not significantly different during exercise (178.8 ± 14.3 mmHg *vs.* 173.4 ± 11.7 mmHg, PL *vs.* NA, respectively, p = 0.089) or post-exercise (125.7 ± 9.2 *vs.* 125.9 ± 9.8 mmHg, PL *vs.* NA, respectively, p = 0.960) (Figure 2-7). Diastolic blood pressure was similar in both treatments during exercise (75.1 ± 10.7 mmHg *vs.* 75.9 ± 11.0 mmHg, PL *vs.* NA, respectively, p = 0.808) and post-exercise (79.7 ± 7.9 mmHg *vs.* 80.6 ± 7.7 mmHg, PL *vs.* NA, respectively, p = 0.755) (Figure 2-7).

There was a significant treatment effect on total peripheral resistance as NA was significantly lower than PL during exercise (470.5 ± 122.4 dyne/sec/cm^5 *vs.* 425.2 ± 95.9 dyne/sec/cm^5, PL *vs.* NA, respectively, p = 0.027) and post-exercise (750.6 ± 191.4 dyne/sec/cm^5 *vs.* 610.2 ± 98.2 dyne/sec/cm^5, PL *vs.* NA, respectively, p = 0.014) (Figure 2-8). A summary of percent changes in hemodynamic variables is presented in Figure 2-

9. There were no significant differences in core temperature (37.0 ± 0.6 °C *vs.* 37.0 ± 0.7 °C, p = 0.757) or skin temperature (30.1 ± 0.4 °C *vs.* 30.1 ± 1.0 °C, p = 0.906) between treatments.

Sweating Analysis Results

Dry bulb (DB) and wet bulb (WB) temperature and relative humidity (RH) were not different between sweating analysis trials (35.4 ± 0.7 °C *vs.* 35.4 ± 0.5 °C DB, p = 0.938; 28.8 ± 0.8 °C ± *vs.* 29.0 ± 0.8 °C WB, p = 0.687; 50.1 ± 6.8 % *vs.* 53.3 ± 2.8 % RH, p = 0.128). Sweat sodium concentration was ~9 % higher during sodium chloride supplementation compared to placebo (64.5 ± 21.7 *vs.* 70.4 ± 19.5 mEq/L, PL *vs.* NA, respectively, p = 0.044) (Figure 2-10) and displayed a similar coefficient of variation to prior investigations (77). Sweating rate was not different between treatments (1.15 ± 0.25 L/h *vs.* 1.29 ± 0.20 L/h, PL *vs.* NA, respectively, p = 0.078). Sweating rate and sweat sodium concentration were significantly related during NA (r = 0.698, p = 0.036), but not during PL (r = 0.261, p = 0.498). Core temperature (38.1 ± 0.2 °C *vs.* 38.0 ± 0.3 °C, PL *vs.* NA, respectively, p = 0.159) and skin temperature (34.1 ± 0.6 °C *vs.* 34.3 ± 0.7 °C, PL *vs.* NA, respectively, p = 0.117) were also not different between trials.

Mean arterial pressure was not significantly different between treatments at 20 min while exercising in the heat during the sweating analysis trial (98 ± 9.6 mmHg *vs.* 96 ± 8.5 mmHg, PL *vs.* NA, respectively, p = 0.555). Systolic and diastolic blood pressure were also similar between treatments, respectively (159 ± 18.0 mmHg *vs.* 160 ± 15.7 mmHg, PL *vs.* NA, respectively, p = 0.718; 67.4 ± 9.9 mmHg *vs.* 64.3 ± 9.3 mmHg, PL *vs.* NA, respectively, p = 0.230).

DISCUSSION

Three days of increased sodium chloride intake resulted in a very small, yet statistically significant increase in serum sodium concentration at rest and during exercise. Sodium chloride supplementation also resulted in an increase in plasma and blood volume at rest (5.9 and 3.2 %, respectively) and during exercise (8.6 and 4.3 %, respectively). Based on an average blood volume of 70 ml/kg body mass in untrained males (27, 63) plasma and blood volume would have increased ~ 170 and 210 ml, at rest and during exercise, respectively, compared to a placebo.

While we didn't measure extracellular sodium content, we did measure serum sodium concentration and plasma and blood volume changes. As serum sodium concentration is a calculation of sodium content and extracellular intravascular volume, and both serum sodium concentration and extracellular vascular volume increased, sodium content also increased. Based on an average blood volume of 70 ml/kg body mass in untrained males (27, 63) and the presently measured serum sodium concentration and plasma volume changes, estimated intravascular and ECF sodium content increased ~26 and 105 mEq, respectively, at rest, an estimated retention of ~13%. While these values are estimates, they are similar to calculated sodium balance increases of ~17-33% at rest in previous studies with longer sodium supplementation protocols using lower levels of supplementation (58.5-215 mEq Na/d) (50, 107).

Stroke volume also increased ~10 % during exercise and ~20 % during recovery at rest following sodium chloride supplementation. Stroke volume was also better maintained during exercise in NA compared to PL. While during NA, SV was maintained at the higher level in NA from 5 to 14 min of exercise, stroke volume during

PL significantly declined. The percent difference in stroke volume between treatments almost doubled from 5 to 14 minutes during exercise (7.7 % and 13.2 %, 5 and 15 min, respectively). The higher exercising stroke volume due to the increase in plasma volume from sodium supplementation was similar to the effects of plasma volume expansion via intravenous infusion in untrained males (57, 63). Hopper et al. infused 400 ml of dextran, a plasma volume expander, in untrained males which resulted in an increase of 11 % in stroke volume during exercise at ~56 % of VO_2peak (57). Krip et al. also increased stroke volume by ~10 % in untrained males with 550 mL of infusate (63). To our knowledge, only 1 other investigation has compared the effects of varying levels of chronic daily sodium chloride ingestion on stroke volume during exercise. Both older heart failure patients and healthy age matched (~57y) male controls increased sodium intake for 7 days (29). Both groups increased stroke volume index during exercise from day 1 to day 7. The age-matched controls increased exercising stroke volume ~5-7 % compared to pre supplementation measurements. Their investigation was different from the current one as the subjects ingested a very low (70 mmol/d) or very high sodium diet (250 mmol/d) over the course of 7 days. Furthermore, the exercise task was a ramp cycling protocol to exhaustion, not steady state. While our supplementation period was less than half as long, and we compared a normal *vs.* high sodium chloride ingestion, our crossover design investigation yielded a larger increase in exercising stroke volume (10 % *vs.* ~6 %) and plasma volume (9 % *vs.* 7 %) during the NA compared to PL trials. Possible differences include adaptation to increased sodium ingestion over 7 days, subject age, or different exercise protocols.

In our investigation, cardiac output also increased during exercise (~8 %) and during recovery at rest (~17 %) compared to PL. The increases in stroke volume and cardiac output occurred during a small insignificant change in heart rate (~2-4 %) and without a change in oxygen consumption. While the young healthy untrained males in our study responded to sodium chloride supplementation with an increase in blood volume, there was no change in mean arterial pressure. This result was due to a decrease in total peripheral resistance during exercise (~11 %) and at rest following exercise (~23 %). This decrease in TPR is similar to prior reports of TPR at rest during increased sodium chloride ingestion (29, 107).

Sweat sodium concentration increased ~9 % while exercising in the heat at 50 % VO_2peak as a result of sodium chloride supplementation. To our knowledge this is the first investigation using a crossover design which compared the effects of normal *vs.* high daily sodium chloride ingestion on sweat sodium losses during exercise in the heat in non heat-acclimatized males. Prior investigations which found a relationship between sodium ingestion and sweat sodium loss either used sodium depletion protocols (66, 82) or measured sweat sodium changes during a heat acclimation protocol, which results in sodium conservation effects (7). Costa et al. did find a relationship between sodium intake and loss during normal or high sodium ingestion, but they did not use a cross-over design (23). As sweat sodium loss is extremely variable between individuals (77, 78, 89, 106), interpretation of sodium ingestion or individual differences in their investigation cannot be made. In contrast to the previous studies, Allsopp et al. found no relationship between sodium chloride ingestion and sweat sodium concentration during an 8 day

supplementation period, which may due to the very long, 12 h, collection period or adaptation to the increased sodium intake (4).

Interestingly, during PL, there was no relationship between sweating rate and sweat sodium concentration ($p = 0.498$) while there was a relationship during NA ($p = 0.036$). Sweating rate was not significantly different between treatments, although it was ~12 % higher in NA. As sweating rate was correlated with sweat sodium concentration in NA, similar to prior investigations (3, 87), the trend for a higher sweating rate may have partially contributed to the increased sweat sodium concentration between treatments. Another potential reason for the increase in sweat sodium may be due to a decrease in aldosterone which is known to occur during high sodium ingestion (29, 107). As aldosterone decreases, the sodium conserving effects it exhibits on sweat glands would be diminished (86), resulting in a higher sweat sodium concentration. In a study of low, moderate, and high sodium intake, Allsopp et al. (4) found a significantly lower aldosterone response to heat exposure during heat acclimation in high *vs.* moderate sodium intake. This coincided with a large, yet non-significant, 24 % increase in sweat sodium concentration during increased sodium ingestion (4). While the small increase in sweat sodium concentration (~6 mEq/L) in the current investigation is likely partially due to a decrease in aldosterone, this is unclear, as we did not measure plasma aldosterone levels.

As all of our exercising measures of cardiac output were made during moderate intensity exercise, it is unclear whether increases in blood volume and stroke volume would also occur at higher exercise intensities in young untrained males. It is also unclear whether maximal cardiac output or maximal oxygen consumption would be

increased via sodium chloride supplementation. However, as the effects of sodium chloride supplementation are similar to plasma volume expansion via intravenous infusion, maximal oxygen consumption may also increase as it does with intravenous infusion of a plasma volume expander (27).

Our participants included untrained/recreationally active, non heat-acclimatized males. We chose this population, as they are known to have sub-optimal exercising blood volumes compared to endurance trained athletes (57, 63). Thus, it is not clear whether sodium chloride supplementation would be beneficial to endurance trained athletes, assuming that blood volume expansion is the reason for the increase in stroke volume and cardiac output.

In summary, plasma and blood volume increased ~4-9 % during exercise following 3 days of increased sodium intake (~15 g NaCl/d) in untrained males. Stroke volume and cardiac output also significantly increased (10 and 8 %, respectively) following sodium chloride supplementation while oxygen consumption and mean arterial pressure was unchanged. Thus, oral sodium chloride supplementation afforded similar cardiovascular benefits of an invasive intravenous infusion of plasma volume expander. Furthermore, the large daily increase of sodium chloride resulted in a 9 % higher sweat sodium concentration during exercise in the heat.

FIGURE LEGENDS

Figure 2-1. Oxygen consumption (L/min) during exercise at 60% VO_2peak and at rest following 3 days of sodium chloride supplementation (3.5 mEq/kg/d; NA) or placebo (PL) (n = 9).

Figure 2-2. Stroke volume (ml/beat) during exercise at 60% VO_2peak and post-exercise following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d; NA) or placebo (PL) (n = 9). '*' indicates significantly different from PL, $p < 0.05$. '†' indicates significantly different from 5 minutes of exercise, $p < 0.05$. '__*__' indicates significant treatment effect, $p < 0.05$.

Figure 2-3. Heart rate (beats/min) during exercise at 60% VO_2peak and post-exercise following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d; NA) or placebo (PL) (n = 9).

Figure 2-4. Stroke volume (ml/beat) *vs.* heart rate (beats/min) relationship during exercise at 60% VO_2peak following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d; NA) or placebo (PL) (n = 9).

Figure 2-5. Cardiac output (L/min) during exercise at 60% VO_2peak and post-exercise following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d; NA) or placebo

(PL) (n = 9). '*' indicates significantly different between treatments, $p < 0.05$. '__*__' indicates significant treatment effect, $p < 0.05$.

Figure 2-6. Percent difference, compared to when ingesting a placebo, in heart rate (HR, beats/min), stroke volume (SV, ml/beat), and cardiac output (CO, L/min) during exercise at 60% VO_2peak following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d) (n = 9). '*' indicates significantly different between treatments, $p < 0.05$.

Figure 2-7. Systolic blood pressure (SBP, mmHg), mean arterial pressure (MAP, mmHg), and diastolic blood pressure (DBP, mmHg) during exercise at 60% VO_2peak and post-exercise following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d; NA) or placebo (PL) (n = 9).

Figure 2-8. Total peripheral resistance (dyne/sec/cm^5) during exercise at 60% VO_2peak and post-exercise following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d; NA) or placebo (PL) (n = 9). '__*__' indicates significant treatment effect, $p < 0.05$.

Figure 2-9. Percent change in systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), and total peripheral resistance (TPR, dyne/sec/cm^5) during exercise at 60% VO_2peak following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d) compared to when ingesting a placebo (n = 9). '*' indicates significantly different between treatments, $p < 0.05$.

Figure 2-10. Sweat sodium concentration in individual subjects during exercise at 50% VO_2peak in the heat (35 °C, 50% RH) following 3 days of sodium chloride supplementation (3.5 mEq Na/kg/d) or placebo (n = 9). The dark bar displays the mean response.

Figure 2-1

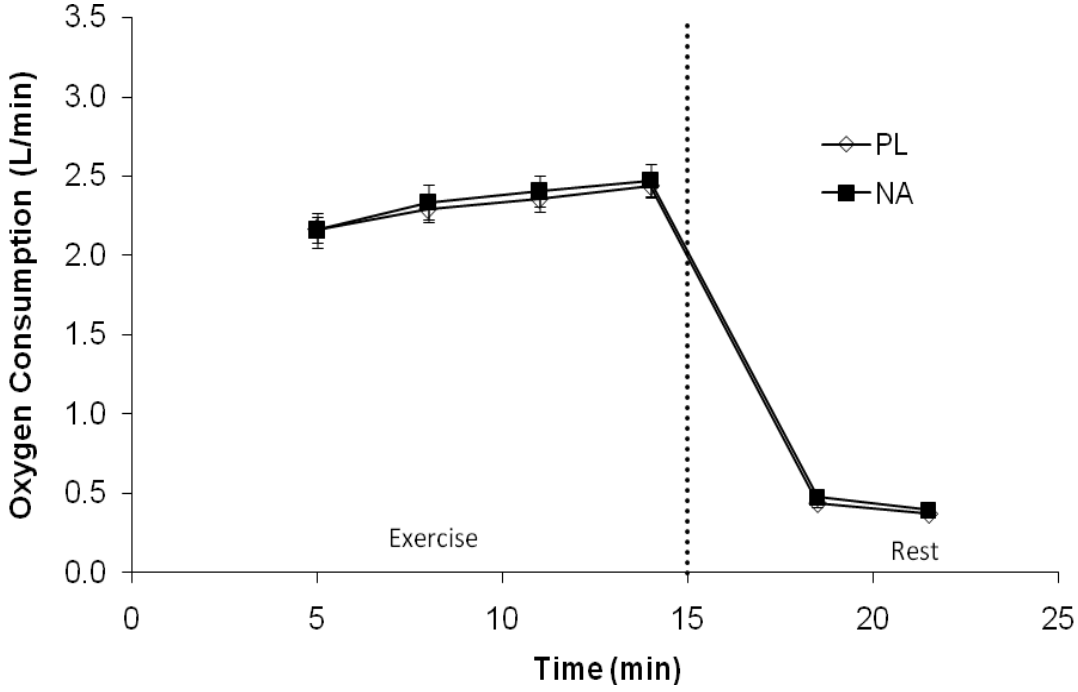
Oxygen Consumption (L/min)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Time (min)
PL
NA
Exercise
Rest

Figure 2-2

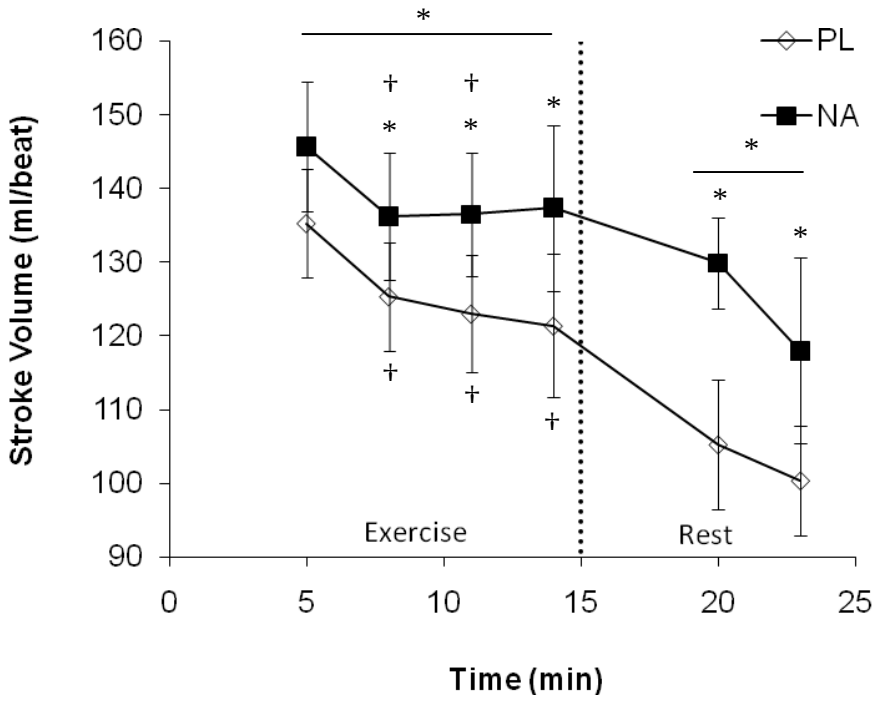
Stroke Volume (ml/beat)
160
150
140
130
120
110
100
90
0
5
10
15
20
25
Time (min)
Exercise
Rest
PL
NA
*
†

Figure 2-3

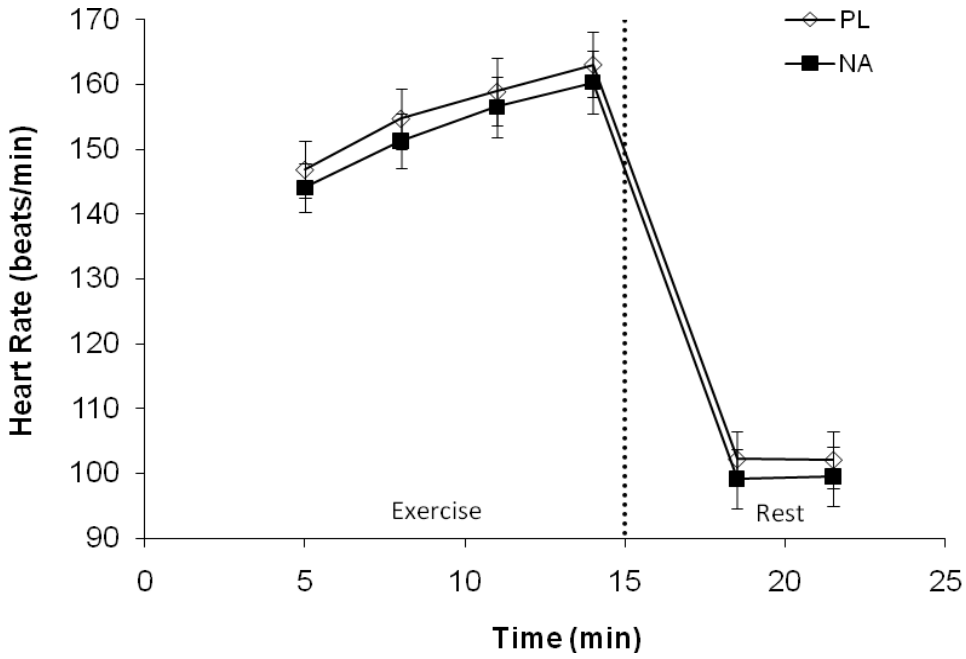
PL
NA
Heart Rate (beats/min)
170
160
150
140
130
120
110
100
90
Exercise
Rest
0
5
10
15
20
25
Time (min)

Figure 2-4

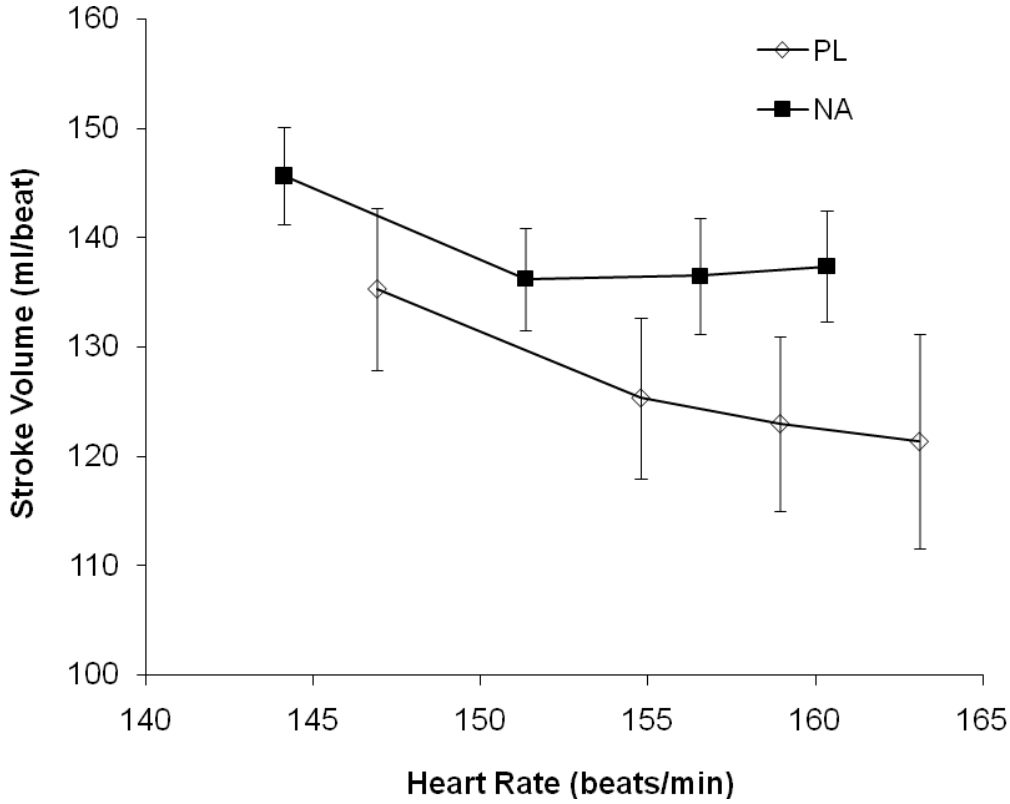
160
150
140
130
120
110
100
Stroke Volume (ml/beat)
140
145
150
155
160
165
Heart Rate (beats/min)
PL
NA

Figure 2-5

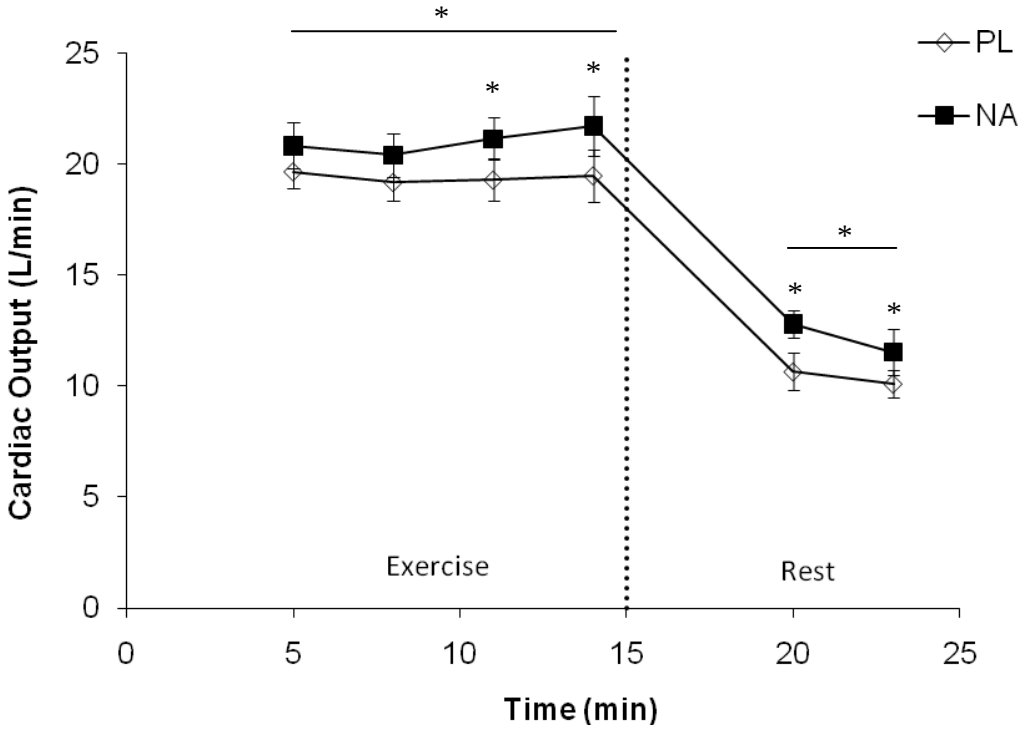
PL
NA
Cardiac Output (L/min)
Time (min)
Exercise
Rest
0
5
10
15
20
25
*

Figure 2-6

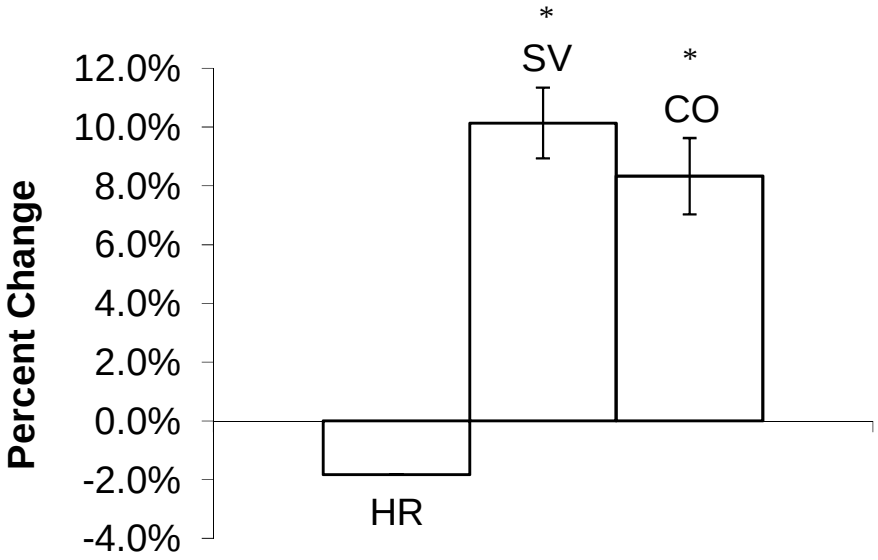

Figure 2-7

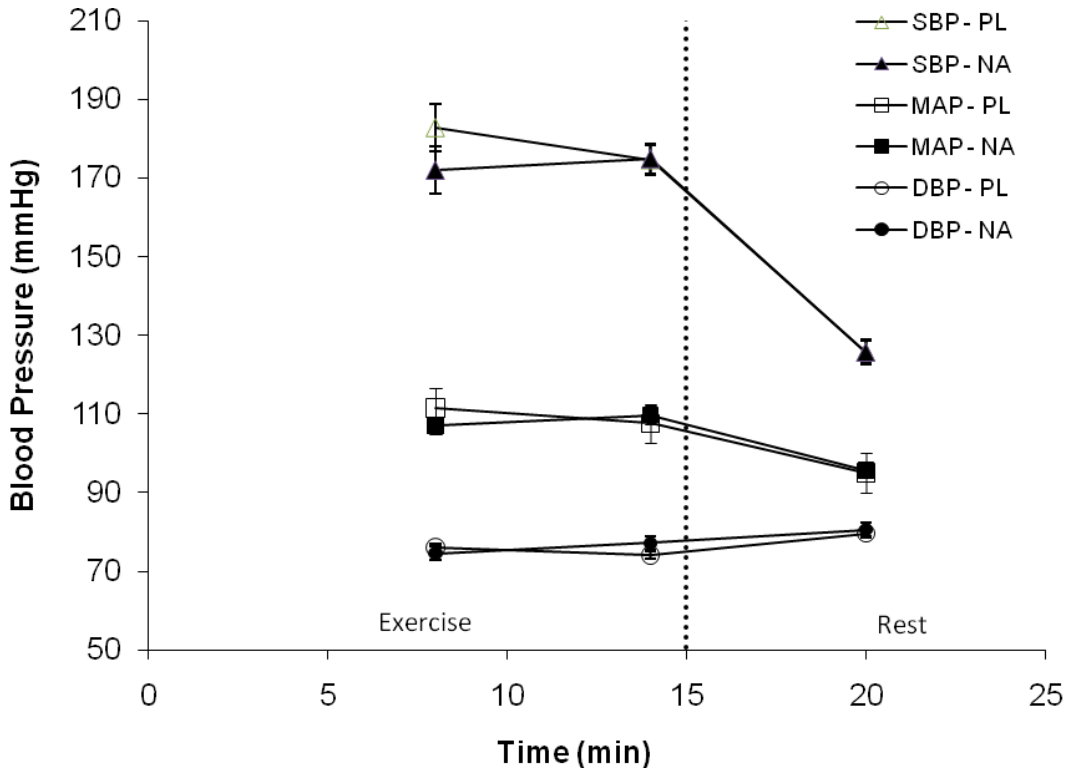
Blood Pressure (mmHg)
210
190
170
150
130
110
90
70
50
0
5
10
15
20
25
Time (min)
Exercise
Rest
SBP - PL
SBP - NA
MAP - PL
MAP - NA
DBP - PL
DBP - NA

Figure 2-8

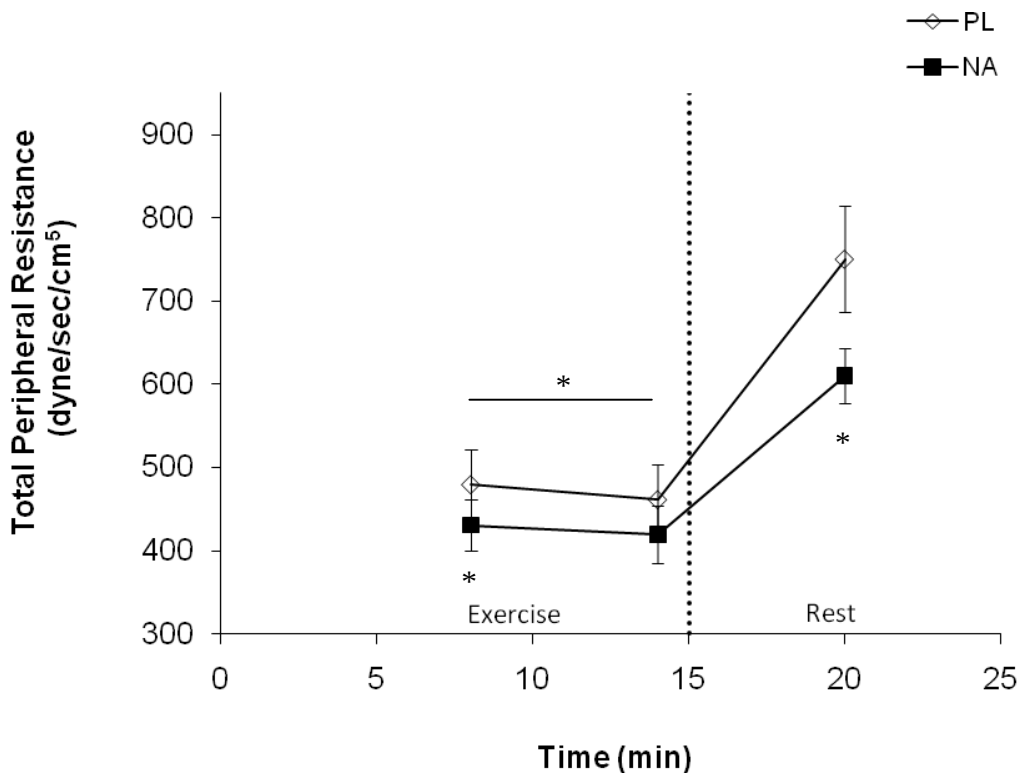
PL
NA
Total Peripheral Resistance (dyne/sec/cm5)
900
800
700
600
500
400
300
0
5
10
15
20
25
Time (min)
Exercise
Rest
*
*
*

Figure 2-9

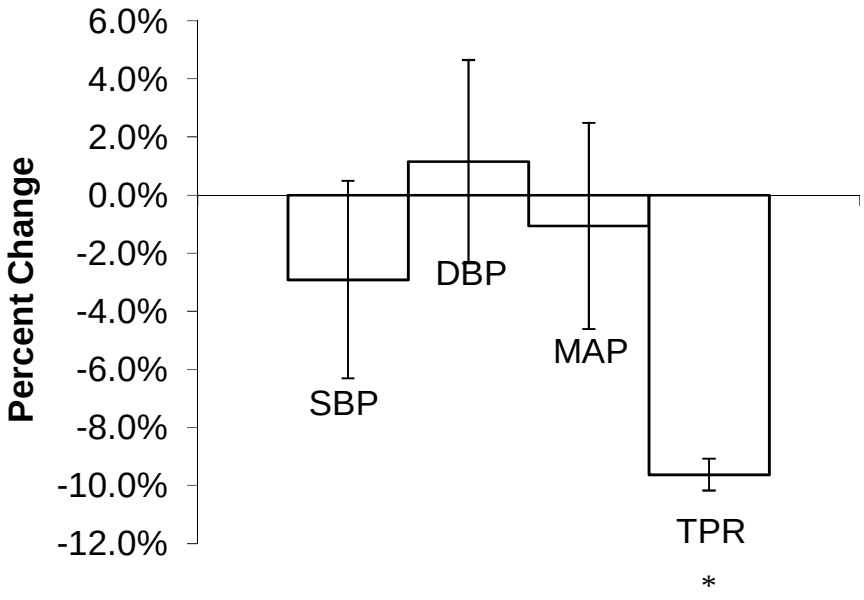

Figure 2-10

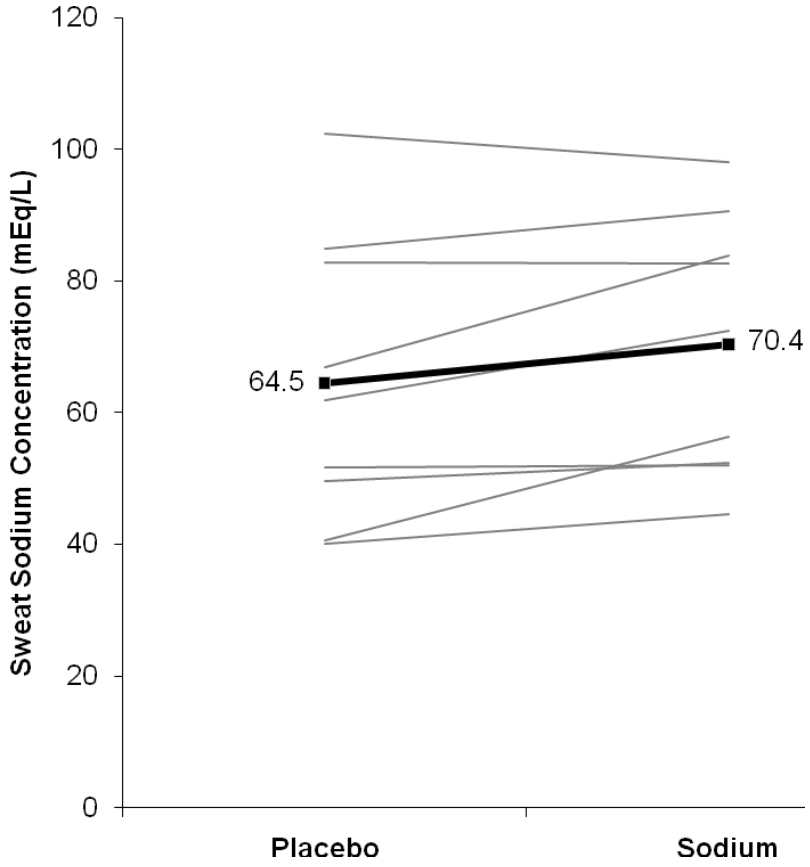
120
100
80
60
40
20
0
Sweat Sodium Concentration (mEq/L)
64.5
70.4
Placebo
Sodium

Table 2-1. Blood markers measured at rest and during exercise at 60% VO2peak during placebo (PL) and sodium chloride supplementation (NA) (n = 9).

		Rest	Exercise	Post-Exercise
Serum Sodium (mEq/L)	PL	144.4 ± 1.4	147.2 ± 1.0	144.8 ± 0.7
	NA	145.0 ± 1.5	148.0 ± 1.0†	145.5 ± 0.6†
Hematocrit (%)	PL	46.8 ± 2.6	49.3 ± 3.2	48.3 ± 3.2
	NA	45.5 ± 3.0	47.4 ± 3.3†	47.1± 3.4†
Hemoglobin (mg/dL)	PL	15.1 ± 1.0	16.3 ± 1.2	15.8 ± 1.2
	NA	14.6 ± 0.9	15.6 ± 1.1†	15.4 ± 1.1*

† significantly different *vs.* placebo at $p < 0.01$, * significantly different *vs.* placebo at $p < 0.05$.

Chapter VI - Review of Literature

Introduction

Body mass is composed of approximately 50-60 % water which is sub-divided between the extracellular and intracellular fluid compartments (34). Fluid and sodium balance can be altered during conditions of physical stress when sweating is prolonged and replenishment strategies are inappropriate (43, 53, 54). While declines in sodium to 130 mEq/L (mild hyponatremia) are typically asymptomatic, large declines in serum sodium to levels <125 mEq/L (severe hyponatremia) are generally symptomatic. A large decline in serum sodium concentration mainly occurs by an excessive consumption of water which dilutes the sodium stores. However, excessive sodium losses in sweat during prolonged exercise have also been included in models as a possible contributor to decreasing serum sodium levels and the development of hyponatremia (69, 70). While the incidence of clinical hyponatremia appears to be more common during ultra-endurance exercise events (31, 53, 59, 94) compared to shorter distance exercise, it is also present in workers undertaking manual labor tasks under heat stress (2), as well as in military personnel (8). As sweat sodium losses are highly variable between individuals (77), those individuals with higher losses would be at a greater risk for decreasing serum sodium concentration according to hypothetical models (69, 70). While the effects of clinical hyponatremia are clear, very little is known about the effects of smaller declines in serum sodium concentration on physical and cognitive performance. Clinically, small changes in serum sodium are typically asymptomatic, but there have been clinical cases where smaller declines in serum sodium concentration (~128 mEq/L) have shown

decrements in cognitive and postural performance (32). Furthermore, the effects of sodium chloride supplementation on maintaining serum sodium concentration and physical performance are not well studied.

Blood volume is a determinant of stroke volume. In untrained individuals plasma volume is suboptimal during exercise compared to trained athletes (57). This lower plasma volume is partially responsible for the higher exercising stroke volume in trained versus untrained individuals. Invasive methods, such as intravenous infusion, have been used to exogenously increase plasma volume in untrained people (57, 63). These efforts have resulted in an increase in stroke volume during rest (61) and during exercise (27, 57). As fluid follows tonicity (90), increasing extracellular osmolality via sodium chloride ingestion also results in an increase in plasma volume. Sodium ingestion has successfully been utilized to increase plasma and stroke volume at rest (28), but it has not been evaluated during exercise in young healthy individuals. Furthermore, the effects of dietary sodium ingestion on sweat sodium concentration have not been evaluated during exercise in the heat in non heat-acclimatized individuals.

1 – Determinants of Fluid and Sodium Balance

1a. *Total body water and fluid compartments*

Over half of a human's body mass (~61 % in males) is composed of water (34). The intracellular fluid (ICF) accounts for approximately two-thirds of total body water and extracellular fluid (ECF) accounts for the remainder (34). The ECF is further subdivided into interstitial fluid (ISF, ~75% of ECF) and plasma volume (~25% of ECF). Water distribution is due to tonicity and water moves freely when tonicity is altered.

Tonicity is primarily determined by sodium in the ECF (~140 mmol/L) and potassium in the ICF (~150 mmol/L). Alterations in the total body water, ECF, and ICF occur when fluid, sodium, or a combination of the two are gained by or lost from the body.

1b. *Increases in Fluid and Sodium Content*

As sodium stores in the body are minimal outside of the ECF, ECF sodium content is increased via external sources, such as food, fluid, or supplements. The average daily intake of sodium in the Western world is approximately 100-200 mmol Na/day (6-12 grams NaCl) (110).

There is currently controversy as to the efficacy of sodium chloride supplementation on altering serum sodium concentration during endurance exercise. Some researchers have reported that sodium chloride supplementation has no significant effect on lessening the decline or on increasing serum concentration during prolonged exercise (14, 52, 85, 96). Others have suggested that sodium chloride supplementation will attenuate the decline in serum sodium concentration during endurance exercise (104, 108). Reasons for the discrepancies may be due to experimental methodology. The investigators who reported that sodium chloride supplementation was not a factor on altering serum sodium concentration provided small amounts of sodium (22-30 mEq/h) (14, 96) or did not record other means of sodium intake (96) or loss. As sweat sodium losses were not measured, it is possible that significantly more than 20-30 mEq/h of sodium could have been lost via sweating and urination (78, 89, 106), or 29 mEq/h from ingesting a saline solution may not have been sufficient to attenuate the decline in serum sodium concentration versus water alone (14). A major shortcoming in the field studies

during ultra-distance triathlons is that sodium ingestion from food or fluid was not measured, thus it is challenging to interpret the data (52, 96). It should be noted that the studies which reported the maintenance or less of a decline in serum sodium concentration with supplementation also have confounding factors. One study which reported an attenuated decline in serum sodium concentration and a trend for a longer time to exhaustion while consuming an 18 mEq/L drink versus water was confounded by the use of a sports drink containing carbohydrate. In addition, there was no mention of level of heat acclimatization which may have affected sweating rates and sweat sodium concentration. In a field investigation which reported a benefit of sodium supplementation on maintaining serum sodium concentration the environmental conditions ranged from 5.3 ˚C and snow to 19.0 ˚C and sun. Had the environmental conditions been controlled (104) or a more concentrated sodium and energy matched beverage given (108), the results might have been more valid.

1c. *Decreases in Fluid and Sodium Content*

The primary means for fluid loss include respiration, urination and sweating. During exercise, fluid loss due to respiration is primarily dependant on ventilation and exercise intensity. At approximately 65% of maximal oxygen consumption, respiration losses are approximately 0.075L/hr (68, 79). This value is comparable to the fluid gains from metabolism and it is quite small in comparison to potential urinary and sweat losses. Urine volume and sweat losses can be affected by many factors, including: exercise intensity, degree of heat acclimation, environmental conditions, and hydration status (12, 22, 38, 39, 45, 64, 102). Urine volume losses during exercise are variable between

subjects, but have been reported at approximately 0.02L/hr at 60% of maximal aerobic capacity at 30 ˚C, 50 % RH (25). Normal urine sodium has been reported from 100 – 140 mEq/L in cool weather to as low as 10 mEq/L in hot weather when sodium conservation mechanisms are near maximal (21, 64, 85).

Sweating accounts for not only the largest fluid loss during exercise, but it is also the major means for sodium loss during exercise. Sweating rates can range from under 0.5 L/hr to almost 4 L/hr (10, 24, 49). Sweat is hypotonic in nature and its principal ions, sodium and chloride, are derived from the ECF (24). A wide range of sweat sodium concentrations (less than 20 to over 70 mEq/L) have been reported (30, 77, 78, 83, 89, 106). Sweating rate and composition appear to be quite variable, not only due to changing external conditions, but also inter-individual variability under similar conditions (14, 24, 77, 78, 89, 106). The reasons for inter-individual variability are not entirely clear, but sweating rate and sweat sodium concentration are influenced in part by core and skin temperatures, heat-acclimatization status, training status, aerobic capacity, and hydration level (1, 3, 11, 30, 40, 41, 67, 71).

2 - The Effects of Daily Sodium Ingestion on Sweat Sodium Concentration

Dietary sodium ingestion has been implicated as having a potential effect on sweat sodium losses, but there are mixed results. The conflicting results may be due to comparing sodium deficiency vs. high dietary intake, introducing heat acclimation, sample size, environment, exercise vs. passive heating, and/or sweat testing methodology.

McCance et al. (66) studied the effect of salt deficiency on sweat electrolyte losses during heat acclimation with passive heating in two men over 5 to 8 days. Salt deficiency resulted in a large decline in sweat sodium concentration from 65 mEq/L to 25 mEq/L in one individual while the other man saw smaller declines. When sodium intake exceeded losses, there was an increase in sweat sodium losses while at rest. Robinson et al., (82) also studied chloride loss in men during heat acclimation and found when salt deficient, the chloride concentration in the sweat declined. They also found some evidence for a small increase in chloride loss when salt intake exceeded urinary and sweat losses for 3 days in 2 subjects. Similarly, Armstrong et al., (7) showed that during heat acclimation, high vs. low sodium chloride intake (399 *vs.* 98 mEq NaCl/day) resulted in a higher sweat sodium loss in the high group at 5 and 8 days of acclimation. While on the high sodium diet, sweat sodium losses progressively increased from 41 mEq/L to 58 mEq/L. During the low sodium diet, sweat sodium losses declined during the heat acclimation protocol. However, these results should be reviewed with caution, as the sweating rate was quite low. These very small sweat samples would be challenging to measure, as the researchers used the whole body and clothing washdown technique (7).

Costa et al. (23) fed men either a “space diet”, with moderate-high sodium, or a normal sodium diet (243 mEq Na/d *vs.* 150 mEq Na/d, respectively) in a parallel study design which did not have a heat acclimatization component. Subjects then completed an interval cycling task in a temperate environment (25 °C) for 30 minutes. On average, the sweating rate was 0.7 L/h and sweat sodium losses measured via patch, arm-bag, and bath were higher while consuming the higher sodium “space diet”. While this study

lends the strongest support for an implication of dietary sodium intake on altering sweat sodium loss, it does have limitations. The investigators did not use a crossover design. As sweat sodium losses are extremely variable (77, 89), and only 6 subjects were in each group, the intra-subject variability could have resulted in a difference in sweat sodium losses. Additionally, subjects only lost ~350 ml of total sweat during the trial. A larger sweat volume would result in a more accurate determination in sweat sodium concentration.

While sweat sodium concentration may decline due to an increase in aldosterone during periods of low sodium intake and negative sodium balance (20, 92), investigators have also found no relationship between sweat sodium excretion and sodium intake (4, 30). Davies et al. (30) found no effect of daily saline loading (0.75L 1% saline per day for 11 days) pre and post heat acclimatization. However, during this study dietary food intake was not controlled, thus it is not possible to distinguish the actual sodium intake of the participants (30). In another study where sweat sodium excretion was not affected by sodium intake, participants ingested a moderate or high sodium intake diet (174 *vs.* 348 mEq/d, respectively). However, when participants were switched to a very low sodium diet (66 mEq sodium/d) sweat sodium excretion declined, similar to previous studies. Thus a possible conclusion from this study is that a sodium deficiency is required for sodium intake to alter sweat sodium loss. It should be noted that one major potential source of error in this study was the method of sweat sodium determination. As the investigators utilized a 12h wash-down, it is possible that a large sampling error was introduced in the sodium loss calculations (4). As mentioned previously, limitations exist

in the studies presented above, thus it is not possible to discern the effects of an increased sodium intake on sweat sodium concentration.

3 - Altered Fluid and Sodium Balance

Fluid turnover occurs during prolonged exercise as water and sodium are lost in the sweat and partial or complete fluid is restored. Low serum sodium concentration can result when fluid and sodium balance is mismatched. Normal serum sodium levels typically range from 140-145 mEq/L (110). While the term "asymptomatic" hyponatremia is currently being questioned (32), when serum sodium concentration levels are more than 130mEq/L it is considered asymptomatic and symptomatic when less than 130 mEq/L (94). Mild symptoms of decreased serum sodium concentration include confusion, nausea and fatigue. As these symptoms can also result from hypoglycemia or dehydration induced by prolonged or intense exercise, the condition can be misdiagnosed. In more severe cases, low serum sodium concentration can result in seizures, coma, and death. The severe medical complications are generally caused by pulmonary or cerebral edema as water shifts from the extracellular compartment to the intracellular compartment due to a decline in extracellular sodium concentration (51, 58). Cerebral edema is of particular concern as intracerebral swelling results in an increase in intracranial pressure due to the confinement of the cranium (13). Clinical hyponatremia is more common during ultra-endurance exercise (31, 53, 59, 93), but it is also present in workers undertaking manual labor tasks under heat stress (2), as well as in the military (8). Typically, serum sodium concentration is lowered by an excessive consumption of fluid which is greater than sweat and urinary fluid losses. The result is an increase in

body mass, total body water, and a dilution of first the ECF and then both the ECF and ICF as the excess fluid redistributes throughout the body (94).

Models of factors which alter serum sodium concentration also include excessive sweat sodium loss as a potential contributor (69). In a field study investigating the sweat sodium losses and changes in serum sodium concentration during an ultra-distance triathlon, we found that declines in serum sodium concentration were significantly and negatively correlated with sweat sodium losses in males (77). While overhydration can lead to a decline in serum sodium concentration, lowering serum sodium concentration via sweat sodium losses while maintaining hydration levels has also been proposed. When fluid is consumed to match fluid losses during prolonged sweating, but sodium is not completely replaced, fluid balance is maintained, but serum sodium concentration declines (e.g. euvolemic hyponatremia). As fluid follows tonicity, and sodium content has declined, fluid shifts from the extracellular to intracellular compartment which results in cell swelling. As this means of lowering serum sodium concentration requires losing large amounts of sodium in sweat those with high sweat sodium losses will be at a greater risk. As mentioned previously, sweat sodium losses are quite variable. In a group of endurance athletes (n=71) we reported a very large coefficient of variation (54%) in the rate of sweat sodium loss with a mean value of 62 mEq/h (77). Eighteen percent of the subjects had a very high rate of sweat sodium losses (>90 mEq/h; >2000 milligrams/h) which would account for roughly 4.5% of estimated extracellular sodium content lost each hour. These subjects would be the ones most at risk for decreasing serum sodium concentration should they not replace their sweat sodium losses.

One of the most common fluid disturbances, dehydration, occurs when fluid consumption does not sufficiently match fluid lost. While sweat contains both water and sodium, it is hypotonic, thus the concentration of sodium in the sweat is lower than blood sodium levels which results in a contraction of total body water. Upon equilibration of fluid spaces, total body water will decline from both compartments, but depending on sodium losses in the sweat, there will be a disproportionate amount of fluid decline in the ECF which results in an increase in serum sodium concentration. However, it is also possible to have a decreased serum sodium concentration and also be hypovolemic. This could occur during very prolonged sweating with high sweat sodium losses with only partial fluid replacement with sodium free fluid or very low sodium fluid which would result in dehydration. However, the sodium losses would have to be substantial enough to overcome the resultant increase in serum sodium concentration due to ECF contraction from dehydration.

4 - Effects of Decreased Serum Sodium Concentration on Performance

4a. *Endurance Performance*

As mentioned previously, excessive declines in serum sodium concentration result in mental confusion, nausea, weakness, seizures and possibly death. However, very little is known about the effects of small declines in serum sodium concentration on performance. In one study where cyclists were given sodium-free fluid or fluid with sodium there was a negative correlation between serum sodium levels and cycling performance, but it is difficult to interpret the data (108). Subjects were given the fluid

replacement beverages to match sweating rate, but the sodium beverage also included carbohydrate. Therefore, it is not possible to determine if the effects were due to the carbohydrate ingestion or to sodium supplementation. Twerenbold et al conducted the only study to date to investigate the effects of several levels of sodium chloride content in beverages on changes in serum sodium concentration and endurance performance (104). The authors altered serum sodium concentration by providing beverages with high (680 mg/L), moderate (410 mg/L), or no sodium during a 4 hour run. They concluded that the 3 levels of serum sodium concentration in the athletes during a 4 hour endurance run did not affect performance, but sodium supplementation would be beneficial to maintain serum sodium concentration when overhydrated. The major flaw with the study is that the tests were performed in three vastly different environmental conditions on an outdoor track. During one trial, the temperature was 5 ˚C and it was snowing, in another trial, it was 19 ˚C degrees and sunny, and in the third trial it was 13.9 ˚C degrees and raining. While the trials were randomized, the environmental effects may have affected the sweating data. Furthermore, sweat sodium losses were not measured. As sweat sodium concentration and losses are quite variable, the results may have been affected as each subject was given the same amount of electrolyte replacement even though there losses may have been dramatically different.

4b. *Maximal Neuromuscular Power*

The effects of gradual declines in serum sodium concentration on maximal neuromuscular power, motor function, and muscle activation have not been investigated in humans. In animal models, skinned muscle fibers have shown a decline in excitability

with a large decline in extracellular sodium (16, 19, 72). This may be due to the alterations in t–tubule sodium concentration which is necessary for the normal development of twitch, tetanus (15), and force (42) during exercise. However, it appears that smaller changes in ECF sodium have no effect on excitability. It is possible that ECF sodium and ICF potassium together may be important for these alterations to occur (17). It should also be noted that fatigued and non-fatigued muscle may also confound results (19). Therefore performance declines accompanied by small changes in serum sodium concentration in humans, may be more likely to occur via other factors, as substantial loss of extracellular sodium would be necessary to decrease power based on animal models. Furthermore, a skinned muscle animal model is not appropriate to draw conclusions to human intact muscle fibers. One additional possibility is that nerve conduction velocity has been shown to slow in a hyponatremic patient (6), but once again, serum sodium concentration was quite low.

4c. *Cognition and Balance*

While small decreases in serum sodium concentration can result in nausea, fatigue, and confusion, very little laboratory data exists on the effects on cognition and balance. Cognitive function and postural balance were evaluated in clinical patients admitted to emergency departments with a moderate lowering of serum sodium concentration (mean 126 mEq/L) (81). Of the 122 patients admitted, 21 % were admitted with falls indicating decreased balance. When compared to 244 matched controls also admitted to the emergency department, the patients with decreased serum sodium levels had 4-fold more falls than the controls. Sixteen of these patients participated in cognitive

testing and 12 participated in postural examination prior to and after treatment. Mean response times were significantly slower (~9 %) when presented with decreased serum sodium concentration compared to after treatment. Balance, measured as center of pressure displacement during 3 steps in tandem, was also significantly worse, as the subjects had a 28% greater displacement in the center of pressure. The larger sway in center of pressure may have accounted for the greater incidence in falling. Based on these findings, there is a questioning of the term "asymptomatic" hyponatremia, as it appears that a systematic investigation of these patients resulted in the detection of physical and mental decrements in performance (32).

5 – Effects of Plasma Volume Expansion on Cardiovascular Function

5a. *Training status and PV*

Cardiac output is important as it is responsible for delivering oxygen and nutrients, as well as removing heat and waste during exercise. An increased blood volume is partially responsible for increased cardiac preload which in turn results in increasing stroke volume and potentially cardiac output. An increase in plasma volume is one of the early adaptations to endurance training (26, 46). In untrained males blood and plasma volume (PV) are sub-optimal for achieving maximal stroke volume (SV) during exercise. Plasma volume expansion via intravenous infusion increases SV by ~10-15 % in untrained males (57).

5b. *Plasma volume expansion – Intravenous infusion*

Moderate PV expansion (~14 %) via intravenous infusion of dextran and saline during upright sub-maximal exercise in untrained males results in an increase in exercising BV (~8 %). An expansion of PV results in an increase in SV (~11 %) during sub-maximal exercise in untrained males (57). Cardiac improvements from the infusion also include increased diastolic filling rate, left ventricular ejection time increases, and enhanced emptying due to Frank Starling mechanisms due to the enhanced diastolic filling rate (63). Not only does SV increase with infusion, but heart rate decreases at the same absolute intensity, and small increases in VO_2max can also occur (27) in untrained males. The effects of PV expansion on SV at rest are mixed as some have found no differences in SV (63, 84) and others have found increased SV at rest, in recreationally trained individuals (61). While plasma volume expansion via intravenous infusion is an effective means to increase SV during exercise, intravenous infusions are invasive, thus their use is not practical. As sodium chloride ingestion may also raise PV, it may also be possible to utilize this non-invasive means to increase SV.

5c. *Acute sodium supplementation*

Acute sodium supplementation via ingesting a hypertonic beverage at rest minutes to hours prior to exercise has also been investigated as an aid to cardiovascular (e.g. PV, SV, HR) and exercise performance (47, 48, 62, 80, 91). As described above, alterations in extracellular sodium content will result in alterations in ECF volume. As the intravascular space, plasma volume, is part of the ECF, this fluid compartment is also affected. Varying amounts, concentrations, and volumes of sodium citrate, sodium

bicarbonate, and sodium chloride pills and solutions have mainly been used to provide acute sodium supplementation in an attempt to hyper-hydrate or re-hydrate untrained and trained males and females (80, 91). Some researchers have shown benefits to PV and exercise performance in supine and upright positions (47, 91), while others have shown no effect (62). One limitation to acute supplementation of sodium prior to exercise is that some people experience gastric upset.

5d. *Dietary sodium intake*

Dietary or chronic sodium chloride intake has been investigated for its effects on heat acclimatization, fluid retention, and hypertension (7, 9, 37, 88, 99-101, 109). While much data exists on daily sodium chloride intake on resting cardiovascular variables, mostly in relation to hypertension, little data exists on its effects during exercise. Normal sodium intake in the United States is ~170 mEq sodium/d (~3.9 g sodium/d), however the recommended daily intake is 100 mEq sodium/day (~2.3 g sodium/d) (105). However, additional sodium may be warranted for an athletic population (36). Varying levels of daily sodium intake have been studied from nearly no sodium intake to over 660 mEq NaCl/d (29, 50).

When ingesting low vs. high dietary sodium (70 vs. 250 mEq NaCl/d, respectively), PV increases in the seated (~10%) and supine positions (~8%) at rest (28). Cardiac output (CO) and SV are also higher in the seated position (10% and 19%, respectively) and resting heart rate is lower (6%) while on a higher sodium diet (28). In a study by Heer et. al (50), 6 healthy males consumed a diet which included 220 mEq NaCl/d for 8 days, then 440 mEq/d, and then 600 mEq/d for 8 days. Resting PV

increased by 8% while on the 440 mEq/d diet and 11 % on the 660 mEq/d diet vs. the 220 mEq/d diet. BV also increased by ~ 11 % on the 660 mEq/d diet. In a follow-up study by the same group, 4 groups of males were assigned to a 50, 200, 400, and 550 mEq/d diet for 7 days. PV was related to sodium intake, while PV at rest increased by 4 %, 8.7 %, and 13.8 % from day 1 to day 7 in the 200, 400, and 550 mEq/d diets vs. the low 50 mEq/d diet (50).

The cardiovascular adaptations in middle age normal men and heart failure patients consuming normal and high sodium diets were investigated while in resting conditions. They consumed a diet of 100 mEq sodium/d for 5 days and then supplemented for 6 days with an additional 150 mEq sodium/d while fluid was restricted to 1.5-1.8 L/d. By the 3rd day of supplementation, left ventricular end-diastolic volume, ejection fraction, CO, and SV were significantly higher (~15 %, 5 %, 24 %, and 2 %, respectively), total peripheral resistance was ~15 % lower in the normal males, and heart rate was not significantly different from baseline. By day 6, total peripheral resistance continued to decline an additional 9 % while there were small increases in left ventricular end-diastolic diameter, ejection fraction, CO, and SV due to the peripheral compensation (107).

Cardiovascular and exercise performance in older heart failure patients and age-matched male controls (57 y) were also investigated while consuming varying amounts of sodium in their diet (29). Seven days of low vs. moderate-high sodium ingestion (70 *vs.* 250 mEq sodium/d) resulted in an increase in PV of 7 % at rest in a seated position. Cardiac index and stroke volume index increased, while total peripheral resistance decreased while at rest. During an incremental exercise test to fatigue (30 watts increase

every 3 min), cardiac index and stroke volume index were also higher during the moderate-high sodium diet at both low (<70 % VO2max) and high intensity (>70 % VO2max) exercise (~7 % and 5 %, respectively). Heart rate was not significantly different and total peripheral resistance was ~6-7 % lower than baseline levels. Time to fatigue during the incremental test was not significantly different. While a short term increase in sodium intake alters cardiovascular function by increasing plasma volume and stroke volume at rest and in older individuals during exercise, the effects on young, healthy, untrained males during exercise have not been investigated.

5e. *Homeostatic Responses*

In healthy humans, mean arterial pressure is the primary variable that is regulated during exercise. Thus an excess fluid load in the extracellular compartment will result in compensation, such as decrease in total peripheral resistance to prevent an increase in mean arterial pressure during exercise (28, 88). Ultimately, the extra fluid and sodium load will be excreted via the kidneys to maintain homeostasis in healthy humans and the fluid volume and sodium content will return to normal.

The attempt to maintain mean arterial pressure during increased sodium chloride intake to offset increases in PV, SV, and CO by decreasing total peripheral resistance occurs in healthy sodium resistant individuals (28, 88). However in sodium sensitive, hypertensive, individuals, total peripheral resistance does not decline, thus mean arterial pressure increases (88, 99). In addition to the alterations in total peripheral resistance, additional homeostatic responses include an increase in daily urinary sodium excretion in order to return the body fluid balance to normal levels. Typically, daily urinary sodium

excretion doubles within 48 hours of increasing sodium ingestion (28, 107). Thus, an increase in plasma volume would most likely be maintained for shorter periods of time, unless the renal system could not compensate for them.

Chapter VII: General Discussion

These studies determined the effects of sodium chloride supplementation on serum sodium concentration, cardiovascular function and cognitive and physical performance. Additionally, we investigated the effects of chronic sodium chloride supplementation on sweat sodium concentration.

Study 1 determined that sweat sodium losses, alone, decreased serum sodium concentration ~6 mEq/L in euhydrated endurance athletes with high sweat sodium losses while exercising in the heat. This finding is in agreement with previously published models of factors altering serum sodium concentration (69, 70). While prior investigations have found varying degrees of success altering serum sodium concentration with sodium chloride supplementation (52, 96, 104), ours is the first to find that sodium chloride supplementation matching individual sweat sodium losses maintains serum sodium concentration. Our methodology of first identifying those athletes most at risk of decreasing serum sodium levels via sweat, those with high sweat sodium losses, and then supplementing to match losses, was critical in determining this relationship as sweat sodium losses are extremely variable (77).

While supplementing with sodium chloride matching sweat sodium losses, serum sodium concentration was maintained. The maintenance of serum sodium concentration resulted in an improved response time during the Stroop Test from pre- to post-exercise while response time did not change when serum sodium concentration declined. Post-exercise balance was also more stable while supplementing with sodium chloride compared to when a placebo was ingested. While cognitive and postural tests have not

been made in athletes with low serum sodium levels, decrements have been seen in patients hospitalized with hyponatremia (56). Additionally, we found small benefits to physical performance compared to when serum sodium concentration declined during 3 hours of profuse sweating. Possible reasons for finding only small changes in physical performance may be due to either fatigue of the 3 h exercise bout masking differences due to decreased serum sodium concentration, only a moderate difference in serum sodium concentration, or possibly the choice of performance task.

Study 2 determined that 3d of sodium chloride supplementation (~15 g NaCl/d) compared to a placebo resulted in a higher plasma and blood volume at rest and during exercise in healthy untrained males. Furthermore, stroke volume and cardiac output were also higher during moderate intensity exercise with an increased sodium chloride ingestion compared to a normal dietary intake. These results are similar to those found following intravenous infusion of a plasma volume expander (57, 63). Prior investigations of very low *vs.* high daily sodium chloride intake have also resulted in plasma and blood volume expansion at rest (28, 29, 50, 107). The effects of increased dietary sodium chloride supplementation on exercising stroke volume have only been investigated in 1 study of older men (~57 y) (29). Our results in young healthy males (20 - 35y) were similar as we found an expansion of plasma volume (~8 %), blood volume (~4 %), and stroke volume (~10 %) during steady state exercise. However, our supplementation period was half as long (3 *vs.* 7 d), we used a steady state *vs.* ramp protocol, and we compared normal *vs.* high daily intake, not a very low *vs.* moderate-high intake.

Three days of sodium chloride supplementation also resulted in a 9 % increase in sweat sodium concentration during exercise in the heat. The effect of daily sodium intake on sweat sodium concentration has been mixed in previous studies (4, 7, 23, 66). Prior investigations have compared very low *vs.* very high intake, investigated during a heat acclimation protocol, or the sweat collection periods were very long and sweat losses were small. All of these variables may also result in independent effects on sweat sodium concentration, large sampling errors, or unrealistic dietary conditions. Our protocol investigated normal *vs.* high intake in non heat-acclimatized males so that we could determine the contributions of 3d of sodium chloride ingestion on sweat sodium losses. While we did not measure aldosterone, prior studies have shown that large increases in dietary sodium intake results in a decrease in plasma aldosterone (50). As aldosterone has a sodium conservation effect, a decrease in aldosterone may have been responsible for the increases in sweat sodium concentration in our study. This may seem to contradict our 1st investigation of acute sodium supplementation as there were no differences in sweat sodium concentration when serum sodium concentration declined by 6 mEq/L *vs.* when serum sodium was maintained via sodium supplementation. However, in prior studies of acute changes in serum sodium concentration during exercise there were no significant differences in plasma aldosterone compared to when serum sodium concentration was maintained (95, 108). Therefore, sweat sodium concentration would likely not be altered by an acute lowering of serum sodium concentration, as occurred in our investigation.

There are several areas of further investigation which could be undertaken. One avenue of exploration could be to further investigate physical performance with a larger

decline in serum sodium concentration, as our investigation only had a decline of ~ 6 mEq/L. Women could also be studied, as they typically have less total body water as a percent of total body mass. Thus, lower sodium content would exist in the smaller ECF compared to males with the same serum sodium concentration and similar sweat sodium losses would result in greater declines in similar sodium concentration. In terms of chronic sodium loading, a time course and dose response could be undertaken in order to determine the optimal supplementation protocol. Additionally, performance tasks could be studied such as a time trial, or possibly the measurement of VO2max, as this has been known to increase in untrained males with intravenous infusion of plasma volume expander (27). Lastly, while trained subjects already have an expanded blood volume, this subject population could be investigated as acute sodium supplementation may have an effect on performance (91).

In summary, these studies determined that when hydration status is maintained, sodium chloride supplementation matching sweat sodium losses is necessary to maintain serum sodium concentration during prolonged exercise when sweat sodium losses are high. Without sodium supplementation, serum sodium concentration declines ~ 6 mEq/L in subjects with high sweat sodium losses exercising in the heat for 3 hours. Maintaining serum sodium levels by matching sweat sodium losses with sodium chloride supplementation results in an improved cognitive function from pre- to post-exercise, as response time improves ~11 %, while there is no change when serum sodium declines. Post-exercise balance is improved 11-17 % while maintaining serum sodium concentration compared to when serum sodium concentration declines. Additionally, maximal power non-significantly declines by 3 % from pre- to post-exercise when

supplementing with sodium chloride to maintain serum sodium concentration, but the 4 % decrease in power is significant when serum sodium concentration falls. Furthermore, in healthy males with a suboptimal exercising blood volume (e.g. non-endurance trained, non heat-acclimated), 3d of sodium chloride supplementation results in an expanded plasma volume (8 %). Stroke volume, cardiac output, and sweat sodium concentration are also 8-10 % higher during exercise compared to a normal dietary intake. Therefore, acute and chronic sodium supplementation positively alters fluid and sodium balance which results in beneficial effects on physical and cognitive performance and cardiovascular function during exercise.

APPENDIX A: General Exercise and Performance Tests

Steady state oxygen consumption and lactate threshold determination (Study 1)

Subjects cycled continuously for 5 stages, 5 minutes in length on an electromagnetically braked ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Each successive stage increased approximately 20 to 50 watts depending on the training status and characteristics of the subject. Oxygen consumption was collected throughout the 25 minute exercise period and averaged during the final minute of each stage. Blood samples were taken at the end of edge stage from a finger stick for the determination of lactate (Lactate Pro, Arkray, Japan). Lactate threshold was determined as a 1 mmol increase in lactate above baseline levels.

Peak oxygen consumption

Following the steady state test and a 10 minute rest, peak oxygen consumption (VO_2peak) was determined using an incremental protocol to exhaustion lasting approximately 8 to 12 minutes. The protocol began with a 2 min stage at approximately 75 % of HRmax. Three successive 2 min stages with an increase of 30 to 50 watts followed (depending on subject characteristics and the submaximal test), and then an increase of 20 to 30 watts per minute to exhaustion followed. Oxygen consumption and carbon dioxide production were measured throughout the test. VO2peak was determined as a plateau in oxygen consumption with increasing work rate, and a RER > 1.10.

Cognitive testing (Study 1)

The modified Stroop Color-Word Interference Test (LSA Stroop, FL) consisted of five 45 second modules. The test difficulty level progressively increased during each module. In short, the subjects were presented with a word (red, green, or blue) on a screen. The color of the word either matched the meaning or was incongruent. Subjects selected the color or the meaning depending on the module. In the final module, the subjects selected the color or meaning based on if the word was framed by a box. Accuracy and response time were recorded.

Balance Testing (Study 1)

The balance assessment task consisted of 3 trials of 3 stances while standing barefoot on a 6 degree of freedom force plate (Bertec, Columbus, OH) which was interfaced with a computer (Dell, Austin, TX). The stances included a 2 leg stance with feet together and eyes open, single leg stance with eyes open, and single leg stance with eyes closed. During stances with eyes open, subjects looked at a spot at head level. Each trial lasted 30 seconds and there was a 15 second break between each repetition. The order of stance conditions were a randomized crossover design between subjects and each subject completed the same order for both of their trials. The center-of-pressure amplitude (ACOP) were calculated in the anteroposterior (COPap) and mediolateral (COPml) planes with custom software (Matlab, The Mathworks, Inc., Natick, MA).

Maximal neuromuscular power (Study 1)

Maximal neuromuscular power per pedal revolution, torque, and revolutions per minute were measured on the PowerCycle prior to the 3 h ride and at 60, 120 min, and following the 3 h ride. Subjects completed 4 all out efforts lasting 3-4 seconds each during each testing session. Subjects remained seated on the ergometer for a 1 min rest period between each trial.

Time Trial Task (Study 1)

Subjects completed a 20 min time trial in a thermoneutral environment. The workrate was fixed for the first 5 min at a power output that elicited an oxygen consumption that was 10 % above lactate threshold. After the first 5 min of the time trial, subjects were free to alter the workrate at 30 sec intervals for the remaining 15min. The same researcher provided verbal encouragement during both time trials.

APPENDIX B: Instrumentation & Analysis

Measurement of gas exchange (Study 1)

Inspired air volume was measured with a pneumotach (model 4813, Hans Rudolph, Shawnee, KS) and expired gases were continuously sampled from a 4 L mixing chamber (Vacumed, Ventura, CA) and analyzed for oxygen (S-3A/I, Ametek, Pittsburgh, PA) and carbon dioxide (CD-3A, Ametek, Pittsburgh, PA). The analyzers were interfaced to a computer for calculation of the rate of oxygen consumption and rate of carbon dioxide production (Max II, AEI Technologies, Pittsburgh, PA).

Breath by breath gas measurement (Study 2)

Subjects breathed through a pneumotachometer (Hans Rudoloph, Kansas City, MO) and two-way non rebreathing valve (2700 Series, Hans Rudolph, Shawnee, KS). Oxygen and carbon dioxide gases were continuously sampled at the mouthpiece via a 6 ft capillary tube. Gas concentrations were determined by a mass spectrometer (Perkin Elmer MGA 1100, St. Louis, MO) interfaced with a computer for calculation of breath by breath oxygen consumption and carbon dioxide production (Beck Integrated Physiological Testing System).

Open circuit acetylene wash-in (Study 2)

Open circuit acetylene wash-in for the determination of cardiac output was performed as described by Johnson et al (60). Briefly, at the end of a full expiration, the participants breathed for a minimum of 8 breaths through a mouthpiece connected to a

bag filled with mixed gases, including 0.7% acetylene, 9.0% helium, 21% oxygen, and balance nitrogen (all gases are medical grade and are safe for use with human participants). The concentrations of acetylene and helium were monitored by continuous sampling at the mouthpiece using a mass spectrometer. As the helium curve served as an indication of the complete mixture of the gases in the system, no sooner was the equilibrium of the helium reached than the region of the acetylene curve was made to calculate cardiac output. From the wash-in curve of acetylene, the cardiac output was calculated according to a single alveolar one-compartment lung model.

Core and Skin Temperatures

Rectal temperature was measured using a thermistor (YSI 401, Yellow Springs Instruments, OH) inserted 12 cm past the anal sphincter. Skin temperature was measured with surface thermistors (YSI 409A, Yellow Springs Instruments, OH) attached to the skin at six sites (upper arm, forearm, chest, back, thigh, and calf) via an elastic strap or athletic tape.

Blood measures

Hematocrit was measured in duplicate (Study 1) or triplicate (Study 2) following microcentrifugation for 15 minutes. Hemoglobin was measured in duplicate (Study 1) or triplicate (Study 2) using the cyanmethemoglobin method (35). Plasma volume change was determined via the method of Dill and Costill (33). Whole blood was stored at room temperature until clotting occurred and then centrifuged for 15 min.

Serum, sweat, and urine electrolyte analysis

Serum, sweat, and urine sodium were measured via electrochemistry (NOVA 5, Waltham, MA). This system uses ion selective electrodes, calibration solutions, and a reference electrode in order to determine the concentration of electrolytes in solution.

Sweating rate and sweat sodium concentration analysis

Sweating rate (L/h) was calculated as the change in nude body mass (Ohaus Champ, ModelCQ250XL11W, Pinebrook, NJ) immediately prior to and after the sweating analysis period (30-60 minute), accounting for fluid consumption and urine loss. A waterproof "sweat patch", composed of 7.6 x 7.6 cm gauze sponge (Johnson & Johnson Medical, Arlington, TX) and 10 x 12 cm Tegaderm® bandage (3M Health Care, St Paul, MN), was applied to the mid-posterior right forearm, right scapula, right mid-anterior thigh, and calf for regional sweat collection. Prior to patch application, the area was cleaned with 70 % isopropyl alcohol and rinsed with de-ionized water. When necessary, these areas were gently shaved prior to cleaning and patch application. Upon removal of the sweat patches, the gauze sponge was immediately separated from each Tegaderm® bandage and placed into a plastic syringe (Study 1) or into a centrifuge filter-less separation tube (Study 2). The sweat content of the sponges in the syringes was "squeeze plunged" or obtained via centrifugation and pipette into four 5 ml plastic test tubes and capped. Sweat electrolyte concentration was measured with a Nova 5 Analyzer (Waltham, MA) with a manufacturer reported CV of 2 % for sodium analysis. A modified weighted equation was used to calculate whole body sweat sodium concentration (sweat sodium concentration = 0.11([Arm]) + 0.276([Back]) +

0.299([Thigh]) + 0.315([Calf])) (98). Whole body sweat sodium loss is the product of whole body sweating rate and weighted sweat sodium concentration.

Serum and sweat osmolality (Study 1)

Serum and sweat osmolality was measured via freezing point depression methods (3MO, Advanced Instruments, Needham Heights, MA).

Urine specific gravity

Urine specific gravity was measured via refractometry to ensure euhydration status pre-exercise. All subjects were required to have a urine specific gravity of less than 1.020 in order to commence the trial.

Maximal Power (Study 1)

The inertial load ergometer uses the resistance created by the moment of inertia of the flywheel to represent the force that the subject must accelerate during the test. Power was calculated as the product of inertia, angular velocity and angular acceleration. Flywheel angular velocity and acceleration were determined by an optical sensor and micro-controller based computer interface which measures time (± 1 microsecond) and allows power to be calculated instantaneously (IP) every 3 degrees of pedal crank revolution or averaged over one complete revolution of the pedal cranks (Pmax). The inertial load of the ergometer used in this study was 7.52 kgm^2. Inertial load is equal to one-half the product of the moment of inertia (0.94 kgm^2) and the gear ratio (4.00:1) squared. The information that was collected by the optical sensor was converted from

analog to digital data prior to the transfer to the computer where it is stored as an ASCII file. This file was then converted and analyzed using Microsoft Excel. This procedure was developed by Martin et al. (65). Pmax values are calculated as the mean of the trials with the highest Pmax of 4 efforts.

Time Trial Performance (Study 1)

Work was calculated every 30 seconds (J = Watts * 30). Total work completed during the 20 minute time trial was calculated as the sum of 30 second intervals.

APPENDIX C: INDIVIDUAL DATA

Body Mass (kg)

Study 1

PL

Time (min)	Pre	0	30	60	90	120	150	180
AG	84.17	85.11	85.75	85.35		85.80	85.70	84.65
BA	84.45	84.70	84.90	84.90	85.50	86.00	85.70	84.95
CC	73.88	74.65	74.90	75.05	75.20	75.50	75.25	74.30
EH	86.35	86.80	87.15	87.25	87.35	87.45	87.50	86.85
JC	82.65	83.10	83.50	83.75	84.00	84.25	84.15	83.60
JS	83.75	84.50	84.75	85.10	85.25	85.50	84.70	84.00
JT	62.52	63.25	63.45	63.50	63.40	63.55	63.20	62.60
KK	82.35	83.00	83.30	83.00	82.95	82.85	82.75	82.45
KS	83.25	83.60	84.25	84.05	84.15	84.30	83.15	82.20
RF	90.60	91.25	91.35	91.05	90.95	91.00	91.40	90.95
ZF	77.75	78.65	78.80	78.65	78.60	79.40	79.18	78.80
MEAN	81.07	81.69	82.01	81.97	81.74	82.33	82.06	81.40
SE	2.3	2.2	2.3	2.2	2.5	2.2	2.3	2.3

NA

Time (min)	Pre	0	30	60	90	120	150	180
AG	85.85	86.70	86.90	86.75	86.60	86.50	86.15	85.30
BA	84.25	84.40	84.50	84.20	84.80	85.40	85.00	84.10
CC	73.76	74.20	74.55	74.60	74.60	74.70	74.40	73.50
EH	87.25	87.90	88.30	88.35	88.50	88.60	88.60	88.00
JC	84.00	84.72	85.00	84.95	85.00	85.00	84.55	83.90
JS	85.40	85.85	86.50	86.20	86.30	86.30	85.30	84.50
JT	62.62	63.25	63.35	63.40	63.40	63.50	63.15	62.50
KK	80.70	81.48	81.90	81.80	81.80	81.60	81.65	81.20
KS	81.42	82.35	82.75	83.25	83.20	83.50	82.35	81.40
RF	90.65	91.20	91.10	90.70	90.60	90.70	91.35	90.70
ZF	78.18	79.05	79.10	79.35	79.50	79.80	79.63	79.20
MEAN	81.28	81.92	82.18	82.14	82.21	82.33	82.01	81.30
SE	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3

Serum Sodium Concentration (mEq/L)

Study 1

PL

Time (min)	0	60	120	180
AG	144.5	141.5	138.0	137.5
BA	147.0	143.0	139.0	139.0
CC	142.5	137.0	135.5	137.0
EH	142.0	139.0	136.0	136.0
JC	145.5	142.0	140.5	137.5
JS	145.0	141.5	139.0	138.0
JT	142.0	140.0	140.0	139.0
KK	144.0	139.0	135.5	135.5
KS	142.0	139.0	136.0	137.0
RF	142.0	140.7	140.0	137.0
ZF	142.0	139.0	134.0	135.0
MEAN	143.5	140.2	137.6	137.1
SE	0.5	0.5	0.7	0.4

NA

Time (min)	0	60	120	180
AG	143.0	142.0	141.0	145.0
BA	146.0	143.0	140.0	144.0
CC	142.0	140.0	138.0	141.0
EH	143.0	142.0	141.0	138.0
JC	144.0	139.0	140.0	143.0
JS	144.0	142.0	142.0	147.0
JT	143.0	140.0	138.0	142.0
KK	144.0	142.0	138.0	144.0
KS	140.5	137.0	137.0	141.0
RF	143.5	142.0	143.0	140.0
ZF	144.5	143.0	140.0	141.0
MEAN	143.4	141.1	139.8	142.4
SE	0.4	0.6	0.6	0.8

Serum Potassium (mEq/L)

Study 1

PL

Time (min)	0	60	120	180
KS	4.1	4.4	4.7	4.8
JS	4.3	5.3	5.4	5.6
BA	4.2	4.8		4.7
AG	4.0	4.8	4.9	4.9
CC	3.8	4.3	4.7	4.4
KK	4.1	5.4	5.9	5.6
JC	3.9	4.4	4.6	4.7
RF	4.2	4.9	4.9	4.9
ZF	3.9	5.3	4.9	4.5
EH	4.7	4.8	4.7	4.4
JT	4.1	4.1	4.4	5.8
MEAN	4.1	4.8	4.9	4.9
SE	0.1	0.1	0.1	0.2

NA

Time (min)	0	60	120	180
KS	3.9	4.9	4.7	5.0
JS	4.7	5.3	5.8	5.3
BA	4.3	4.9	4.8	4.1
AG	4.2	4.5	4.8	4.7
CC	3.7	4.4	4.6	4.5
KK	4.2	5.2	5.1	5.2
JC	4.0	4.7	4.7	4.7
RF	4.2	5.1	5.1	4.5
ZF	4.6	5.3	5.1	5.3
EH	4.2	5.0	4.7	4.9
JT	3.6	4.3	4.3	4.3
MEAN	4.1	4.9	4.9	4.8
SE	0.1	0.1	0.1	0.1

Serum Chloride (mEq/L)

Study 1

PL

Time (min)	0	60	120	180
KS	104.0	102.0	98.0	98.0
JS	104.0	105.5	102.0	102.0
BA	105.5	104.0		96.5
AG	104.0	103.5	97.0	100.5
CC	103.0	99.0	97.0	95.0
KK	118.0	114.5	110.0	109.0
JC	107.0	104.0	101.5	100.0
RF	119.0	117.0	117.0	115.0
ZF	119.0	116.0	112.0	112.0
EH	115.0	113.0	110.0	107.0
JT	107.0	105.0	103.0	120.0
MEAN	109.6	107.6	104.8	105.0
SE	2.0	1.9	2.2	2.5

NA

Time (min)	0	60	120	180
KS	102.0	99.5	98.0	101.0
JS	110.0	107.0	120.0	112.0
BA	107.0	106.0	106.0	105.0
AG	103.0	102.0	105.0	107.0
CC	102.0	101.0	100.0	103.0
KK	123.0	119.0	119.0	123.0
JC	118.0	115.0	116.0	119.0
RF	117.0	117.0	119.0	121.0
ZF	119.0	118.0	117.0	115.0
EH	117.0	114.0	113.0	114.0
JT	103.0	102.0	100.0	104.0
MEAN	111.0	109.1	110.3	111.3
SE	2.4	2.3	2.6	2.3

Serum Osmolality (mOsm/L)

Study 1

PL

Time (min)	0	60	120	180
AG	292	284	277	274
BA	295	284	279	275
CC	287	277	277	278
EH	292	283	280	277
JC	288	277	274	270
JS	292	284	279	277
JT	285	276	277	274
KK	290	281	276	277
KS	293	286	276	277
RF	290	287	285	278
ZF	293	287	278	278
MEAN	290.4	282.3	277.8	275.7
SE	0.9	1.2	0.9	0.7

NA

Time (min)	0	60	120	180
AG	290	285	287	292
BA	293	288	280	289
CC	289	286	281	278
EH	290	288	284	279
JC	288	283	283	289
JS	289	287	286	293
JT	285	280	278	284
KK	292	288	289	289
KS	285	277	276	281
RF	289	287	292	287
ZF	293	290	284	282
MEAN	289.4	285.4	283.6	285.7
SE	0.8	1.2	1.4	1.6

Hematocrit (%)

Study 1

PL

Time (min)	0	60	120	180
AG	48.4	51.1	50.9	49.8
BA	46.0	46.1	46.0	45.9
CC	46.0	47.6	47.4	48.0
EH	43.0	44.4	45.1	45.7
JC	46.4	47.7	47.7	46.2
JS	42.3	42.5	42.6	43.4
JT	43.7	44.3	43.1	43.0
KK	46.7	46.7	48.5	49.0
KS	47.6	47.3	49.8	50.6
RF	48.4	50.2	50.1	49.1
ZF	43.8	46.2	46.6	47.1
MEAN	45.7	46.7	47.1	47.1
SE	0.7	0.8	0.8	0.8

NA

Time (min)	0	60	120	180
AG	48.6	49.3	48.0	45.5
BA	44.9	45.5	43.7	43.7
CC	45.1	46.1	45.2	42.5
EH	44.1	46.5	46.4	43.9
JC	43.8	46.0	45.6	45.4
JS	39.3	39.6	39.8	39.5
JT	44.7	43.5	42.9	43.1
KK	43.7	46.1	43.8	44.1
KS	50.3	51.3	49.7	50.2
RF	51.9	50.4	50.9	49.2
ZF	46.8	48.1	46.9	45.3
MEAN	45.7	46.6	45.7	44.8
SE	1.1	1.0	1.0	0.9

Hemoglobin (g/dL)

Study 1

PL

Time (min)	0	60	120	180
AG	15.3	16.4	16.4	16.4
BA	17.9	18.2	15.8	15.0
CC	14.3	14.5	14.2	14.7
EH	13.2	14.6	14.1	14.9
JC	14.2	14.5	14.9	14.8
JS	12.6	12.8	12.9	13.1
JT	15.7	16.2	15.9	15.2
KK	14.8	14.7	14.7	14.7
KS	15.3	15.7	15.7	15.8
RF	14.9	15.3	15.6	15.3
ZF	16.0	14.6	14.5	15.0
MEAN	14.9	15.2	15.0	15.0
SE	0.4	0.4	0.3	0.2

NA

Time (min)	0	60	120	180
AG	15.8	15.8	15.1	15.5
BA	13.9	14.0	13.7	13.7
CC	14.3	14.1	13.9	13.7
EH	13.2	14.9	14.6	15.0
JC	14.4	15.2	15.1	15.0
JS	12.7	12.4	12.8	12.8
JT	17.1	16.5	16.4	16.8
KK	14.2	14.6	14.3	14.4
KS	16.4	16.5	15.8	15.8
RF	16.1	15.7	16.3	16.3
ZF	14.8	15.5	14.9	14.9
MEAN	14.8	15.0	14.8	14.9
SE	0.4	0.4	0.3	0.4

Mean Corpuscular Hemoglobin Concentration (g/dL)

Study 1

PL

Time (min)	0	60	120	180
AG	31.5	32.1	32.3	32.8
BA	38.8	39.6	34.2	32.6
CC	31.0	30.6	29.9	30.7
EH	30.7	32.9	31.3	32.5
JC	30.6	30.4	31.2	31.9
JS	32.1	33.1	31.5	31.2
JT	36.0	36.6	36.8	35.2
KK	31.6	31.4	30.3	30.0
KS	32.1	33.1	31.5	31.2
RF	30.8	30.4	31.2	31.1
ZF	36.5	31.6	31.2	31.7
MEAN	32.9	32.9	31.9	31.9
SE	0.9	0.9	0.6	0.4

NA

Time (min)	0	60	120	180
AG	32.5	32.1	31.5	34.0
BA	30.9	30.8	31.3	31.4
CC	30.1	30.5	30.8	32.1
EH	33.1	32.1	31.4	34.1
JC	32.8	32.9	33.0	33.0
JS	32.7	32.2	32.6	31.4
JT	38.3	37.9	38.1	38.9
KK	32.6	31.7	32.6	32.7
KS	32.7	32.2	31.9	31.6
RF	31.0	31.1	32.0	33.0
ZF	31.6	32.2	31.8	32.9
MEAN	32.6	32.3	32.5	33.2
SE	0.6	0.6	0.6	0.6

Urine Volume (ml)

Study 1

PL

Time (min)	0	30	60	90	120	150	180	Total
KS	475		665	432	400	449	303	2724
JS		230		185		125		540
BA	271	270	342	375	470	445	400	2573
AG			478		500	268	211	1457
CC		212	195	240		352	107	1106
KK		150	380	380	360	360	285	1915
JC	140	90	130	128	110	190	120	908
RF		308	532	510	342	447	340	2479
ZF		210	190	307	240	240	200	1387
EH	50	90	121	162	80	50	40	593
JT	312	247	305	442	290	408	100	2104
MEAN	249.6	200.8	333.8	316.1	310.2	303.1	210.6	1616.9
SE	73.1	25.4	57.7	41.7	48.8	41.8	37.6	238.5

NA

Time (min)	0	30	60	90	120	150	180	Total
KS		305		533	337	520	400	2095
JS	185	162	395	387	355	387	240	2111
BA	371	395	415	450	465	455	430	2981
AG		425	600	638	590	595	219	3067
CC	332	172	260	310	275	325	205	1879
KK		160	400	420	405	420	100	1905
JC		150	230	270	290	520	230	1690
RF		470	562	445	355	135	525	2492
ZF		230	318		325	330	228	1431
EH	25	30	220	172	120	0	0	567
JT		330	285	416	265	429	255	1980
MEAN	228.3	257.2	368.5	404.1	343.8	374.2	257.5	2018.0
SE	78.7	41.5	41.8	41.7	36.1	52.6	44.6	210.2

Urine Sodium Loss (mEq)

Study 1

PL

Time (min)	0	30	60	90	120	150	180	Total
AG			12.4					12.4
BA	16.7	4.1	5.1	7.1	9.4	6.7	4.0	53.0
CC		17.7	1.6	0.0				19.3
EH	4.3	1.0	1.0	1.6				7.9
JC	1.3							1.3
JS		6.9		3.2		1.8		11.9
JT	6.9	3.2	4.3	6.4	4.2	6.7	1.0	32.7
KK		7.5						7.5
KS	19.2		8.3	4.3	5.0	5.4	3.0	45.3
RF		18.3	10.6	6.1	2.7	3.1		41.0
ZF		10.3	2.3					12.6
MEAN	9.7	8.6	5.7	4.1	5.3	4.7	2.7	22.3
SE	3.5	2.3	1.5	1.0	1.4	1.0	0.9	5.3

NA

Time (min)	0	30	60	90	120	150	180	Total
AG		8.1	5.4	5.7	7.7	10.7	8.1	45.7
BA	5.9	5.5	7.9	8.6	9.3	8.2	6.9	52.3
CC	25.2	3.5	3.3	4.3	3.3	2.9		42.6
EH	3.2	1.8	2.4	1.9				9.3
JC		5.0	3.7	3.8	4.6	9.1	3.5	29.6
JS	28.1	8.4	11.1	9.7	8.9	9.7	8.9	84.7
JT		10.4	3.6	6.0	3.3	6.4	2.7	32.4
KK		15.3	9.8	5.9	6.1	8.4	1.8	47.2
KS					5.0	5.4	4.8	15.2
RF		19.7	7.3	6.0	3.9	2.8	6.8	46.6
ZF		8.3	3.5		3.3			15.0
MEAN	15.6	8.6	5.8	5.8	5.5	7.1	5.4	38.2
SE	6.4	1.7	1.0	0.8	0.7	1.0	0.9	6.5

Urine Sodium Concentration (mEq/L)

Study 1

PL

Time (min)	0	30	60	90	120	150	180
AG			26		0	0	0
BA	62	15	15	19	20	15	10
CC		84	8	0		0	0
EH	85	11	8	10	0	0	0
JC	9	0	0	0	0	0	0
JS		30		18		14	
JT	22	13	14	15	15	17	10
KK		50	0	0	0	0	0
KS	41		13	10	13	12	10
RF		60	20	12	8	7	0
ZF		49	12	0	0	0	0
MEAN	43.7	34.6	11.6	8.3	6.1	5.9	3.0
SE	13.7	9.2	2.6	2.4	2.6	2.1	1.5

NA

Time (min)	0	30	60	90	120	150	180
AG		19	9	9	13	18	37
BA	16	14	19	19	20	18	16
CC	76	21	13	14	12	9	0
EH	127	60	11	11	0	16	0
JC		33	16	14	16	18	15
JS	152	52	28	25	25	25	37
JT		32	13	15	13	15	11
KK		96	25	14	15	20	18
KS		0	0	0	11	12	12
RF		42	13	14	11	21	13
ZF		36	11		10	0	0
MEAN	92.8	36.7	14.2	13.4	13.2	15.5	14.4
SE	30.1	7.8	2.3	2.0	1.9	2.0	3.9

Urine Potassium Concentration (mEq/L)

Study 1

PL

Time (min)	0	30	60	90	120	150	180
AG			37		20	18	20
BA	17	9	8	0	10	11	11
CC		27	10	0		14	18
EH	94	30	24	23	30	33	34
JC	15	21	18	19	20	20	17
JS		25		26		36	
JT	9	10	0	8	8	14	17
KK		50	19	18	21	20	19
KS	0		0	0	0	0	9
RF		36	17	15	16	15	16
ZF		47	30	23	28	21	24
MEAN	27.1	28.2	16.3	13.2	17.0	18.4	18.4
SE	17.0	4.8	3.8	3.3	3.2	3.0	2.2

NA

Time (min)	0	30	60	90	120	150	180
AG		47	22	19	20	22	41
BA	10	10	11	10	11	11	11
CC	12	12	9	9	14	14	16
EH	79	68	19	20	25	68	0
JC		28	17	17	17	16	19
JS	41	31	17	15	14	14	37
JT		19	10	8	12	20	16
KK		49	19	0	0	17	16
KS		0	0	9	10	11	13
RF		39	10	20	22	41	23
ZF		39	22		22	27	31
MEAN	35.9	31.0	14.0	12.7	15.1	23.6	20.2
SE	16.2	6.0	2.0	2.1	2.1	5.2	3.6

Urine Chloride Concentration (mEq/L)

Study 1

PL

Time (min)	0	30	60	90	120	150	180
AG			51		21	20	19
BA	59	0	0	0	20	0	0
CC		128	0	9		0	0
EH	173	32	23	0	0	0	0
JC	0	20	0	0	0	0	20
JS		46		31		31	
JT	21	0	0	16	18	0	0
KK		92	0	0	0	0	0
KS	38		0	0	0	0	0
RF		86	26	19	0	0	0
ZF		77	26	0	0	0	0
MEAN	58.1	53.3	12.6	7.5	6.6	4.6	3.9
SE	30.3	14.9	5.7	3.5	3.3	3.2	2.6

NA

Time (min)	0	30	60	90	120	150	180
AG		53	22	20	27	30	61
BA	0	0	0	23	23	21	23
CC	82	27	0	0	18	0	0
EH	220	156	0	20	21	68	0
JC		51	22	19	21	19	20
JS	188	69	34	29	28	28	44
JT		42	0	18	20	20	21
KK		147	30	12	14	25	21
KS		0	0	0	0	0	0
RF		64	19	19	0	37	0
ZF		64	0		18	18	26
MEAN	122.4	60.9	11.5	15.9	17.2	24.0	19.5
SE	50.4	15.2	4.1	3.0	2.8	5.5	5.9

Urine Osmolality (mOsm/L)

Study 1

PL

Time (min)	0	30	60	90	120	150	180
AG			218		107		112
BA		123	100	107	105	102	88
CC		539	122	97		112	141
EH	621	227	199	151	208	216	242
JC	144	184	153	164	149	141	149
JS		331		249		294	
JT	89	88	81	81	84		94
KK		555	140	112	113	114	107
KS			86	74	78	76	83
RF		351	135	116	123	115	114
ZF		707	227	181	193	194	213
MEAN	284.3	344.9	146.0	133.0	128.6	151.4	134.0
SE	168.8	71.5	16.7	17.0	15.2	23.3	17.0

NA

Time (min)	0	30	60	90	120	150	180
AG		231	107	96	99	116	217
BA	89	123	99	107	109	106	107
CC	346	172	101	94	103	95	110
EH	705	572	148	144	173		386
JC		226	115	108	114	106	117
JS	571	301	166	149	142	148	192
JT		167	83	78	83	80	89
KK		691	190	110	108	140	126
KS		77	0	83	81	78	83
RF		274	110	129	146	220	129
ZF		414	185		167	164	169
MEAN	427.6	295.1	118.3	109.5	120.2	125.1	156.7
SE	135.0	57.7	16.2	7.6	9.6	13.8	26.2

Heart Rate (beats/min)

Study 1

PL

Time(min)	0	30	45	60	75	90	105	120	135	150	165	180
AG	109	116	115	115	115	123	121	116	120		130	133
BA	117	119	124	117	122	124	123	124	124	124	122	124
CC	120	129	130	165	130	130	134	130	134	137	136	139
EH		130	141	135	149	150	150	145	146	147	150	158
JC	126	132	135	139	142	135	135	131	138	142	140	141
JS	115	117	120	123	124	123	116	122	126	127	126	125
JT	141	136	136	140	141	138	139	142	150	144	151	147
KK	127	135	132	132	127	135	133	129	136	132	132	
KS	115	115	114	115	111	114	114	118	116	122	121	122
RF	123	128	127	130	130	137	132	131	137	137	164	136
ZF	119	119	137	139	143	139	139	138	142	142	140	140
MEAN	121.2	125.1	128.3	131.8	130.4	131.6	130.5	129.6	133.5	135.4	137.5	136.5
SE	2.8	2.4	2.7	4.4	3.7	3.0	3.3	2.8	3.3	2.8	4.0	3.5

NA

Time(min)	0	30	45	60	75	90	105	120	135	150	165	180
AG	115	121	120	120	118	121	118	123	128	128		127
BA	111	130	120	125	128	123	126	131	127	130		132
CC	124	129	130	160	124	130	132	128	127	131	136	138
EH	133	145		139	140	146	146	144	148	152	151	153
JC	101	130	127		131	129	134	136	139		143	144
JS	115	120	118	117	114	119	121	121	118	119	124	126
JT	147	147	147	139	142	141	142	142	150	146	151	154
KK	140	144	137	144	145	143	143	147	142	148	147	
KS	108	108	110	114	112	113	114	114	114	113	116	118
RF	127	127	131	132	139	138	140	140	150	141		145
ZF	127	130	131	126	132	131	134	132	132	138	135	138
MEAN	122.5	130.1	127.1	131.6	129.5	130.4	131.8	132.5	134.1	134.6	137.9	137.4
SE	4.2	3.5	3.3	4.5	3.5	3.2	3.3	3.1	3.8	4.0	4.5	3.7

Core Temperature(°C)

Study 1

PL

Time (min)	0	15	30	45	60	75	90	105	120	135	150	165	180
AG	36.9	37.5	37.3	37.5	37.5	37.4	37.5	37.4	37.4	37.4	37.6	37.7	37.8
BA	37.0	37.7	37.6	37.5	37.6	37.7	37.8	37.9	37.9	37.8	37.9	37.9	37.9
CC	37.0	37.6	37.6	37.6	37.6	37.7	37.7	37.7	37.7	37.7	37.7	37.7	37.8
EH	38.1	38.4	38.6	38.6	38.6	38.5	38.6	38.4	38.5	38.4	38.5	38.5	38.6
JC	37.3	37.7	37.9	38.0	38.1	38.4	38.3	38.3	38.3	38.2	38.3	38.3	38.2
JS	37.5	37.4	37.9	38.1	38.1	38.0	38.1	38.1	38.1	37.7	38.0	38.1	38.1
JT	38.0	38.0	38.0	38.1	37.6	38.1	37.8	38.2	37.6	38.1	37.9	38.1	38.2
KK	37.4	37.7	37.8	37.9	37.9	37.8	38.0	38.0	38.0	37.9	38.1	37.9	37.9
KS	37.9	27.7	37.9	37.7	37.8	37.8	37.8	38.0	38.0	37.8	37.9	38.0	38.0
RF	37.3	38.1	37.7	37.8	37.7	37.8	37.8	37.7	37.8	37.8	37.9	72.0	37.8
ZF	37.3	38.0	37.7	37.8	37.7	37.8	37.8	37.8	37.9	37.7	37.5	37.9	37.9
MEAN	37.4	36.9	37.8	37.9	37.8	37.9	37.9	38.0	37.9	37.9	37.9	41.1	38.0
SE	0.1	0.9	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	3.1	0.1

NA

Time (min)	0	15	30	45	60	75	90	105	120	135	150	165	180
AG	36.8	37.4	37.5	37.5	37.5	37.5	37.5	37.6	37.7	37.6	37.7	37.8	37.8
BA	37.2	37.4	37.8	37.8	37.9	38.0	37.9	37.8	37.9	37.9	37.7	37.8	37.9
CC	37.0	37.8	37.9	37.8	37.9	37.7	37.8	37.9	37.9	37.9	38.1	37.9	38.1
EH	37.6	38.2	38.4	38.3	38.4	38.4	38.5	38.4	38.5	38.4	38.6	38.5	38.6
JC	37.1	37.6	37.8	37.9	38.1	38.1	38.2	38.1	38.2	38.2	38.4	38.3	38.3
JS	37.4	37.5	37.8	37.9	37.9	38.1	38.0	38.0	37.9	38.0	38.0	38.0	38.0
JT	37.6	37.9	38.0	38.1	37.6	38.0	38.0	38.1	37.6	38.0	38.0	38.1	38.2
KK	37.8	37.7	37.9	37.9	37.8	37.8	37.8	37.8	37.9	38.1	38.1	38.1	38.1
KS	37.4	37.9	37.9	37.9	37.9	37.9	38.0	38.0	38.0	37.9	37.9	38.0	38.0
RF	37.5	37.5	37.8	37.8	37.8	37.7	37.8	37.8	37.9	37.9	37.8	37.8	37.9
ZF	37.3	38.4	37.8	37.9	37.9	37.8	37.9	37.9	37.9	37.8	37.5	37.7	37.8
MEAN	37.3	37.8	37.9	37.9	37.9	37.9	37.9	37.9	37.9	38.0	38.0	38.0	38.1
SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Mean Arterial Pressure (mmHg)

Study 1

PL

Time (min)	45	105	165
AG	118.7	111.7	
BA	114.7	99.3	96.0
CC	68.7	100.0	103.3
EH	98.7	104.7	99.3
JC	106.7	104.7	102.0
JS	89.3	95.3	94.7
JT	103.3	86.7	83.3
KK	105.0		
KS	106.3	102.7	104.0
RF	100.0	107.3	70.9
ZF	117.3	169.3	109.3
MEAN	102.6	108.2	95.9
SE	4.3	7.1	4.0

NA

Time (min)	45	105	165
AG	111.3	110.7	110.0
BA	90.0	99.3	95.3
CC	67.6	92.0	94.0
EH	92.0	91.3	88.0
JC	117.3	108.0	94.7
JS	96.0	95.3	98.0
JT	86.7	83.3	80.0
KK	109.3	110.7	114.7
KS	105.3	102.0	102.0
RF		107.3	72.5
ZF	119.3	128.0	123.3
MEAN	99.5	102.5	97.5
SE	5.1	3.7	4.5

Rating of Perceived Exertion

Study 1

PL

Time (min)	0	75	120	150	180
AG	11	11	13	12	13
BA	9	11	12	13	14
CC	13	12	12	12	13
EH	11	12	12	12	13
JC	12	12	13	13	14
JS	12	11	12	12	13
JT	10	12	13	15	15
KK	11	12	12	13	14
KS	9	10	11	12	12
RF	13	13	14	14	15
ZF	9	11	12	12	13
MEAN	10.9	11.5	12.4	12.7	13.5
SE	0.5	0.2	0.2	0.3	0.3

NA

Time (min)	0	75	120	150	180
AG	12	12	13	13	15
BA	7	10	11	15	16
CC	12	12	12	12	13
EH	11	12	13	12	13
JC	12	12	13	13	14
JS	11	11	11	11	13
JT	11	12	13	15	15
KK	13	13	13	13	14
KS	8	10	12	13	13
RF	14	15	15	15	16
ZF	9	11	12	12	13
MEAN	10.9	11.8	12.5	13.1	14.1
SE	0.6	0.4	0.3	0.4	0.4

Sweating Rate (L/h)

Study 1

PL

Time (min)	0-30	30-60	60-90	90-120	120-150	150-180
AG	1.47	1.87	1.82	1.82	1.90	1.88
BA	1.21	1.46	1.40	1.41	1.45	1.32
CC	1.36	1.60	1.51	1.59	1.42	1.90
EH	1.33	1.78	1.70	1.86	1.59	1.86
JC	1.17	1.39	1.39	1.43	1.56	1.59
JS	1.40	1.66	1.69	1.86	1.71	1.70
JT	1.36	1.54	1.57	1.37	1.70	1.85
KK	1.42	2.16	1.66	1.80	1.86	0.60
KS	1.47	1.84	1.71	1.67	2.10	1.80
RF	1.59	1.94	1.59	2.02	1.68	1.52
ZF	1.98	1.27	2.18	1.96	2.17	2.36
MEAN	1.43	1.68	1.66	1.71	1.74	1.67
SE	0.1	0.1	0.1	0.1	0.1	0.1

NA

Time (min)	0-30	30-60	60-90	90-120	120-150	150-180
AG	1.52	1.87	1.80	1.79	1.75	1.48
BA	1.36	1.52	1.35	1.22	1.53	1.56
CC	1.24	1.77	1.67	1.64	1.48	1.59
EH	1.36	1.68	1.58	1.88	1.69	1.84
JC	1.29	1.59	1.51	1.57	1.60	1.67
JS	0.74	2.17	1.39	1.65	1.59	1.52
JT	1.56	1.58	1.62	1.52	1.66	1.44
KK	1.16	1.72	1.48	1.65	1.70	1.30
KS	1.36	1.77	1.81	1.50	1.77	1.80
RF	1.67	2.08	1.82	1.80	1.80	1.55
ZF	2.14	1.56	1.76	1.55	1.79	1.31
MEAN	1.40	1.76	1.62	1.61	1.67	1.55
SE	0.1	0.1	0.1	0.1	0.0	0.1

Sweat Sodium Concentration (mEq/L)

Study 1

PL

Time (min)	30-60	120-150
AG	65.4	69.9
BA	78.2	91.6
CC	46.0	51.5
EH	80.5	81.2
JC	61.5	72.8
JS	85.0	89.1
JT	42.4	42.2
KK	83.2	107.7
KS	65.6	74.8
RF	40.7	43.7
ZF	58.0	54.8
MEAN	64.2	70.8
SE	4.9	6.4

NA

Time (min)	30-60	120-150
AG	55.4	61.0
BA	66.3	71.2
CC	63.7	68.4
EH	100.8	87.6
JC	72.0	80.9
JS	77.4	91.6
JT	47.2	47.9
KK	93.0	104.1
KS	59.9	67.0
RF	45.0	48.1
ZF	42.7	48.7
MEAN	65.8	70.6
SE	5.7	5.7

Sweat Sodium Loss (mEq/h)

Study 1

PL

Time (min)	30-60	120-150
AG	122.3	133.0
BA	114.2	132.8
CC	73.6	73.3
EH	143.1	129.2
JC	85.4	113.5
JS	141.0	152.4
JT	65.3	71.9
KK	180.1	199.9
KS	120.9	157.2
RF	79.0	73.3
ZF	73.7	119.0
MEAN	109.0	123.2
SE	11.1	12.0

NA

Time (min)	30-60	120-150
AG	103.6	106.7
BA	100.8	108.9
CC	112.7	101.1
EH	169.3	148.0
JC	114.3	129.1
JS	168.1	145.6
JT	74.6	79.6
KK	160.4	176.5
KS	106.1	118.4
RF	93.8	86.8
ZF	66.5	87.3
MEAN	115.5	117.1
SE	10.7	9.1

Sweat Sodium Concentration (mEq/L)

Study 1

PL

Time (min)	30-60 Arm	30-60 Back	30-60 Thigh	30-60 Calf	120-150 Arm	120-150 Back	120-150 Thigh	120-150 Calf
AG	62	80	60	60	64	89	51	74
BA	75	88	70	79	81	90	83	105
CC	43	74	36	32	49	80	36	43
EH	76	113	70	64	85	108	72	66
JC	61	83	54	50	65	76	85	61
JS	72	99	72	90	83	95	80	95
JT	31	60	39	35	34	58	40	35
KK	90	101	82	67	93	104	84	139
KS	62	88	59	54	66	87	64	78
RF	40	55	34	36	41	54	42	38
ZF	58	58	56	60	54	57	51	58
MEAN	60.7	81.5	57.4	56.9	64.8	81.4	62.4	71.8
SE	5.3	5.7	4.8	5.5	5.8	5.6	5.8	9.5

NA

Time (min)	30-60 Arm	30-60 Back	30-60 Thigh	30-60 Calf	120-150 Arm	120-150 Back	120-150 Thigh	120-150 Calf
AG	54	76	47	46	61	82	49	55
BA	64	78	63	61	83	75	60	75
CC	67	97	52	45	74	101	53	53
EH	139	137	80	76	95	120	76	68
JC	79	87	71	58	94	89	76	75
JS	51	89	59	95	67	93	73	117
JT	41	73	39	35	43	71	41	36
KK	98	106	92	82	108	110	101	102
KS	59	78	53	52	57	76	53	76
RF	40	61	38	41	48	59	43	44
ZF	39	54	40	37	45	56	43	50
MEAN	66.2	84.8	57.4	56.9	70.3	84.4	60.6	68.1
SE	9.0	6.9	5.3	5.9	6.6	6.1	5.6	7.4

Sweat Chloride Concentration (mEq/L)

Study 1

PL

Time (min)	30-60 Arm	30-60 Back	30-60 Thigh	30-60 Calf	120-150 Arm	120-150 Back	120-150 Thigh	120-150 Calf
AG	55	72	54	52	58	84	45	67
BA	58	72	52	55	71	82	73	93
CC	39	69	31	28	44	76	34	38
EH	66	106	58	50	73	99	60	52
JC	44	66	37	25	66	60	75	45
JS	67	94	67	87	78	89	75	92
JT	22	46	26	23	30	52	33	28
KK	86	97	76	60	85	95	74	143
KS	56	82	52	46	67	82	58	73
RF	35	48	28	30	37	48	36	33
ZF	53	50	48	51	48	50	44	51
MEAN	52.7	72.8	48.0	46.0	59.5	74.0	54.9	64.8
SE	5.3	6.1	4.8	5.7	5.4	5.6	5.2	10.2

NA

Time (min)	30-60 Arm	30-60 Back	30-60 Thigh	30-60 Calf	120-150 Arm	120-150 Back	120-150 Thigh	120-150 Calf
AG	51	73	43	42	54	76	43	46
BA	57	69	53	50	77	69	53	65
CC	61	92	44	37	66	94	44	44
EH	74	128	63	57	66	94	64	55
JC	73	79	62	48	88	80	67	65
JS	48	89	60	102	66	91	72	127
JT	35	66	32	28				
KK	93	100	83	71	103	106	98	97
KS	53	73	47	44	0	0	0	0
RF	35	57	31	34	35	44	30	34
ZF	34	48	33	30	37	45	33	40
MEAN	55.5	79.2	49.9	49.2	59.2	69.8	50.2	57.2
SE	5.6	6.7	4.8	6.4	9.3	10.1	8.5	11.0

Stroop Color-Word Interference Task

Study 1

PL

	Time to correct response (sec)		% Correct	
	Pre	Post	Pre	Post
AG	1.14	1.10	96.0	98.2
BA	1.21	1.13	98.8	99.6
CC	0.99	1.00	99.0	94.4
EH	1.03	1.16	100.0	98.4
JC	1.16	1.31	100.0	100.0
JS	1.05	0.82	98.2	98.8
JT	1.35	1.73	96.6	88.8
KK	1.25	1.22	98.8	99.6
KS	1.27	1.23	99.4	99.4
RF	1.18	1.24	99.2	98.4
ZF	0.88	0.83	93.8	89.0
MEAN	1.14	1.16	98.16	96.78
SE	0.04	0.07	0.58	1.26

NA

	Time to correct response (sec)		% Correct	
	Pre	Post	Pre	Post
AG	1.14	0.81	96.0	97.8
BA	1.26	1.21	98.8	100.0
CC	0.92	0.84	99.2	97.0
EH	1.11	0.98	100.0	99.2
JC	1.47	1.26	97.0	98.4
JS	0.82	0.93	99.6	99.0
JT	1.30	1.13	98.6	97.0
KK	1.18	1.19	99.6	97.8
KS	1.55	1.46	98.2	93.8
RF	1.39	1.24	98.4	100.0
ZF	1.23	0.91	85.0	88.4
MEAN	1.22	1.09	97.3	97.1
SE	0.07	0.06	1.3	1.0

Center of Pressure Amplitude in the Anteroposterior (ap) Direction (m)

Study 1

PL

	Stance 1 Pre	Stance 1 Post	Stance 2 Pre	Stance 2 Post	Stance 3 Pre	Stance 3 Post
BA	0.030	0.028	0.086	0.083	0.111	0.077
EH	0.034	0.029	0.063	0.043	0.112	0.087
JC	0.029	0.027	0.043	0.040	0.123	0.088
JS	0.034	0.031	0.042	0.033	0.056	0.059
RF	0.021	0.017	0.040	0.036	0.124	0.097
ZF	0.020	0.025	0.048	0.039	0.109	0.088
MEAN	0.028	0.026	0.054	0.046	0.106	0.082
SE	0.002	0.002	0.007	0.008	0.010	0.005

NA

	Stance 1 Pre	Stance 1 Post	Stance 2 Pre	Stance 2 Post	Stance 3 Pre	Stance 3 Post
BA	0.035	0.029	0.149	0.074	0.127	0.060
EH	0.024	0.024	0.040	0.036	0.060	0.100
JC	0.024	0.022	0.044	0.032		0.134
JS	0.026	0.032	0.044	0.039	0.068	0.084
RF	0.016	0.016	0.033	0.031	0.085	0.062
ZF	0.016	0.031	0.039	0.037	0.115	0.094
MEAN	0.023	0.026	0.058	0.041	0.091	0.089
SE	0.003	0.002	0.018	0.007	0.013	0.011

Center of Pressure Amplitude in the Mediolateral (ml) Direction (m)

Study 1

PL

	Stance 1 Pre	Stance 1 Post	Stance 2 Pre	Stance 2 Post	Stance 3 Pre	Stance 3 Post
BA	0.020	0.023	0.064	0.065	0.076	0.075
EH	0.024	0.025	0.028	0.038	0.057	0.056
JC	0.019	0.021	0.028	0.032	0.043	0.046
JS	0.023	0.027	0.033	0.027	0.046	0.048
RF	0.014	0.014	0.028	0.026	0.202	0.112
ZF	0.015	0.018	0.024	0.028	0.056	0.067
MEAN	0.019	0.021	0.034	0.036	0.080	0.067
SE	0.002	0.002	0.006	0.006	0.025	0.010

NA

	Stance 1 Pre	Stance 1 Post	Stance 2 Pre	Stance 2 Post	Stance 3 Pre	Stance 3 Post
BA	0.020	0.026	0.088	0.058	0.088	0.065
EH	0.022	0.022	0.028	0.028	0.053	0.057
JC	0.022	0.017	0.026	0.023	6.387	0.049
JS	0.024	0.023	0.026	0.031	0.053	0.051
RF	0.015	0.018	0.024	0.023	0.073	0.051
ZF	0.017	0.020	0.027	0.022	0.089	0.087
MEAN	0.020	0.021	0.037	0.031	1.124	0.060
SE	0.002	0.001	0.010	0.006	1.053	0.006

Work completed during final 15 min of Time Trial (J)

Study 1

PL

Time (min)	5-10	10-15	15-20	Total	Mean Watts
AG	102900	91500	107850	292200	325
BA	66000	73200	77100	212200	236
CC	78000	78000	85800	234000	260
EH	79200	74400	84240	229200	255
JC	93450	91500	93600	285300	317
JS	88800	84000	84000	256800	285
KK	85800	85800	106680	267600	297
RF	108000	108000	118800	324000	360
ZF	89100	84000	94200	257700	286
MEAN	87917	85600	94697	262111	291
SE	4281	3562	4582	11616	13

NA

Time (min)	5-10	10-15	15-20	Total	Mean Watts
AG	117300	119400	116850	355050	395
BA	63000	63000	73500	195400	217
CC	81000	81000	93300	238500	265
EH	82800	82200	98940	254400	283
JC	95400	94500	95400	278550	310
JS	83400	79200	75600	238200	265
KK	85800	85800	101580	263100	292
RF	108000	112200	121200	333000	370
ZF	96000	96000	107100	288900	321
MEAN	90300	90367	98163	271678	302
SE	5345	5794	5427	16443	18

Maximal Power (watts)

Study 1

PL

Time (min)	0	60	120	180
AG	1205	1179	1211	1252
BA	1149			1096
CC	1002	1026	1042	964
EH	1205	1148	1060	1047
JC	1285	1313	1228	1169
JS	1312	1337	1357	1247
JT	1163	1157	1132	1094
KK	1255	1219	1152	1231
KS	1235	1256	1231	1153
RF	1486	1463	1477	1487
ZF	1490	1525	1485	1474
MEAN	1253.4	1262.3	1237.5	1201.3
SE	42.8	47.9	49.7	49.3

NA

Time (min)	0	60	120	180
AG	1263	1228	1225	1252
BA	1119	1080	1069	1054
CC	1002	997	1029	956
EH	1113	1139	1118	1055
JC	1282	1289	1303	1294
JS	1335	1258	1301	1247
JT	1203	1094	1054	1097
KK	1220	1295	1232	1295
KS	1194	1293	1262	1211
RF	1474	1456	1388	1399
ZF	1452	1425	1435	1416
MEAN	1241.5	1232.2	1219.6	1206.9
SE	42.9	43.2	41.2	44.8

Torque at Pmax (Nm)

Study 1

PL

Time (min)	0	60	120	180
AG	88.6	87.4	89.2	91.8
BA	96.5			90.5
CC	85.2	81.0	81.6	77.6
EH	90.2	87.8	88.9	82.6
JC	104.8	100.0	91.8	88.6
JS	101.3	109.4	105.6	97.8
JT	97.7	98.0	96.4	93.9
KK	97.5	100.7	92.6	96.1
KS	87.5	93.2	92.0	88.3
RF	118.2	115.4	116.7	118.0
ZF	114.6	116.9	115.6	115.4
MEAN	98.4	99.0	97.0	94.6
SE	3.3	3.8	3.7	3.7

NA

Time (min)	0	60	120	180
AG	91.3	95.8	95.5	104.7
BA	85.7	89.2	83.2	82.7
CC	85.2	79.2	85.5	81.4
EH	96.6	87.4	86.4	83.1
JC	94.4	102.0	96.8	95.2
JS	109.3	98.7	102.1	97.6
JT	100.1	94.0	90.9	94.2
KK	99.5	98.5	102.2	99.0
KS	94.5	100.6	99.8	95.8
RF	115.9	114.8	111.6	105.5
ZF	114.2	111.6	112.5	110.5
MEAN	98.8	97.4	97.0	95.4
SE	3.2	3.1	3.0	2.9

Velocity at Pmax (revolutions/minute)

Study 1

PL

Time (min)	0	60	120	180
AG	129	128	129	130
BA	113			115
CC	112	120	121	118
EH	127	124	113	120
JC	116	125	127	125
JS	123	116	122	121
JT	113	112	112	111
KK	122	115	118	122
KS	134	128	127	124
RF	119	120	120	120
ZF	123	124	122	121
MEAN	121.0	121.2	121.1	120.6
SE	2.2	1.7	1.8	1.5

NA

Time (min)	0	60	120	180
AG	131	122	122	116
BA	124	115	122	121
CC	112	119	114	112
EH	127	124	123	121
JC	129	120	128	129
JS	116	121	121	123
JT	114	111	110	111
KK	116	125	114	124
KS	120	122	102	120
RF	121	120	118	126
ZF	121	121	121	122
MEAN	121.0	120.0	117.7	120.5
SE	1.9	1.2	2.2	1.7

Study 2: Raw Data

Body Mass (kg)
Study 2
PL

	CV - Pre	Sweat - Pre	Sweat - Post
CL	61.05	60.55	60.40
JM	86.55	85.70	85.35
JW	83.50	82.50	82.50
LH	76.45	74.15	73.90
MD	71.15	70.60	70.60
NY	79.85	79.45	79.30
PT	80.35	79.55	79.20
SP	72.20	70.40	70.25
TS	77.75	77.20	77.05
MEAN	76.54	75.57	75.39
SE	2.5	2.5	2.5

NA

	CV - Pre	Sweat - Pre	Sweat - Post
CL	61.95	61.25	61.10
JM	86.75	86.15	85.80
JW	86.60	85.90	85.60
LH	75.70	74.05	73.70
MD	70.95	70.35	70.15
NY	80.30	79.90	79.80
PT	81.00	80.05	79.65
SP	70.76	71.75	71.55
TS	77.50	76.95	76.75
MEAN	76.83	76.26	76.01
SE	2.7	2.6	2.6

Serum Sodium Concentration (mEq/L)

Study 2

PL

Time (min)	0	5	10	15	20
CL	144.0	146.5	149.0	149.0	145.0
JM	144.5	145.0	148.0	148.0	145.0
JW	147.0	148.5	148.0	148.0	146.0
LH	146.0	148.0	148.0	149.0	145.0
MD	143.0	144.0	146.0	146.0	144.0
NY	143.0	146.0	146.0	146.5	144.0
PT	144.5	146.0	147.0	148.0	145.0
SP	143.0	145.5	147.0	147.0	144.0
TS	145.0	146.0	149.0	148.5	145.0
MEAN	144.4	146.2	147.6	147.8	144.8
SE	0.5	0.5	0.4	0.4	0.2

NA

Time (min)	0	5	10	15	20
CL	144.0	147.0	149.0	149.0	146.0
JM	144.5	146.0	149.0	149.0	146.0
JW	148.0	148.0	149.0	149.0	146.0
LH	147.0	149.0	150.0	150.0	146.0
MD	144.0	145.0	146.5	147.0	145.0
NY	144.5	148.0	148.0	148.0	145.0
PT	145.0	146.5	147.5	148.0	146.0
SP	144.0	146.5	148.0	146.5	145.0
TS	144.0	147.0	149.0	149.0	144.5
MEAN	145.0	147.0	148.4	148.4	145.5
SE	0.5	0.4	0.3	0.4	0.2

Serum Potassium (mEq/L)

Study 2

PL

Time (min)	0	5	10	15	20
CL	4.2	4.3	4.5	5.0	3.9
JM	3.9	4.4	4.7	5.0	4.2
JW	3.8	4.5	4.6	4.7	4.3
LH	4.0	4.5	4.6	4.6	4.2
MD	4.1	4.6	4.9	5.0	4.5
NY	4.2	4.8	4.8	4.9	4.4
PT	4.2	4.6	5.0	5.1	4.3
SP	4.0	4.9	5.1	5.3	4.3
TS	3.6	4.2	4.5	4.6	4.0
MEAN	4.0	4.5	4.7	4.9	4.2
SE	0.1	0.1	0.1	0.1	0.1

NA

Time (min)	0	5	10	15	20
CL	3.8	5.0	5.0	5.2	3.9
JM	3.7	4.0	4.3	4.8	4.1
JW	4.1	4.5	4.7	4.8	4.2
LH	3.8	4.0	4.3	4.3	3.8
MD	4.3	4.7	5.1	5.2	4.8
NY	4.4	4.6	4.6	4.6	4.2
PT	4.0	4.3	4.6	4.7	4.2
SP	4.2	5.0	5.2	5.3	4.3
TS	3.9	4.3	4.9	4.9	4.1
MEAN	4.0	4.5	4.7	4.8	4.2
SE	0.1	0.1	0.1	0.1	0.1

Serum Chloride (mEq/L)

Study 2

PL

Time (min)	0	5	10	15	20
CL	105.5	108.0	106.0	108.0	105.0
JM	106.0	104.0	107.0	108.0	105.0
JW	109.5	111.0	111.0	112.0	111.0
LH	108.0	108.0	109.0	110.0	108.5
MD	106.0	104.0	106.0	106.5	104.5
NY	106.0	107.0	107.5	108.0	108.0
PT	107.0	108.0	110.0	110.0	108.0
SP	106.0	105.0	108.0	108.0	106.0
TS	109.5	106.5	109.5	108.5	107.0
MEAN	107.1	106.8	108.2	108.8	107.0
SE	0.5	0.8	0.6	0.5	0.7

NA

Time (min)	0	5	10	15	20
CL	107.0	108.0	110.0	109.5	107.0
JM	105.5	105.5	108.0	108.5	107.0
JW	112.0	112.0	113.0	114.0	112.0
LH	110.0	111.0	111.0	111.0	110.0
MD	105.0	105.0	107.0	107.0	105.0
NY	109.5	110.0	111.0	110.0	109.0
PT	108.5	109.0	110.0	111.0	109.0
SP	108.0	108.0	111.0	110.0	108.0
TS	105.5	106.0	108.0	107.5	105.5
MEAN	107.9	108.3	109.9	109.8	108.1
SE	0.8	0.8	0.6	0.7	0.7

Hematocrit (%)

Study 2

PL

Time (min)	0	5	10	15	20
CL	48.0	51.0	52.1	52.7	51.1
JM	50.3	53.0	53.1	53.6	52.0
JW	42.2	44.6	44.3	44.1	42.3
LH	45.7	46.7	47.8	48.2	46.6
MD	49.4	53.2	52.8	53.2	51.4
NY	47.0	48.4	47.5	48.5	48.3
PT	45.2	46.6	46.6	46.2	44.8
SP	48.3	50.8	51.1	51.6	49.3
TS	44.6	45.1	48.7	49.8	48.8
MEAN	46.7	48.8	49.3	49.8	48.3
SE	0.9	1.1	1.0	1.1	1.1

NA

Time (min)	0	5	10	15	20
CL	45.5	49.6	50.5	49.7	49.8
JM	48.9	50.7	52.3	51.6	51.2
JW	41.3	42.3	43.0	42.4	41.2
LH	42.1	44.2	43.1	45.3	44.2
MD	49.5	49.6	51.0	50.6	50.5
NY	46.0	46.7	47.3	46.9	46.2
PT	42.7	44.0	43.5	43.9	44.4
SP	45.7	46.3	48.1	48.4	47.1
TS	47.8	49.0	50.0	49.7	49.3
MEAN	45.5	46.9	47.6	47.6	47.1
SE	1.0	1.0	1.2	1.1	1.1

Hemoglobin (g/dL)

Study 2

PL

Time (min)	0	5	10	15	20
CL	16.1	17.3	17.7	18.3	17.3
JM	16.5	17.5	18.1	18.2	17.5
JW	14.0	14.9	15.1	15.2	14.2
LH	14.8	14.8	15.7	16.0	15.1
MD	15.9	16.8	17.3	17.2	16.7
NY	14.3	15.5	15.5	15.3	15.1
PT	14.1	14.7	14.9	14.9	14.3
SP	15.6	16.4	16.8	16.9	16.0
TS	14.5	16.0	16.4	16.9	16.1
MEAN	15.1	16.0	16.4	16.5	15.8
SE	0.3	0.4	0.4	0.4	0.4

NA

Time (min)	0	5	10	15	20
CL	15.0	16.8	17.1	17.1	16.5
JM	16.0	16.6	17.2	17.1	16.7
JW	13.2	14.1	14.3	14.2	13.8
LH	14.0	14.5	14.7	15.2	14.6
MD	15.4	16.1	16.4	16.5	15.9
NY	14.7	15.0	15.3	15.2	14.9
PT	13.5	14.2	14.3	14.4	14.1
SP	14.5	14.7	15.8	15.5	15.2
TS	15.3	16.3	17.0	17.0	16.7
MEAN	14.6	15.4	15.8	15.8	15.4
SE	0.3	0.4	0.4	0.4	0.4

Mean Corpuscular Hemoglobin Concentration (g/dL)

Study 2

PL

Time (min)	0	5	10	15	20
CL	33.5	33.9	34.0	34.6	33.9
JM	32.8	33.0	34.1	34.0	33.6
JW	33.2	33.4	34.1	34.5	33.6
LH	32.4	31.7	32.9	33.2	32.4
MD	32.2	31.6	32.8	32.3	32.5
NY	30.5	32.0	32.7	31.6	31.3
PT	31.3	31.5	31.9	32.3	31.9
SP	32.2	32.2	32.9	32.7	32.4
TS	32.4	35.4	33.7	33.9	33.0
MEAN	32.3	32.7	33.2	33.2	32.7
SE	0.3	0.4	0.3	0.4	0.3

NA

Time (min)	0	5	10	15	20
CL	33.0	33.9	33.8	34.4	33.2
JM	32.8	32.7	32.8	33.2	32.6
JW	32.0	33.2	33.1	33.4	33.4
LH	33.3	32.8	34.1	34.6	33.0
MD	31.1	32.5	32.2	32.6	31.5
NY	32.0	32.2	32.4	32.4	32.3
PT	31.7	32.3	32.9	32.8	31.7
SP	31.8	31.8	32.8	32.0	32.2
TS	32.0	33.3	34.0	34.2	33.9
MEAN	32.2	32.7	33.1	33.3	32.6
SE	0.2	0.2	0.2	0.3	0.3

Plasma Volume Change (%)
Study 2

Time (min)	0	5	10	15	20
CL	12.5	5.9	7.0	13.8	7.6
JM	6.2	10.7	7.6	10.9	6.7
JW	7.4	10.6	7.9	10.1	4.6
LH	12.7	7.5	16.1	10.7	6.1
MD	3.1	12.8	9.8	10.2	6.8
NY	0.0	6.4	1.6	4.0	5.5
PT	9.5	8.7	9.9	8.3	2.3
SP	12.3	21.8	10.2	15.9	9.9
TS	-10.8	-0.9	-5.9	-0.4	-4.3
MEAN	5.9	9.3	7.1	9.3	5.0
SE	2.5	2.0	2.1	1.6	1.4

Blood Volume Change (%)
Study 2

Time (min)	0	5	10	15	20
CL	7.3	3.0	3.5	7.0	4.9
JM	3.2	5.5	5.6	6.4	4.8
JW	5.7	6.1	5.5	6.9	2.7
LH	5.7	2.6	6.5	4.9	3.5
MD	3.2	4.7	5.6	4.6	4.9
NY	-2.6	3.0	1.2	0.9	1.4
PT	4.7	3.5	3.9	3.8	1.6
SP	7.0	11.4	3.9	8.8	5.3
TS	-5.4	-2.1	-3.5	-0.6	-3.4
MEAN	3.2	4.2	3.6	4.8	2.8
SE	1.5	1.2	1.0	1.0	0.9

Urine Electrolyte Concentration (mEq/L)
Study 2
PL

	Na	K	Cl
CL	73.0	38.4	69.5
JM	111.0	47.9	130.5
JW	100.5	34.7	107.0
LH	89.0	16.1	93.0
MD	67.0	52.5	110.5
NY	44.5	36.3	81.0
PT	68.5	23.1	81.5
SP	0.0	32.5	23.0
TS	69.5	37.3	97.0
MEAN	69.2	35.4	88.1
SE	10.9	3.7	10.2

NA

	Na	K	Cl
CL	52.0		46.0
JM	39.0		32.0
JW	112.0		126.5
LH	107.5	28.3	155.0
MD	76.5	25.0	87.0
NY	44.0	18.5	58.0
PT	82.5	24.0	103.0
SP	31.5	12.6	35.0
TS	109.0	27.0	114.5
MEAN	72.7	22.6	84.1
SE	10.7	2.4	14.6

Heart Rate (beats/min)

Study 2

PL

Time (min)	-5	5	8	11	14	20	23
CL	62	160	166	173	178	106	104
JM	70	169	175	178	183	118	117
JW	76	138	145	145	148	92	87
LH	74	129	137	137	145	98	95
MD	69	147	160	167	170	103	102
NY	78	131	137	140	145	95	97
PT	75	143	148	155	156	94	103
SP	69	149	155	160	164	88	87
TS	76	156	170	176	179	126	127
MEAN	72.1	146.9	154.8	158.9	163.1	102.2	102.1
SE	1.7	4.4	4.7	5.3	5.1	4.2	4.4

NA

Time (min)	-5	5	8	11	14	20	23
CL	64	147	154	162	168	96	100
JM	71	166	174	176	180	109	113
JW	76	138	141	143	146	91	92
LH	67	132	135	138	143	95	89
MD	69	142	156	165	165	96	99
NY	75	133	143	147	150	95	95
PT	68	138	145	152	155	98	97
SP	54	143	147	150	153	82	82
TS	73	158	167	176	183	131	129
MEAN	68.6	144.1	151.3	156.6	160.3	99.2	99.6
SE	2.2	3.8	4.2	4.6	4.8	4.6	4.6

Cardiac Output (L/min)

Study 2

PL

Time (min)	-5	5	8	11	14	20	23
CL	7.1	14.8	13.2	12.9	13.3	7.3	7.8
JM	13.0	19.4	18.2	18.2	18.5	10.7	10.9
JW	8.5	21.7	20.0	20.0	21.6	10.2	9.3
LH	6.4	20.6	21.1	21.1	24.5	10.0	11.2
MD	7.8	17.8	19.0	18.1	17.8	7.8	7.3
NY	6.2	19.7	19.4	18.7	19.7	9.8	9.2
PT	11.1	20.3	19.3	21.5	21.6	11.0	11.6
SP	13.4	19.7	20.6	20.5	15.8	14.0	12.5
TS	9.6	23.0	21.9	22.7	22.7	15.1	11.3
MEAN	9.2	19.7	19.2	19.3	19.5	10.7	10.1
SE	0.9	0.8	0.8	1.0	1.2	0.8	0.6

NA

Time (min)	-5	5	8	11	14	20	23
CL	7.1	15.0	14.1	14.7	15.0	10.2	7.8
JM	9.2	20.9	20.5	20.4	22.1	12.7	11.8
JW	10.9	23.6	24.2	22.7	23.2	12.6	10.9
LH	7.4	25.1	22.4	23.2	28.7	14.7	14.9
MD	8.9	17.5	19.3	21.9	19.2	11.2	7.8
NY	8.2	19.8	19.4	19.8	18.3	10.9	7.8
PT	10.6	22.2	23.1	24.8	26.2	14.7	14.2
SP	11.7	21.3	20.5	21.8	21.7	12.4	14.5
TS	9.9	22.3	20.3	21.1	21.2	15.8	14.1
MEAN	9.3	20.8	20.4	21.1	21.7	12.8	11.5
SE	0.5	1.0	1.0	1.0	1.4	0.6	1.0

Stroke Volume (ml/beat)

Study 2

PL

Time (min)	-5	5	8	11	14	20	23
CL	114.4	92.8	79.7	74.6	74.9	69.3	75.3
JM	185.9	115.0	104.1	102.1	101.1	90.8	92.8
JW	111.6	157.0	137.9	138.0	145.9	110.5	106.4
LH	87.1	159.8	153.7	153.9	168.8	102.0	117.6
MD	113.4	121.4	118.6	108.6	104.6	75.6	71.7
NY	79.8	150.0	141.8	133.8	135.7	103.2	94.6
PT	147.3	142.0	130.7	138.9	138.1	117.4	112.6
SP	194.2	132.5	132.8	128.1	96.3	159.0	143.6
TS	126.5	147.2	129.0	129.1	126.9	119.7	89.0
MEAN	128.9	135.3	125.4	123.0	121.4	105.3	100.4
SE	13.3	7.4	7.4	8.0	9.8	8.9	7.5

NA

Time (min)	-5	5	8	11	14	20	23
CL	110.8	101.8	91.5	90.4	89.4	106.7	77.7
JM	129.3	126.1	117.6	116.0	122.9	116.2	104.8
JW	143.0	170.9	171.7	158.7	159.0	138.5	118.0
LH	111.1	190.0	165.6	167.9	200.3	154.6	167.8
MD	129.1	123.5	123.8	132.5	116.6	116.8	79.0
NY	109.1	148.5	135.5	134.6	121.7	114.2	82.0
PT	155.3	160.5	159.5	163.2	169.3	150.0	146.3
SP	216.9	148.7	139.5	145.2	141.5	151.5	177.1
TS	135.8	141.3	121.7	120.1	115.8	120.8	109.3
MEAN	137.8	145.7	136.3	136.5	137.4	129.9	118.0
SE	11.2	8.9	8.7	8.4	11.3	6.2	12.6

Oxygen Consumption (L/min)

Study 2

PL

Time (min)	-5	5	8	11	14	20	23
CL	0.25	1.75	1.86	1.89	1.99	0.37	0.24
JM	0.35	2.31	2.50	2.49	2.58	0.43	0.39
JW	0.36	2.49	2.61	2.63	2.72	0.53	0.45
LH	0.31	2.07	2.19	2.29	2.46	0.52	0.40
MD	0.29	2.01	2.20	2.35	2.37	0.46	0.44
NY	0.26	1.99	2.08	2.11	2.27	0.45	0.38
PT	0.41	2.24	2.28	2.38	2.44	0.38	0.39
SP	0.28	2.46	2.58	2.61	2.66	0.31	0.26
TS	0.26	2.16	2.33	2.45	2.45	0.49	0.40
MEAN	0.31	2.16	2.29	2.36	2.44	0.44	0.37
SE	0.02	0.08	0.08	0.08	0.07	0.02	0.02

NA

Time (min)	-5	5	8	11	14	20	23
CL	0.23	1.69	1.85	1.90	1.92	0.41	0.35
JM	0.37	2.63	2.86	2.87	2.88	0.56	0.51
JW	0.41	2.49	2.60	2.63	2.65	0.46	0.43
LH	0.33	2.00	2.18	2.31	2.31	0.56	0.32
MD	0.24	1.70	1.90	2.08	2.22	0.49	0.29
NY	0.35	2.04	2.23	2.28	2.33	0.52	0.41
PT	0.32	2.29	2.50	2.59	2.71	0.39	0.41
SP	0.24	2.28	2.39	2.46	2.48	0.30	0.28
TS	0.33	2.34	2.51	2.56	2.76	0.60	0.55
MEAN	0.31	2.16	2.34	2.41	2.47	0.47	0.39
SE	0.02	0.11	0.11	0.10	0.10	0.03	0.03

Arterial Venous Oxygen Difference (ml O2/L)

Study 2

PL

Time (min)	-5	5	8	11	14	20	23
CL	3.5	11.8	14.0	14.7	14.9	5.1	3.1
JM	2.7	11.9	13.7	13.7	13.9	4.0	3.6
JW	4.3	11.5	13.0	13.1	12.6	5.2	4.9
LH	2.7	9.4	10.6	10.8	10.8	3.2	3.5
MD	4.2	10.1	10.7	11.3	11.6	4.6	4.1
NY	4.8	10.0	10.4	10.8	10.1	5.2	3.6
PT	3.7	11.0	11.8	11.0	11.3	3.5	3.3
SP	2.1	12.5	12.5	12.8	16.8	2.2	2.1
TS	3.7	11.2	11.6	12.9	13.3	5.9	6.0
MEAN	3.51	11.06	12.05	12.36	12.81	4.33	3.80
SE	0.29	0.33	0.46	0.47	0.72	0.39	0.37

NA

Time (min)	-5	5	8	11	14	20	23
CL	3.2	11.3	13.1	13.0	12.8	4.0	4.5
JM	4.0	12.5	14.0	14.1	13.0	4.4	4.3
JW	3.7	10.6	10.7	11.6	11.4	3.6	4.0
LH	3.3	10.5	12.3	12.1	13.0	3.8	3.9
MD	4.2	10.3	11.5	11.5	12.8	4.7	5.3
NY	4.4	8.0	9.8	9.9	8.1	3.8	2.1
PT	3.0	10.3	10.8	10.4	10.3	2.7	2.9
SP	2.0	10.7	11.7	11.3	11.5	2.4	1.9
TS	2.7	9.7	9.8	9.5	11.5	4.3	3.7
MEAN	3.40	10.43	11.53	11.49	11.60	3.75	3.62
SE	0.26	0.41	0.48	0.48	0.54	0.26	0.37

Mean Arterial Pressure (mmHg)

Study 2

PL

Time (min)	-8	8	14	20
CL	97.3	130.7	120.7	96.3
JM	94.3	117.3	114.0	98.7
JW	85.3	103.0	102.3	101.3
LH	78.7	103.3	100.0	81.3
MD	92.7	119.0	114.7	100.0
NY	90.7	110.3	111.0	95.7
PT	95.7	108.7	107.3	93.7
SP	89.7	108.7	107.3	90.7
TS	93.7	103.7	91.3	97.3
MEAN	90.9	111.6	107.6	95.0
SE	1.9	3.1	2.9	2.0

NA

Time (min)	-8	8	14	20
CL	91.0	114.7	118.7	94.3
JM	95.3	117.0	113.0	105.7
JW	86.0	98.0	99.0	86.3
LH	91.7	104.7	112.0	97.0
MD	101.3	103.7	108.3	98.3
NY	92.7	111.3	118.7	97.3
PT	98.0	108.3	112.3	96.7
SP	88.3	108.7	107.7	92.7
TS	94.0	97.0	98.3	92.7
MEAN	93.1	107.0	109.8	95.7
SE	1.6	2.3	2.5	1.7

Systolic Blood Pressure (mmHg)

Study 2

PL

Time (min)	-8	8	14	20
CL	116	208	186	131
JM	115	184	168	120
JW	122	175	173	144
LH	126	204	180	122
MD	124	205	194	134
NY	112	167	177	115
PT	119	162	158	119
SP	111	176	176	120
TS	121	165	160	126
MEAN	118.4	182.9	174.7	125.7
SE	1.8	6.1	3.9	3.1

NA

Time (min)	-8	8	14	20
CL	113	180	178	115
JM	116	179	173	129
JW	124	164	173	121
LH	129	176	166	141
MD	140	189	211	139
NY	112	168	172	122
PT	124	155	163	118
SP	111	172	177	116
TS	124	165	161	132
MEAN	121.4	172.0	174.9	125.9
SE	3.2	3.4	4.9	3.3

Diastolic Blood Pressure (mmHg)

Study 2

PL

Time (min)	-8	8	14	20
CL	88	92	88	79
JM	84	84	87	88
JW	67	67	67	80
LH	55	53	60	61
MD	77	76	75	83
NY	80	82	78	86
PT	84	82	82	81
SP	79	75	73	76
TS	80	73	57	83
MEAN	77.1	76.0	74.1	79.7
SE	3.4	3.7	3.7	2.6

NA

Time (min)	-8	8	14	20
CL	80	82	89	84
JM	85	86	83	94
JW	67	65	62	69
LH	73	69	85	75
MD	82	61	57	78
NY	83	83	92	85
PT	85	85	87	86
SP	77	77	73	81
TS	79	63	67	73
MEAN	79.0	74.6	77.2	80.6
SE	2.0	3.4	4.3	2.6

Total Peripheral Resistance (dyne/sec/cm5)

Study 2

PL

Time (min)	-8	8	14	20
CL	1098	790	724	1049
JM	580	515	493	737
JW	805	412	379	797
LH	977	393	327	651
MD	948	502	516	1027
NY	1166	455	451	781
PT	693	450	398	679
SP	535	422	543	518
TS	779	378	322	516
MEAN	842.2	479.6	461.5	750.6
SE	73.6	41.7	42.2	63.8

NA

Time (min)	-8	8	14	20
CL	1200	651	632	737
JM	831	457	408	667
JW	633	324	341	548
LH	985	375	313	528
MD	910	430	451	702
NY	906	460	520	718
PT	742	375	342	526
SP	603	424	398	597
TS	758	382	371	469
MEAN	840.9	430.8	419.6	610.2
SE	61.8	31.2	33.9	32.7

Core Temperature (°C)
Study 2 - Sweat
Analysis

PL

Time (min)	0	15	30
CL	38.1	38.3	38.6
JM	37.5	37.9	38.4
JW	38.0	38.3	38.7
LH	37.9	38.1	38.2
MD	37.7	37.8	38.0
NY	37.8	38.1	38.3
PT	38.0	38.3	38.5
SP	37.8	38.2	38.3
TS	38.0	38.3	38.5
MEAN	37.9	38.1	38.4
SE	0.1	0.1	0.1

NA

Time (min)	0	15	30
CL	37.7	38.0	38.2
JM	37.4	37.8	38.1
JW	37.7	37.8	38.1
LH	37.8	38.0	38.3
MD	37.7	37.9	38.0
NY	37.9	38.1	38.3
PT	37.9	38.3	38.5
SP	37.6	37.7	37.8
TS	38.3	38.5	38.8
MEAN	37.8	38.0	38.2
SE	0.1	0.1	0.1

Forearm Skin Temperature (°C)
Study 2 - Sweat Analysis
PL

Time (min)	0	15	30
CL	34.7	34.6	34.4
JM	34.7	33.5	34.0
JW	34.4	34.3	33.6
LH	35.5	34.0	33.1
MD	35.0	32.7	32.8
NY	35.0	34.3	33.9
PT	34.8	34.8	34.1
SP	34.9	34.8	34.7
TS	35.4	35.2	34.9
MEAN	34.9	34.2	33.9
SE	0.1	0.3	0.2

NA

Time (min)	0	15	30
CL	35.1	34.7	34.2
JM	35.3	34.7	34.6
JW	35.8	34.9	34.1
LH	35.2	33.9	34.7
MD	35.5	33.5	33.6
NY	35.1	34.3	34.8
PT	33.9	33.3	34.4
SP	34.0	34.0	34.5
TS	36.5	35.0	35.8
MEAN	35.2	34.3	34.5
SE	0.3	0.2	0.2

Upperarm Skin Temperature (°C)

Study 2 - Sweat Analysis

PL

Time (min)	0	15	30
CL	33.7	34.1	33.5
JM	34.9	32.9	33.4
JW	34.7	34.4	34.9
LH	35.9	33.7	33.3
MD	34.3	34.4	33.9
NY	32.6	34.4	33.3
PT	35.0	34.8	33.6
SP	34.5	34.2	34.0
TS	34.9	35.4	34.9
MEAN	34.5	34.3	33.9
SE	0.3	0.2	0.2

NA

Time (min)	0	15	30
CL	34.6	33.5	33.7
JM	34.4	34.1	32.8
JW	37.7	34.4	34.5
LH	35.3	34.1	34.8
MD	35.4	34.3	34.5
NY	33.5	32.9	32.8
PT	35.4	33.7	34.5
SP	34.7	34.4	34.6
TS	35.9	35.4	35.1
MEAN	35.2	34.1	34.1
SE	0.4	0.2	0.3

Chest Skin Temperature (°C)

Study 2 - Sweat Analysis

PL

Time (min)	0	15	30
CL	32.0	31.8	32.3
JM	34.4	33.2	33.3
JW	35.7	35.0	35.5
LH	35.1	32.6	33.6
MD	35.1	33.0	33.3
NY	33.3	33.7	31.6
PT	33.7	34.4	32.9
SP	32.2	32.9	32.2
TS	35.7	34.6	34.1
MEAN	34.1	33.5	33.2
SE	0.5	0.3	0.4

NA

Time (min)	0	15	30
CL	32.4	29.5	31.2
JM	34.7	34.9	33.8
JW	35.6	35.5	35.3
LH	34.2	32.7	32.8
MD	34.1	33.8	33.8
NY	34.3	33.0	33.5
PT	34.6	32.2	32.2
SP	32.2	33.6	33.6
TS	35.8	34.0	34.3
MEAN	34.2	33.2	33.4
SE	0.4	0.6	0.4

Back Skin Temperature (°C)

Study 2 - Sweat Analysis

PL

Time (min)	0	15	30
CL	33.0	31.4	31.6
JM	32.7	30.9	31.3
JW	35.0	35.2	35.4
LH	34.3	31.5	31.6
MD	34.2	33.2	34.0
NY	33.7	34.1	33.6
PT	33.7	33.7	32.1
SP	33.9	33.6	32.8
TS	35.0	34.7	34.3
MEAN	33.9	33.1	33.0
SE	0.3	0.5	0.5

NA

Time (min)	0	15	30
CL	33.0	31.3	32.2
JM	33.5	32.2	32.2
JW	35.5	34.7	34.8
LH	35.4	32.6	33.3
MD	35.3	33.4	33.4
NY	35.7	34.4	34.6
PT	34.1	31.6	32.3
SP	33.2	32.5	32.5
TS	35.6	34.7	35.0
MEAN	34.6	33.0	33.4
SE	0.4	0.4	0.4

Thigh Skin Temperature (°C)
Study 2 - Sweat Analysis

PL

Time (min)	0	15	30
CL	35.1	34.6	34.8
JM	33.7	34.9	34.9
JW	35.7	35.4	34.1
LH	35.3	35.1	35.4
MD	34.2	32.9	32.8
NY	34.7	35.2	35.5
PT	34.6	34.6	34.3
SP	34.3	35.2	35.0
TS	35.4	36.0	35.7
MEAN	34.8	34.9	34.7
SE	0.2	0.3	0.3

NA

Time (min)	0	15	30
CL	35.3	35.3	34.6
JM	35.4	35.2	35.3
JW	35.4	35.2	34.6
LH	35.5	34.7	34.4
MD	35.0	34.5	35.0
NY	35.2	35.5	35.4
PT	34.4	34.6	34.9
SP	33.9	34.3	34.3
TS	36.2	35.4	36.1
MEAN	35.1	35.0	35.0
SE	0.2	0.1	0.2

Calf Skin Temperature (°C)

Study 2 - Sweat Analysis

PL

Time (min)	0	15	30
CL	34.0	34.9	35.1
JM	33.7	34.4	34.9
JW	34.5	34.0	33.7
LH	35.2	35.4	35.6
MD	34.3	33.6	33.6
NY	34.7	34.6	34.3
PT	34.0	34.9	33.6
SP	34.8	34.3	34.7
TS	35.6	35.7	35.0
MEAN	34.5	34.6	34.5
SE	0.2	0.2	0.2

NA

Time (min)	0	15	30
CL	33.9	34.2	34.6
JM	34.0	34.1	34.2
JW	35.6	35.0	34.7
LH	35.8	35.0	35.3
MD	34.5	34.3	34.8
NY	35.1	34.7	35.1
PT	34.9	33.8	34.6
SP	34.1	34.0	34.6
TS	36.0	36.1	36.4
MEAN	34.9	34.6	34.9
SE	0.3	0.2	0.2

Mean Weighted Skin Temperature (°C)

Study 2 - Sweat Analysis

PL

Time (min)	0	15	30
CL	33.6	33.3	33.5
JM	33.8	33.3	33.6
JW	35.2	34.9	34.6
LH	35.1	33.6	33.8
MD	34.5	33.2	33.4
NY	34.0	34.4	33.7
PT	34.2	34.4	33.3
SP	33.9	34.1	33.8
TS	35.4	35.2	34.8
MEAN	34.4	34.1	33.8
SE	0.2	0.2	0.2

NA

Time (min)	0	15	30
CL	33.9	32.8	33.2
JM	34.5	34.2	33.9
JW	35.7	35.0	34.8
LH	35.2	33.7	34.0
MD	34.9	34.0	34.2
NY	34.9	34.3	34.5
PT	34.5	33.1	33.6
SP	33.5	33.7	33.9
TS	36.0	35.0	35.4
MEAN	34.8	34.0	34.2
SE	0.3	0.3	0.2

Sweating Rate (L/h)
Study 2 - Sweat Analysis

PL

Time (min)	0-30
CL	1.10
JM	1.50
JW	0.85
LH	1.30
MD	0.80
NY	1.10
PT	1.50
SP	1.10
TS	1.10
MEAN	1.15
SE	0.1

NA

Time (min)	0-30
CL	1.10
JM	1.50
JW	1.40
LH	1.40
MD	1.20
NY	1.00
PT	1.60
SP	1.20
TS	1.20
MEAN	1.29
SE	0.1

Sweat Sodium Concentration (mEq/L)

Study 2 - Sweat Analysis

PL

	Arm	Back	Thigh	Calf	Weighted Mean
CL	44.0	89.0	37.0	35.5	51.6
JM	97.0	100.0	78.0	74.0	84.9
JW	77.5	98.5	68.5	84.5	82.8
LH	63.5	89.0	52.5	46.5	61.9
MD	51.5	69.0	24.0	27.5	40.5
NY	42.5	63.5	28.0	30.0	40.0
PT	77.5	78.5	57.0	62.5	66.9
SP	48.5	88.0	33.5	31.5	49.6
TS	108.5	120.0	93.5	93.5	102.5
MEAN	67.8	88.4	52.4	53.9	64.5
SE	8.0	5.7	8.0	8.5	7.2

NA

	Arm	Back	Thigh	Calf	Weighted Mean
CL	46.0	79.5	42.5	39.0	52.0
JM	95.0	108.0	83.0	81.0	90.6
JW	83.0	105.0	75.0	70.0	82.6
LH	69.5	100.5	63.5	57.5	72.5
MD	35.5	82.5	40.0	56.5	56.4
NY	46.5	66.0	35.0	34.5	44.7
PT	76.0	108.0	63.0	85.5	83.9
SP	53.5	82.0	39.0	39.0	52.5
TS	100.5	122.0	82.0	91.5	98.1
MEAN	67.3	94.8	58.1	61.6	70.4
SE	7.7	6.0	6.5	7.2	6.5

Sweat Chloride Concentration (mEq/L)
Study 2 - Sweat
Analysis

PL

	Arm	Back	Thigh	Calf
CL	33.0	75.0	27.0	24.5
JM	80.5	85.0	62.5	57.0
JW	62.0	84.5	55.0	69.0
LH	48.0	71.5	37.0	30.0
MD	45.0	62.0	21.5	24.0
NY	36.5	52.5	23.0	25.0
PT	57.0	58.0	40.0	42.0
SP	35.5	71.5	22.0	19.0
TS	98.0	110.0	81.5	81.0
MEAN	55.1	74.4	41.1	41.3
SE	7.4	5.8	7.0	7.5

NA

	Arm	Back	Thigh	Calf
CL	34.0	66.0	31.5	27.0
JM	79.0	93.5	68.0	64.5
JW	78.0	98.0	66.0	60.5
LH	54.0	86.0	49.0	42.0
MD	29.0	71.0	31.5	45.0
NY	39.0	56.5	28.0	27.0
PT	65.0	98.5	55.0	74.5
SP	42.0	68.5	28.5	27.0
TS	90.5	114.0	72.0	79.0
MEAN	56.7	83.6	47.7	49.6
SE	7.4	6.3	6.1	6.9

Sweat Sodium Loss (mEq/h)

Study 2 - Sweat Analysis

PL

Time (min)	0-30
CL	57
JM	127
JW	70
LH	80
MD	32
NY	44
PT	100
SP	55
TS	113
MEAN	75.5
SE	10.8

NA

Time (min)	0-30
CL	57
JM	136
JW	116
LH	101
MD	68
NY	45
PT	134
SP	63
TS	118
MEAN	93.1
SE	11.7

Blood Pressure (mmHg)
Study 2 - Sweat
Analysis

PL

	SBP	DBP	MAP
CL	154	74	101
JM	137	57	84
JW	161	60	94
LH	192	55	101
MD	172	75	107
NY	174	80	111
PT	148	58	85
SP	138	70	93
TS	154	78	103
MEAN	158.8	67.4	97.6
SE	6.0	3.3	3.2

NA

	SBP	DBP	MAP
CL	161	58	92
JM	136	54	81
JW	150	60	90
LH	188	52	97
MD	173	77	109
NY	166	78	107
PT	165	65	98
SP	144	70	95
TS	156	65	95
MEAN	159.9	64.3	96.2
SE	5.2	3.1	2.8

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