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FLUID AND SODIUM BALANCE DURING EXERCISE

IN SPECIAL POPULATIONS

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ABSTRACT

Fluid and sodium imbalances are implicated in the impairment of both physiological function and exercise/sport performance. Older adults are at an increased risk for fluid and sodium imbalances due to an impaired thirst sensitivity and renal water- and sodium-conserving ability. Overdrinking and hyponatremia (low blood sodium concentration) tend to occur most commonly in endurance athletes, especially small non-elite female athletes. Basketball players can lose large volumes of sweat due to the high-intensity and prolonged nature of the game. The unique characteristics of these populations of athletes render them susceptible to the negative consequences of fluid and sodium imbalances during exercise. Therefore, the purpose of this series of studies was to 1) elucidate the role of fluid intake behavior, changes in body mass, sodium consumption, and sweat sodium loss on fluid and sodium balance in younger and older adult men and women during prolonged exercise in a warm environment and 2) determine the effects of dehydration on performance during prolonged running and a simulated basketball game.

The purpose of the first study was to compare the voluntary fluid intake behavior of older men and women (54-70 yr) when provided cold, palatable beverages and ample opportunity to drink between repeated bouts of exercise in the heat. Thirteen men and 14 women performed 4 bouts of 15 min cycling at 65% VO_{2peak} followed by 15 min rest at 30°C and 50% rh. In separate trials, subjects drank either a carbohydrate-electrolyte solution (CES) or water *ad libitum* during the rest periods and were unaware that their fluid intake was being measured. Fluid intake behavior was repeatable (intraclass correlation coefficient = 0.75) and subjects drank enough of either beverage to match sweating rates and maintain their body mass (BM). Fluid intake per kg BM was greater with CES (18.7±2.2 vs. 15.1±2.1 mL/kg; P< 0.05) and plasma volume (PV) was better maintained during the CES trials (-1.3±1.1 vs. -4.2±1.1% during the second half of the session). Women drank significantly more water than the men on a per kg basis (17.2±2.9 vs. 12.8±1.7 mL/kg BM) and one woman (BM = 45.7 kg) became hyponatremic ($S_{[Na+]}$ = 126 mmol/L) with symptoms during the water trial. In conclusion, older adults drink enough to maintain fluid balance when palatable fluid is

readily available; however, CES promotes greater voluntary fluid intake and restores PV losses faster than water. In addition, older women drink more water than men during interval exercise in the heat which may put smaller women at an increased risk for developing hyponatremia.

In the second study we compared the measured serum $[Na^+]$ $(S_{[Na+]})$ with that predicted by the Nguyen-Kurtz equation by manipulating ingested beverage $[Na^+]$ and changes in body mass (ΔBM) during prolonged running in a warm environment. Endurance-trained athletes (4 men, 4 women; 22-36 yr) ran for 2 h, followed by a run to exhaustion, and 1 h of recovery. During exercise and recovery, subjects drank a 6% carbohydrate solution without Na^+ (Na^+0), 6% carbohydrate solution with 18 mmol/L Na^+ (Na^+18), or 6% carbohydrate solution with 30 mmol/L Na^+ (Na^+30) to maintain BM, increase BM by 2%, or decrease BM by 2% or 4% in 12 separate trials. Net fluid, Na^+, and K^+ balance were measured to calculate the Nguyen-Kurtz predicted $S_{[Na+]}$ for each trial. The predicted and measured $S_{[Na+]}$ were not significantly different during the 0%, -2%, and -4% ΔBM trials (-0.2 ± 0.2 mmol/L), but were significantly different during the +2% ΔBM trials (-2.6 ± 0.5 mmol/L). Na^+ consumption attenuated the decline in $S_{[Na+]}$ (-2.0 ± 0.5, -0.9 ± 0.5, -0.5 ± 0.5 mmol/L from pre- to post-experiment of the 0% ΔBM trials for Na^+30, Na^+18, and Na^+0, respectively) but the differences among beverages were not statistically significant. Beverage $[Na^+]$ did not affect performance; however, time to exhaustion was significantly shorter during the -4% (8 ± 3 min) and -2% (14 ± 3 min) vs. 0% (22 ± 5 min) and +2% (26 ± 6 min) ΔBM trials. In conclusion, when athletes maintain or lose BM, changes in $S_{[Na+]}$ can be accurately predicted by changes in the mass balance of fluid, Na^+, and K^+ during prolonged running in the heat.

The third study tested the hypothesis that the ΔBM accurately reflects the change in total body water (ΔTBW, deuterium oxide (D_2O) dilution technique) after prolonged exercise. Endurance-trained runners (4 men, 4 women; 22-36 yr; 66 ± 10 kg) completed 2 h of interval running (70% VO_{2max}) in the heat (30ºC) and then sat for a 1 h recovery period to allow fluid compartments to stabilize. During exercise and recovery, subjects drank fluid or no fluid to maintain their BM, increase BM by 2%, or decrease BM by 2% or 4% in separate trials. Three hours before running, subjects ingested 30 g of D_2O. D_2O concentration was measured in pre- and post-experiment blood and urine samples using a

Fourier Transform Infrared Spectrometer. When calculating pre- and post-experiment TBW, corrections were made for D_2O lost in urine, sweat, breath vapor, and nonaqueous hydrogen exchange. The average difference between ΔBM and ΔTBW was 0.09 ± 1.09 kg (paired t-test, $P = 0.58$). The slope and intercept of the relation between ΔBM and ΔTBW were not significantly different from 1 and 0, respectively. The intraclass correlation coefficient between ΔBM and ΔTBW was 0.76, which is indicative of excellent reliability between methods. Measuring pre- to post-exercise ΔBM is an accurate and reliable method to assess the ΔTBW. Athletes can use pre- to post-exercise BM change to obtain a reasonable estimate of their sweat loss and their hydration status.

The purpose of the fourth study was to determine the effect of 1, 2, 3, and 4% dehydration (DEH) vs. euhydration (EUH) on basketball performance in adult male players. Seventeen 17-28 yr old male basketball players completed 3 h of interval treadmill walking (40°C and 20% rh) with or without fluid replacement. Subjects completed six trials in random order: 1) EUH with a CES, 2) EUH control (flavored water with 0% carbohydrate and 18 mmol sodium), 3) 1% DEH, 4) 2% DEH, 5) 3% DEH, and 6) 4% DEH. After a 70-min recovery period, subjects performed a sequence of continuous basketball drills designed to simulate a fast-paced game. Measures of overall skill performance during the 80-min game included: 1) total time to complete basketball-specific movement drills (sprinting, defensive slides, sprinting-defensive slides combination, and repetitive jumping drills) and 2) total number of shots (foul line and baseline jump shots, layups, 3-point, 15-foot, free throws) made per game. Performance during all timed and shooting drills declined progressively as % DEH increased. Total time to complete basketball-specific movement drills was slower (1%: $+7 \pm 6$; 2%: $+20 \pm 5$ ($P<0.05$); 3%: $+26 \pm 7$ ($P<0.005$); 4%: $+57 \pm 9$ ($P<0.0001$) sec) and fewer shots were made during DEH vs. EUH control (1%: -5 ± 1; 2%: -6 ± 2 ($P<0.05$); 3%: -8 ± 2 ($P<0.005$); 4%: -10 ± 1 ($P<0.0001$) shots made). There were no significant differences in performance between CES and EUH control. In conclusion, basketball players experienced a progressive deterioration in performance as DEH progressed from 1 to 4%. The threshold, or % DEH at which the performance decrement reached statistical significance, was 2% for combined timed and shooting drills.

The fifth study tested the hypothesis that DEH impairs attentional vigilance in male basketball players. The Test of Variables of Attention (TOVA; Universal Attention Disorders™) was administered to 11 male basketball players (17-28 yr) at baseline (Test 1), after walking (50% VO_{2max}) in the heat (40°C and 20% rh) (Test 2), and then after a simulated basketball game (Test 3). Tests 2 and 3 were performed while subjects were either DEH (1-4%) or EUH. The TOVA consisted of target-infrequent and target-frequent conditions, simulating static and dynamic (such as a basketball game) environments, respectively. TOVA measures included errors of omission (OE) and commission (CE), response time (RT), and sensitivity. During the target-infrequent half of Test 3, EUH resulted in significantly better sensitivity (+0.4 ± 1.2 vs. -0.9 ± 1.3), faster RT (-8 ± 20 vs. +16 ± 28), and fewer OE (-0.4± 0.7 vs. +1.3 ± 2.4) compared to DEH. During the target-frequent half, EUH resulted in significantly fewer OE (-4 ± 15 vs. +5 ± 7) and CE (-1.9 ± 3.2 vs. 0.6 ± 1.4) in Test 2 and greater sensitivity (+0.7 ± 2.6 vs. -0.7 ± 1.1) and faster RT (-21 ± 28 vs. +5 ± 31) than DEH in Test 3. In conclusion, vigilance-related attention of male basketball players was impaired by DEH, especially during the target-frequent condition of the TOVA. These results suggest that fluid replacement is essential to prevent the decline in vigilance that occurs with DEH in highly dynamic environments. Therefore, basketball players should be advised to maintain EUH for optimal concentration and attentional skills during competition.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Analysis of Variance	ANOVA
Antidiuretic Hormone	ADH
Blood pressure	BP
Body core temperature	T_c
Body mass	BM
Carbohydrate electrolyte solution	CES
Change in the mass balance of sodium and potassium	ΔE
Coefficient of variation	CV
Dehydration	DEH
Deuterium oxide	D_2O
Esophageal temperature	T_{es}
Euhydration	EUH
Exercise-associated hyponatremia	EAH
Fourier Transform Infrared Spectroscopy	FTIR
Heart rate	HR
Hematocrit	Hct
Hemoglobin	Hb
Maximal oxygen uptake	VO_{2max}
Mean arterial pressure	MAP
Plasma volume	PV
Rating of perceived exertion	RPE
Rectal temperature	T_{re}
Relative humidity	rh
Serum glucose concentration	$S_{[gluc]}$
Serum osmolality	S_{osm} or S_{osmol}
Serum protein concentration	$S_{[prot]}$
Serum sodium concentration	$S_{[Na+]}$
Skin temperature	T_{sk}

Sweat loss	SL
Sweat potassium concentration	$Sw_{[K+]}$
Sweat sodium concentration	$Sw_{[Na+]}$
Sweat volume	Sw_{vol}
Test of Variables of Attention	TOVA
Total body water	TBW
Urine color	U_{col}
Urine osmolality	U_{osm} or U_{osmol}
Urine specific gravity	U_{sg}
Urine volume	U_{vol}

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Chapters 3, 6, and 7 are individual articles that have been previously published, and copyright permission has been obtained for inclusion of these three manuscripts in this dissertation. These articles (L.B. Baker, T.A. Munce, and W.L. Kenney, Sex differences in voluntary fluid intake by older adults during exercise, *Med. Sci. Sports Exerc.* 37: 789-796, 2005; L.B. Baker, K.A. Dougherty, M. Chow, W.L. Kenney, Progressive dehydration causes a progressive decline in basketball skill performance, *Med. Sci. Sports Exerc.* 39: 1114-1123, 2007; and L.B. Baker, D.E. Conroy, W.L. Kenney, Dehydration impairs vigilance-related attention in male basketball players, *Med. Sci. Sports Exerc.* 39: 976-983, 2007) have been used with permission from Lippincott Williams & Wilkins.

Chapter 1

INTRODUCTION

Background and Significance

Water is the largest component of the human body and accounts for approximately 45-70% of total body mass (BM) in the average adult. Total body water (TBW) can be divided into two components – the intracellular water (ICW) and the extracellular water (ECW). The ICW accounts for approximately 55% of TBW, while the ECW accounts for the remaining 45%. The ECW can be further divided into the extravascular and intravascular fluid spaces (Edelman and Leibman, 1959). Water in the vascular space (i.e., plasma) plays a central role in the body's cardiovascular and thermoregulatory capacity during exercise. Sodium (Na^+) and its associated anions comprise the most osmotically active components of the plasma. Consequently, Na^+ balance plays a key role in governing the size of the ECW compartment and water movement between the ICW and ECW (Mack and Nadel, 1996). During intense and/or prolonged exercise, body water and electrolytes are lost as a consequence of thermoregulatory sweating. When there is a mismatch between fluid/Na^+ intake and fluid/Na^+ loss this imbalance can have implications for both health and performance.

Throughout this dissertation, the term "euhydration" or "EUH" refers to maintenance of "normal" baseline body water content, while the terms "hypohydration" and "hyperhydration" refer to body water deficits and excesses beyond EUH, respectively. The term "dehydration" or "DEH" is defined as the dynamic loss of body water or the transition from EUH to hypohydration. For simplicity, the more common term DEH will be used to describe both the process of body water loss and hypohydration in this dissertation, unless stated otherwise. Additionally, it will be assumed that an acute BM loss is a reflection of a body water deficit. For example, a 2% BM deficit is defined as 2% DEH. This topic is a matter of debate and will be discussed in more detail later. However, for simplicity, the term DEH will be used to denote a BM and body water deficit throughout this dissertation, unless stated otherwise.

Thirst and Voluntary Fluid Intake

Sweating rates can vary from less than 0.5 to over 2.5 L (17 to over 85 oz) per h depending upon exercise intensity, environmental conditions, amount and type of clothing or equipment, acclimation state, fitness level, and hydration status (Rehrer and Burke, 1996; Sawka, 1992). Because of this considerable variability, it can be difficult to closely match the volume of fluid intake to the volume of sweat output. Moreover, thirst does not provide a good index of body water status because it typically lags behind physiological indicators of DEH. For example, the threshold for the release of antidiuretic hormone (ADH) and renal water conserving mechanisms occurs at lower osmolalities than does thirst. The osmotic threshold for ADH secretion is approximately 286 mOsm/kg whereas the average threshold for thirst is 295 mOsm/kg (Robertson, 1984; Vokes and Robertson, 1987). Therefore, a*d libitum* drinking during and/or after exercise-heat stress usually results in incomplete fluid replacement, a concept known as "voluntary dehydration" (Greenleaf and Sargent, 1965; Rothstein et al., 1947). In fact, it is common for individuals to voluntarily replace only about 50% of their sweat losses and incur $\geq$ 2% DEH during a given bout of exercise in a hot environment (Binkley et al., 2002; Convertino et al., 1996; Passe et al., 2007; Sawka, 1992).

Older Adults and Fluid/Sodium Balance

Older adults (> 60 yr) are particularly susceptible to "voluntary dehydration" because they exhibit decreased thirst sensation and reduced fluid intake in response to hypovolemia compared with young adults (Stachenfeld et al., 1997). Further, older adults are at an increased risk for fluid and electrolyte imbalances due to an impaired renal water- and Na^+-conserving ability (Mack et al., 1994). Fluid replacement studies in young adults have shown that carbohydrate-electrolyte solutions (CES) are more effective than plain water in stimulating voluntary fluid intake, restoring plasma volume, and maintaining fluid/electrolyte balance during exercise-heat stress (Carter and Gisolfi, 1989; Costill and Sparks, 1973; Murray, 1998; Nose et al., 1988; Sawka et al., 2007). However, the efficacy of CES as a fluid replacement beverage for older active men and women during or after exercise-heat stress has not been previously studied. Further, little

data exist on the actual sex-related differences in *ad libitum* fluid intake behavior of older adults during exercise-heat stress.

Exercise-Associated Hyponatremia

Exercise-associated hyponatremia (EAH) is defined as a serum Na^+ concentration ($S_{[Na+]}$) less than 135 mmol/L (Montain et al., 2001). EAH is a rare but potentially life-threatening condition. The incidence of symptomatic EAH in endurance events, such as marathons, ultramarathons, and triathlons, has been reported to range from 0 to 4% of all participants (Almond et al., 2005; Hew, 2005; Hew et al., 2003; Hsieh et al., 2002; Noakes et al., 1990; Noakes et al., 2005; Speedy et al., 1999). The reduction in solute concentration in serum promotes movement of water from the serum and into cells, which can cause swelling in the brain and/or congestion in the lungs (Montain et al., 2001). Symptoms of mild to moderate EAH may include headache, nausea, dizziness, and muscle weakness, while severe EAH (typically $S_{[Na+]} < 125$ mmol/L) is characterized by pulmonary edema, cardiorespiratory arrest, cerebral edema, seizures, and/or coma (Backer et al., 1999, Montain et al., 2001).

Contributing factors to the etiology of EAH include 1) overdrinking of hypotonic fluids at rates that exceed sweating and 2) excessive loss of Na^+ in sweat (Montain et al., 2001; Noakes, 1985; Sawka, 2007). Investigators have used a prediction equation developed by Kurtz and Nguyen (2003) to calculate post-exercise $S_{[Na+]}$ based on changes in the mass balance of water, Na^+, and K^+ (Montain et al., 2006; Weschler, 2005). However, its predictions have not been directly compared to empirical data of post-exercise $S_{[Na+]}$ in athletes with known pre-exercise $S_{[Na+]}$, BM, sweating rates, sweat $[Na^+]$ and $[K^+]$, fluid intake, and Na^+ and K^+ intake in a controlled laboratory setting. Further, EAH tends to occur more commonly in women than men (Almond et al., 2005; Backer et al., 2005; Eichner, 2002). Thus, it would be important to compare measured $S_{[Na+]}$ with that predicted by the Nguyen-Kurtz equation in both male and female endurance-trained athletes after prolonged running to assess the equation's accuracy.

Another matter of uncertainty concerning fluid/Na^+ balance in endurance athletes is the relation between the ΔBM and the actual ΔTBW, i.e., hydration status, during

prolonged exercise. The debate centers on whether endogenous water production (metabolic water production via cellular metabolism and the liberation of water as glycogen is utilized) plays a significant role in the overall ΔTBW during prolonged exercise (Cheuvront et al., 2007; Sawka et al., 2007). Some investigators suggest that a 70-kg athlete can lose ≥ 2.2 kg (≥ 3% of BM) during endurance events without experiencing a net loss in TBW. Further, they propose that endogenous water production contributes significantly to the dilution of $S_{[Na+]}$ and therefore the etiology of EAH in endurance athletes (Hew, 2005; Noakes et al., 2005). The mass of endogenous water gain and non-sweat sources of BM loss (oxidation of glycogen and fatty acids) and their contribution to overall fluid balance during endurance exercise has been calculated previously (Rogers et al., 1997). However, the relation between the ΔBM and the ΔTBW in male and female endurance-trained athletes after prolonged running has not been directly measured.

Dehydration and Endurance Performance

The prevailing view is that DEH negatively affects cardiovascular and thermoregulatory function, the combined effect of which is increased perceived effort and impaired performance during activity which is greatly dependent upon these two systems, i.e., prolonged aerobic exercise (Sawka and Noakes, 2007). However, Noakes challenges this view, suggesting that drinking according to thirst, a practice which is likely to result in ≥ 2% DEH (Binkley et al., 2002; Convertino et al., 1996; Sawka, 1992) will optimize performance (Sawka and Noakes, 2007). Thus, it would be important to test the effects of ≥ 2% DEH compared with EUH on prolonged running performance in endurance-trained male and female athletes.

Dehydration and Basketball Skill Performance

The game of basketball is characterized by intermittent bouts of high-intensity activity repeated over a prolonged period of time and requires the execution of complex sport-specific skills. Further, success in skill sports such as basketball requires optimal

concentration and attentional skills. DEH has been implicated in impaired performance of soccer skills (McGregor et al, 1999) and various aspects of cognitive function, such as arithmetic ability, short-term memory, visuomotor tracking, response time, and coordination (Cian et al., 2001; Cian et al., 2000; Gopinathan et al., 1988; Sharma et al., 1986). However, the impact of DEH on basketball skill performance and measures of attention in adult players has not been investigated. Further, few investigators have tested progressive levels of DEH in a dose-response manner to determine whether a critical level of water deficit exists at which performance is impaired compared to that of EUH.

Summary

The five separate studies that comprise this dissertation were conducted to 1) elucidate the role of fluid intake behavior, ΔBM, sodium consumption, and sweat sodium loss on fluid and Na^+ balance in young adult and older men and women during prolonged exercise in a warm environment (Studies 1, 2, and 3) and 2) to determine the effects of 2 and 4% DEH on prolonged running performance in endurance-trained male and female athletes (Study 2) and progressive 1 to 4% DEH on basketball skill performance and measures of attention in male players (Studies 4 and 5).

Specific Aims and Hypotheses

Specific Aim 1: The purpose of the first study, "Sex differences in voluntary fluid intake by older adults during exercise", was: (1) to compare the voluntary fluid intake behavior of older active men and women when provided ample opportunity to drink during rest periods of a moderate-intensity interval cycling protocol in a hot environment and (2) to compare the efficacy of a CES and water in maintaining fluid and Na^+ balance.

    Hypothesis 1a: Older adults will not drink enough to maintain fluid balance.

    Hypothesis 1b: CES will promote greater voluntary fluid intake than water and consumption of CES will maintain plasma volume and $S_{[Na+]}$ better than water.

Specific Aim 2: The purpose of the second study, "Quantitative analysis of serum sodium concentration after prolonged running in the heat", was 1) to compare measured $S_{[Na^+]}$ with that predicted by the Nguyen-Kurtz equation in male and female endurance-trained athletes after prolonged running in a hot environment, 2) to measure the effects of beverage $[Na^+]$ (0, 18, and 30 mmol/L) and pre-to post-exercise ΔBM (+2%, 0%, -2%, and -4% ΔBM) on measured $S_{[Na^+]}$, and 3) to measure the effects of a BM deficit (-2% and -4% BM) vs. fluid consumption to match sweating rate (0% ΔBM) on prolonged running performance in the heat.

> Hypothesis 2a: The Nguyen-Kurtz equation would accurately predict the measured post-exercise $S_{[Na^+]}$.
>
> Hypothesis 2b: Increased beverage $[Na^+]$ in a CES would attenuate the decline in $S_{[Na^+]}$ compared with a beverage with no Na^+.
>
> Hypothesis 2c: An increase in BM as a result of overdrinking relative to sweat losses would be associated with a decrease in $S_{[Na^+]}$.
>
> Hypothesis 2d: A decrease in BM via underdrinking relative to sweat losses would impair endurance performance compared with maintenance of pre-exercise BM.

Specific Aim 3: The purpose of the third study, "Change in body mass accurately and reliably predicts change in body water", was to determine the relation between the ΔBM and the ΔTBW in male and female endurance-trained athletes after prolonged running.

> Hypothesis 3: The ΔBM would accurately and reliably predict the ΔTBW.

Specific Aim 4: The purpose of the fourth study, "Progressive dehydration causes a progressive decline in basketball skill performance", was (1) to determine the effect of 1 to 4% DEH vs. EUH on performance of basketball-specific shooting and movement drills during a simulated game in highly skilled 17-to 28-yr-old male players and (2) to determine whether addition of carbohydrate enhances basketball performance over EUH with a carbohydrate-free solution.

> Hypothesis 4a: One to 4% DEH will progressively impair basketball performance compared with EUH.

Hypothesis 4b: A CES will improve basketball performance measures compared with water.

Specific Aim 5: The purpose of the fifth study, "Dehydration impairs vigilance-related attention in male basketball players, was (1) to determine the effect of 1 to 4% DEH vs. EUH on attentional vigilance in 17-to 28-yr-old male basketball players at the end of a simulated game and (2) to compare the effects of a CES vs. a carbohydrate-free placebo on attentional performance.

Hypothesis 5a: DEH would result in slower response times and increased omission errors compared with EUH.

Hypothesis 5b: A CES will improve attentional performance measures compared with water.

Chapter 2

REVIEW OF THE LITERATURE

Aging and Hydration

A body water deficit elicits reflex adjustments, including thirst-induced drinking and renal water and Na^+ reabsorption, to restore fluid homeostasis. Older individuals (> 60 yr) have been characterized as being more susceptible to dehydration (DEH) than younger adults due to a deficient thirst response and an impaired ability of their kidneys to conserve water and Na^+ (Miescher and Fortney, 1989; Phillips et al., 1984). The attenuated renal function in older individuals has been attributed to the progressive decline in the number of functioning nephrons with age (Rowe et al., 1976). Additional factors, such as impaired release of, or renal responsiveness to, plasma antidiuretic hormone (ADH) and reduced renin-angiotensin-aldosterone system activity may also contribute to the age-related impairment of Na^+ and water conservation (Rowe et al., 1976; Weidmann et al., 1975).

DEH induced by sweat loss results in both a decrease in the extracellular compartment size and an increase in plasma osmolality (Adolph, 1947; Kozlowski and Saltin, 1964). An increase in plasma osmolality initiates fluid movement from the cellular compartment into the plasma to maintain osmotic balance. This results in cellular DEH, i.e., cell shrinkage and hypertonicity. Increases in cellular tonicity (cellular DEH) are sensed by osmoreceptors in the central nervous system, while decreases in extracellular fluid volume (extracellular DEH) are sensed by the cardiopulmonary (low pressure) baroreceptors. Physiological thirst is stimulated independently by cellular and extracellular DEH and it has been suggested that older individuals demonstrate reduced thirst relative to young adults in response to both stimuli (Phillips et al., 1991; Phillips et al., 1993). However, more recent work by Stachenfeld et al. (1996 and 1997), suggests that the blunted thirst response with aging is due to an attenuated low pressure baroreceptor sensitivity, while osmoreceptor sensitivity is intact. In 1996, Stachenfeld infused older and younger adults with hypertonic saline to induce hyperosmotic hypervolemia and found that both groups voluntarily drank sufficient water

during a 180 min recovery period to restore preinfusion plasma osmolality. In Stachenfeld et al.'s study (1997), older and younger adults dehydrated by overnight water restriction and subsequent exercise-heat stress. Subjects then recovered with or without head-out water immersion (HOI). By forcing blood volume centrally HOI minimizes hypovolemia. In the younger adults, HOI caused an immediate fall in thirst and voluntary fluid intake. However, a similar central blood volume expansion had no effect on thirst in the older adults.

Inadequate fluid intake following dehydration has implications for chronic adaptation to exercise-heat stress. Increased fluid intake during a heat acclimation regimen is critical for plasma volume expansion. Takamata et al. (1999) and Zappe et al. (1996) found that inadequate fluid intake in older men during recovery from 4-6 days of exercise-heat stress contributed to an inability to expand plasma volume. Meanwhile, the younger men replaced significantly more of their fluid losses 2 h after exercise (80% vs. 34%; Takamata et al., 1999) and increased their 24-h fluid intake to a greater extent (45 mL/kg BM/day vs. 32 mL/kg BM/day; Zappe et al., 1996) than the older men. Subsequently, the younger men experienced a ~5-10% increase in plasma volume following repeated exercise-heat exposure, whereas the older men did not (Takamata et al., 1999; Zappe et al., 1996).

Role of Sodium Balance in Hydration

The loss of water due to thermoregulatory sweating is accompanied by a concomitant loss of electrolytes, primarily Na^+. The average $[Na^+]$ of sweat is ~50 mmol/L (Costill, 1977). Highly fit, heat-acclimated athletes may exhibit a sweat $[Na^+]$ of ≤ 20 mmol/L. At the other extreme are some athletes who excrete sweat with a $[Na^+] \geq$ 80 mmol/L (Casa et al., 2005; Maughan, 2001; Stofan et al., 2005). Even those athletes with low or average sweat $[Na^+]$, can accrue a substantial Na^+ deficit by virtue of large sweat losses due to high sweating rates (≥ 2 L/h) or extended periods of strenuous exercise (two-a-day practices or ultraendurance events).

Na^+ balance is important for muscle and nerve function, but is also involved in the regulation of body water content and the distribution of water among the intracellular and

extracellular fluid compartments. Therefore, replacement of sweat Na^+ losses via Na^+ ingestion plays several important roles in the maintenance of fluid balance during and after exercise. For instance, Na^+ helps maintain the osmotic drive to drink, often resulting in greater voluntary fluid consumption (Nose et al. 1988; Rivera-Brown et al., 1999; Wilk and Bar-Or, 1996). Further, Na^+ ingestion provides an osmotic impetus for renal water reabsorption and fluid retention in the vascular space. Thus, the inclusion of Na^+ in a fluid replacement beverage helps reduce urine production (Nose et al., 1988; Vrijens & Rehrer, 1999) and restore plasma volume (Carter and Gisolfi, 1989; Costill and Sparks, 1973; Shirreffs et al., 2007), stimulating more rapid and complete rehydration vs. a Na^+-free beverage during recovery from exercise-induced DEH (Maughan & Shirreffs, 1998).

Putative Mechanisms of Exercise-Associated Hyponatremia

The two primary factors which would cause a decline in serum Na^+ concentration ($S_{[Na+]}$) during exercise are 1) dilution via gross overdrinking of hypotonic fluids and 2) excessive loss of total body Na^+ via sweating. When exercise-associated hyponatremia (EAH) occurs in events lasting < 4 h it is likely the result of drinking copious volumes of hypotonic fluids before, during, and /or after the event (Sawka et al., 2007). In fact, the mathematical model predicts that the typical magnitude of sweat Na^+ losses incurred within 4 h of exercise, even by individuals with high sweat $[Na^+]$ (i.e., $\geq$ 80 mmol/L), would be insufficient to cause symptomatic EAH by itself (Montain et al., 2006). Overdrinking is also the main factor involved in EAH cases that develop during longer duration events, such as the Ironman Triathlon (~11-12 h or longer). However, because of the longer duration of sweating, it is possible that a sufficient sweat Na^+ deficit can accrue to induce EAH in ultraendurance athletes who do not overdrink. In fact, Hiller (1989) reported that a high percentage (~70%) of athletes treated for clinical EAH at the Hawaiian Ironman Triathlon actually finish the race DEH.

It has been suggested that the etiology of EAH involves more than simply overdrinking and/or sweat sodium losses. Instead, some propose that certain athletes have conditions which exacerbate these effects, rendering them susceptible to more

dramatic decreases in $S_{[Na+]}$. For example, it has been suggested that a significant number of athletes who develop EAH carry the heterozygous gene for cystic fibrosis, causing excessive sweat sodium losses (Montain et al., 2001). Another theory is that EAH is fundamentally caused by an inability to excrete free water / inappropriate retention of water following the overconsumption of fluids. Renal damage from non-steriodal anti-inflammatory drugs (Wharam et al., 2006) and rhabdomyolysis (Siegel, 2006) and inappropriate secretion of antidiuretic hormone (Armstrong et al., 1993; Noakes et al., 2005) have all been postulated to play a role. Additionally, Noakes et al. (2005) and Hew (2005) contend that endogenous water gain (via metabolic water production and water released from glycogen) contributes to the dilution of $S_{[Na+]}$, even when athletes have not gained BM from pre-to post-race. Finally, it has recently been suggested that the osmotically-inactive, but exchangeable Na^+ stored in bone and cartilage can serve as reservoir to modulate $S_{[Na+]}$ during exercise. Subsequently, some athletes may inappropriately osmotically-inactivate Na^+ during exercise, contributing to their development of EAH (Noakes et al., 2005). A myriad of theories exist; however, at this time there is little to no direct evidence available to support a role for any of these factors in EAH (Dumke et al., 2007; Nguyen and Kurtz, 2007).

Hydration and Performance

Intermittent High-Intensity Exercise and Skill Sports

Basketball is a sport defined by bursts of high-intensity activity with intermittent rest periods. Although basketball is played indoors in thermoneutral conditions, the high-intensity nature of the sport coupled with the large body sizes of these athletes can lead to heavy sweat losses (Burke, 1997). For instance, sweat losses of > 2 L were reported in just 21 min of playing time in players of the National Basketball Association (NBA) during summer league games (Osterberg et al., 2005). A regulation NBA game is 48 minutes in length so it is possible that very large sweat losses may be incurred by halftime of a regular-season game. In comparison to other stop-and-go sports such as soccer, the sport of basketball provides more opportunities to drink because of closer

proximity to fluids and the greater number of breaks. Nevertheless, the mean fluid intake by players in the NBA field study was less than half of their mean sweat loss, indicating that even under relatively ideal conditions fluid replacement remains a challenge for basketball players (Osterberg et al., 2005).

It is often difficult for athletes to replace high volumes of sweat losses between games or training sessions. In fact, a high percentage (up to 77%) of athletes report to the locker room in a hypohydrated state (Bergeron et al., 2006; Godek et al., 2005; Stover et al., 2006), as estimated by urine specific gravity (Casa et al., 2000). The Osterberg et al. field studies also showed that NBA players were inadequately hydrated prior to pre-season practices (2004) and summer league games (2005). Specific gravity measurements of urine samples collected from NBA players prior to competition indicated that approximately half of the players were $\geq$ 1% DEH (Casa et al., 2000) before the practice or game commenced. Further, players accrued an additional 1-3% DEH because they only replaced about half of their sweat losses throughout the course of practices and games (Osterberg et al., 2004 and Osterberg et al., 2005). These field study results suggest that a combination of inadequate pre-game and in-game hydration practices can lead to up to 4% DEH by the end of competition.

The impact of DEH on performance of various short-duration, high-intensity activities has been tested previously. University and semi-professional soccer players' performance in a soccer skill test following intermittent high-intensity shuttle running was significantly impaired during 2.4% DEH trials compared to *ad libitum* fluid intake (which led to 1.4% DEH) trials (McGregor et al., 1999). Conversely, compared to a control condition (0.6-0.7% DEH), 2.2 and 2.5% DEH were not detrimental to competitive sprint (50 m, 200 m, and 400 m) or power (vertical jump) performance in high school and collegiate track athletes (Watson et al., 2005). Interestingly, Viitasalo et al. (1987) reported improved vertical jump performance following 3.4% and 3.8% DEH in track and field athletes and volleyball players. Viitasalo et al. (1987) suggested that the decrease in body weight by DEH allowed a greater rise in the athletes' center of gravity and thus the improved vertical jump height. The results of these studies indicate some inconsistencies among the literature regarding the impact of various levels of DEH on high-intensity exercise performance.

Only two studies have tested the effects of DEH on basketball-specific skills. Dougherty et al. (2006) showed that shooting percentage and on-court sprinting and lateral movement times within a simulated game context were significantly impaired by prior 2% DEH relative to placebo EUH in 12-15 yr old male basketball players. Conversely, Hoffman et al. (1995) found no difference in basketball shooting performance in 17 yr old boys playing a simulated 2-on-2 full-court basketball game when fluid was restricted (causing progressive 1.9% DEH) vs. EUH.

Vigilance represents an individual's ability to sustain a high level of alertness over an extended period of time. Vigilance is a particularly important skill for athletes, such as basketball players, whose competitive environments impose strong attentional demands because of their complexity, dynamic nature, and extended duration. Continuous performance tests are commonly used to assess vigilance-related attentional performance (Kindlon, 1998). These tests require participants to respond selectively to stimuli presented over an extended time period. Two salient stresses in sport environments that may affect attentional vigilance are fatigue and DEH. Several studies have evaluated the effect of DEH on perceived fatigue and/or cognitive performance (Cian et al., 2000; Cian et al., 2001; Gopinathan et al., 1988; Shirreffs et al., 2004; Sharma et al., 1986; Szinnai et al., 2005); however, no study has tested the effect of DEH on attentional vigilance in basketball players.

Dehydration Threshold

There is some debate regarding the critical level of DEH at which exercise/sport performance becomes significantly impaired compared to that of EUH. For instance, some investigators suggest that performance is impaired when the level of DEH is *equal to* 2% (Murray, 2007); while others believe that performance becomes impaired only when DEH *exceeds* 2% (Cheuvront et al., 2007). Further, Noakes contends that it is not a body water deficit *per se*, but the development of thirst that impairs performance – as part of an anticipatory control. Noakes speculates that a level of DEH even up to 11% might not impair exercise performance, provided the athlete is not thirsty (Sawka and Noakes, 2007; Sharwood et al., 2002; Sharwood et al., 2004).

A few investigators have tested progressive DEH to determine the critical level of body water deficit at which performance is impaired compared to that of EUH. In 1988, Pichan et al. tested 1%, 2%, and 3% DEH and found that all three levels of DEH significantly impaired physical work capacity in men. In a field study with baseball players, Yoshida et al. (2002) induced DEH levels of approximately 1% to 4% and found that the threshold for impaired performance of aerobic (step test) and anaerobic (10 s maximal cycling) exercise after a regular practice session was 2% and 4%, respectively. McConell et al. (1997) tested the effects of 2% and 3% DEH vs. EUH on cycling performance in well-trained men and found that only 3% DEH caused a significant reduction in cycling time to exhaustion compared with that of EUH. One study has tested the DEH threshold for impaired cognitive function. Gopinathan et al. (1988) induced 1%, 2%, 3%, and 4% DEH (in separate trials) in soldiers by combined water restriction and exercise-heat stress and found a significant deterioration in arithmetic ability, short-term memory, and visuomotor tracking at $\geq$ 2% DEH. All four of the aforementioned studies' authors noted a progressive deterioration in performance as % DEH increased. However, the critical level of DEH for significantly impaired performance varies among the different tasks and ranges from 1% to 4% DEH.

Physiological Consequences of Dehydration

Another matter of debate is the physiological mechanism(s) by which a body water deficit would impair endurance and intermittent high-intensity performance. DEH could contribute to fatigue by impairing cardiovascular and/or thermoregulatory responses to exercise-heat stress. Exercise- and/or heat-induced DEH will elicit a state of hyperosmotic (increase in plasma solute concentration because eccrine sweat is hypotonic relative to plasma; Kirby and Convertino, 1986) hypovolemia (overall reduction in blood plasma volume). The primary driving factor in impaired physiological function during DEH is a reduction in plasma volume. Several studies have confirmed that hypovolemia mediates a decline in venous return, cardiac filling pressure, stroke volume, cardiac output, and a compensatory augmentation in heart rate compared to EUH (Montain and Coyle, 1992; Nadel, 1981; Nadel et al., 1980; Sawka et al., 1979). Additionally,

Gonzalez-Alonso et al. (1998) found that blood flow to exercising muscles becomes significantly reduced when cardiac output and systemic vascular conductance decline with DEH during prolonged exercise in the heat.

DEH augments exercise-induced hyperthermia by impairing evaporative and convective cooling capacity. In a series of studies conducted by Fortney et al., the authors observed that the DEH-induced attenuation of sweating rate and skin blood flow is mediated by both hypovolemia and hyperosmolality. In 1981, Fortney et al. found that hypovolemia *per se* (via diuretic administration) increased the esophageal temperature (T_{es}) threshold for cutaneous vascular conductance by 0.4°C and reduced the slope of the relation between sweating rate and T_{es}. In 1984, Fortney et al. found that hyperosmolality *per se* elevated the T_{es} thresholds for both cutaneous vasodilation and sweating. The magnitude of hyperthermia induced by DEH depends on ambient conditions and exercise intensity, but the range is 0.1 to 0.4 ºC (above that of EUH) per 1% DEH (Sawka, 1992). DEH also increases muscle temperature and exacerbates the sympathoadrenal response during exercise (Hargreaves et al., 1996; Gonzalez-Alonso et al., 1997). Further, DEH causes a shift in metabolism; such that muscle glycogen oxidation and blood lactate accumulation occur at a faster rate compared with that of EUH (Hargreaves et al., 1996; Gonzalez-Alonso et al., 1997). Therefore, DEH could contribute to impaired performance via its role in decreasing intramuscular carbohydrate stores (Febbraio, 1999).

Any of these factors could contribute to the early onset of fatigue during exercise; however, the most important may be central nervous system changes that occur as a result of hyperthermia. According to the critical internal temperature hypothesis, individuals exercising in the heat consistently reach the point of exhaustion at the same threshold body core temperature (~40°C) (Cheung and Sleivert, 2004; Gonzalez-Alonso et al., 1999). The concept underlying the critical core temperature hypothesis is that an increased body core and brain temperature is associated with a reduction in cerebral blood flow and altered brain metabolism which decreases the central drive to exercise. The diminished drive to exercise is associated with an increased rating of perceived exertion and reduced motor unit recruitment and firing rate to the exercising muscle,

ultimately resulting in an attenuation of muscular force generation (Nielsen and Nybo, 2003; Nybo et al., 2002; Nybo and Nielsen, 2001).

Chapter 3

**SEX DIFFERENCES IN VOLUNTARY FLUID INTAKE BY OLDER ADULTS
DURING EXERCISE**

Introduction

During exercise in the heat, body water is lost (dehydration) as a consequence of
sweating. Fluid replacement is critical to ameliorate the deterioration in physiological
function and performance that accompanies dehydration (Convertino et al., 1996).
However, over-consumption of fluids (especially sodium-free fluids such as water) in
excess of sweat loss can lead to electrolyte imbalances (Armstrong et al., 1993).

Older adults are at an increased risk for fluid and electrolyte imbalances due to
impaired thirst sensitivity (Kenney and Chiu, 2001; Nadel et al., 1980; Stachenfeld et al.,
1997) and renal sodium- and water-conserving ability (Mack et al., 1994; Phillips et al.,
1991; Phillips et al., 1984). In addition, anecdotal evidence suggests that hyponatremia
(low blood sodium), which usually occurs due to over-consumption of water, most
commonly occurs in women (Ayus et al., 2000; Backer et al., 2003; Davis et al., 2001;
Speedy et al., 1999); however, little data exists on the actual sex-related differences in
voluntary fluid intake behavior of older adults during exercise-heat stress.

Numerous studies looking at fluid replacement in young adults have shown that,
due primarily to the presence of sodium, carbohydrate-electrolyte solutions (CES) are
more effective than water in stimulating voluntary fluid intake (Convertino et al., 1996;
Murray, 1998, Nose et al., 1988), restoring plasma volume (Carter and Gisolfi, 1989;
Costill and Sparks, 1973; Gonzalez-Alonso et al., 1992), maintaining fluid/electrolyte
balance (Costill et al., 1970), and attenuating increases in core temperature (Costill et al.,
1970) during exercise-heat stress. In addition, Bar-Or and Wilk have shown that *ad
libitum* consumption of water leads to voluntary dehydration in children; however, the
addition of flavor, carbohydrates and sodium chloride to water prevents dehydration
when children are allowed to drink *ad libitum* (Bar-Or and Wilk, 1996). For these
reasons, CES is the recommended fluid replacement beverage for children and young

adults during prolonged activity (Bar-Or and Wilk, 1996; Convertino et al., 1996). The
efficacy of CES as a fluid replacement beverage for older active men and women during
or after exercise-heat stress has not been previously studied.

The purpose of the present study was to compare the voluntary fluid intake
behavior of older (defined here as 54-70 years of age) active men and women when
provided ample opportunity to drink during rest periods of a moderate intensity interval
cycling protocol in the heat. A second goal was to compare the efficacy of two
replacement fluids, CES and water, in maintaining fluid/electrolyte balance, restoring
plasma volume, and minimizing thermoregulatory strain associated with exercise-heat
stress in older men and women.

Methods

Subjects. Twenty-seven healthy older recreational exercisers (13 men, 14
women, 54-70 yrs) volunteered to participate in this study. Participants were informed of
the experimental procedures and associated risks prior to providing written informed
consent. This study was approved by the Institutional Review Board for the Protection of
Human Subjects of The Pennsylvania State University. Preliminary screening included a
resting 12-lead electrocardiogram, skin fold measurements to determine adiposity, blood
analysis (CHEM-24), a graded exercise test on a cycle ergometer to determine peak
oxygen uptake (VO_{2peak}), and a physical exam by a physician. Criteria for exclusion
included an abnormal resting or exercise electrocardiogram, smoking, diabetes, coronary
artery disease, inactive lifestyle, elite athlete, or the taking of medications that may
influence thermoregulatory or cardiovascular variables of interest. Volunteers with
medical conditions or on medications not mentioned in the exclusion criteria and that did
not pose a safety risk were included in the study. Subject characteristics are presented in
Table 3.1.

Experimental Procedure. All subjects completed 2 experimental trials in which
they consumed either a commercially available CES (6% carbohydrate and 18.0 mmol/L
NaCl), or distilled water. During an additional trial 14 subjects repeated 1 of the 2 fluids

in order to determine the reproducibility of fluid intake. Experimental trials were scheduled at least one week apart and trials were assigned in random order.

Subjects reported to the laboratory on the morning of test days after having fasted overnight. Immediately upon arrival they were asked to complete Survey 1, a visual-analog rating scale with questions pertaining to subjective feelings of physical and psychological well-being (described later). After collecting a urine sample, emptying their bladder, and being weighed, the subjects had an 18-gauge Teflon catheter placed in an antecubital vein in one arm. Next, the subject inserted a rectal thermistor 8 cm past the anal sphincter, and then entered an environmental chamber set at 30°C and 50% relative humidity. The subject, wearing shorts and a t-shirt, then mounted a cycle ergometer (Monark Ergomedic 818E) and relaxed in a seated position for approximately 15 min while being instrumented with four skin thermocouples (chest, upper arm, thigh, calf), a Polar® heart rate monitor, and blood pressure cuff. A modification was made to the bike seat which included a back rest and retractable padded seat that slid under the subject for the rest periods and then pulled out of the way during the exercise periods. This padded seat allowed for improved comfort during the baseline, rest, and recovery periods without having to change body posture by getting off the bike to sit on a chair.

After instrumentation, the subject sat at rest for a 15 min baseline period. Next, the subject cycled at an intensity of $65 \pm 1\%$ of VO_{2peak} for 15 min, followed by 15 min of rest during which subjects were allowed to drink *ad libitum*. This interval cycling protocol continued until the subject completed four 15-min bouts of cycling, separated by 15-min rest periods. After completing the fourth exercise bout the subject remained in a seated position on the bike and was permitted to drink *ad libitum* during a 30 min recovery period. The subject was asked to complete Survey 2 (which included questions about beverage satisfaction and feelings of physical well-being) at the end of the second rest period (min 70) and then again during the recovery period (min 130).

At the end of the 2.5 h interval cycling protocol the subject exited the chamber, and a post-experiment body mass (BM) and then urine sample were obtained. Finally, the subject was asked to again complete the first survey before leaving the laboratory. A schematic of the study protocol is diagrammed in Figure 3.1.

Ad Libitum Fluid Intake. During the rest periods and recovery, a bottle containing 700 mL of 15°C fluid (CES or water) randomly assigned to that given trial was provided to the subjects while they sat quietly at rest. Subjects were not encouraged to drink, but were told that more fluid was readily available if needed. Volume of fluid intake was measured to the nearest mL after rest periods 1-3 and recovery. The subjects were unaware that their fluid consumption was being measured.

Measurements. Rectal and skin temperature (T_{re} and T_{sk}, respectively) were measured continuously throughout the protocol and recorded as one-minute averages. HR was measured using a Polar® heart rate monitor and blood pressure was measured by brachial auscultation (sphygmomanometry). Ratings of perceived exertion (RPE) were assessed using the Borg scale (Borg, 1973). Oxygen uptake was measured (ParvoMedics TrueOne 2400 Metabolic Measurement System) 5 min into the second exercise bout to verify that the subject was exercising at the target workload of 65% of VO_{2peak}. Pre- and post-experiment BM was measured to the nearest 0.1 kg on a Toledo scale.

Blood and Urine Analysis. Venous blood samples (11 mL each) were drawn without stasis. A 2.5 mL aliquot was transferred into an EDTA-treated test tube and immediately analyzed for hematocrit (Hct) and hemoglobin (Hb) in duplicate using a Beckman Coulter Microdiff 16. The remaining 8.5 mL aliquot was transferred into a serum separator tube, allowed 30-60 minutes to clot, and then centrifuged at 4°C for 15 min. Serum was analyzed for glucose concentration ($S_{[gluc]}$; hexokinase UV method, Olympus Model AU5200), sodium concentration ($S_{[Na+]}$; ion specific electrode method, Olympus Model AU5200), total protein concentration ($S_{[prot]}$; biuret method, Olympus Model AU5200), and osmolality (S_{osmol}; freezing point depression, Advanced DigiMatic Osmometer Model 3D2) in duplicate. Pre- and post-experiment urine osmolality (U_{osmol}) and specific gravity (U_{sg}; Refractometer, Atago A300CL) were also determined in duplicate.

Calculations. T_{sk} was calculated as the weighted sum of 4 sites, chest (T_{ch}), upper arm (T_a), thigh (T_{th}), and calf (T_{leg}) using the following equation (Ramanathan, 1964):

$$T_{sk} = 0.3 \cdot T_{ch} + 0.2 \cdot T_a + 0.3 \cdot T_{th} + 0.2 \cdot T_{leg}$$

Mean arterial pressure (MAP) was calculated as MAP = ⅓ Pulse Pressure + Diastolic BP. Total body sweat loss was calculated from the net ΔBM corrected for fluid consumed and urine excreted. The percent change in plasma volume from the baseline period (ΔPV) was calculated from Hct and Hb (Dill and Costill, 1974):

$$\Delta PV(\%) = 100 \ X \ (Hb_{pre}/Hb_{post}) \ X \ \{[1-(Hct_{post}/100)] \ / \ [1-(Hct_{pre}/100)]\} \ -100$$

Subjective Ratings. Survey 1 was administered to the subject before and after the experiment. Subjective feelings of physical and psychological well-being were assessed by visual analog rating scales. Subjects responded to the following questions, "how alert do you feel?", "how sad do you feel?", "how tense do you feel?", how much of an effort is it to do anything?", "how happy do you feel?", "how weary do you feel?", "how calm do you feel?", and "how sleepy do you feel?" on 100-point scales with responses ranging from "very little"(0) to "very much"(100).

Survey 2 was completed at min 70 and min 130 to determine the subjects overall perception of the beverage consumed during the trial. The subject was instructed to mark an "X" in the box which best described their "overall acceptance" of the beverage and their opinion of its "flavor" and "aftertaste". The questions were on a 9-point scale in which possible responses ranged from "dislike extremely"(#1) to "like extremely"(#9). This questionnaire also included visual analog scales which measured perceived intensities of overall flavor ("weak overall flavor" to "strong overall flavor"), sweetness ("not at all sweet" to "very sweet"), saltiness ("not at all salty" to "very salty"), off-flavor ("no off-flavor" to "strong off-flavor"), aftertaste ("weak aftertaste" to "strong aftertaste"), and whether the beverage was thirst quenching ("not thirst quenching" to "very thirst quenching"). In addition, Survey 2 included visual analogue scales which rated feelings of hunger ("not hungry" to "very hungry"), hotness ("not feeling hot/overheated" to "feeling very hot/overheated"), muscle fatigue ("no muscle fatigue" to "severe muscle fatigue"), difficulty of exercise ("exercise very easy" to "exercise very difficult"), burping ("no burping" to "severe burping"), stomach fullness ("no stomach fullness" to "severe stomach fullness"), and stomach upset ("no stomach upset" to "severe stomach upset"). The subject answered these questions by placing a mark on a 100-point scale between the extreme answers at the opposite ends of the line.

Statistical Analysis. An intraclass correlation coefficient was used to test the reproducibility of fluid intake (Shoukri and Pause, 1999). A Student's *t*-test was used to determine any significant differences between sexes in the subjects' physical characteristics. Variables which were measured through time (e.g. T_{re}, T_{sk}, HR, BP, RPE, blood variables, and responses to subjective questionnaires) were analyzed using two-way analysis of variance (ANOVA) (fluid vs. time) with repeated measures. A one-way ANOVA was used to compare variables measured once per test (e.g. total fluid intake, ΔBM, ΔU_{osmol}, ΔU_{sg}). To detect any association of a subject's sex with the physiological and subjective variables measured throughout the protocol a two-way (fluid vs. sex) ANOVA or three-way (fluid vs. time vs. sex) repeated measures ANOVA was performed where appropriate. The Tukey post-hoc test was performed when main effects were found. The significance level for all statistical tests were set to alpha = 0.05. All data are presented as means ± SE.

Results

Physical characteristics of the participants are shown in Table 3.1. The men had a significantly greater body mass, height, and VO_{2peak} than the women, while women had a significantly higher adiposity than the men.

In the subjective evaluation of the fluids (Figure 3.2) men rated the "overall acceptance" and "flavor" of CES higher than water. By contrast, women rated the "overall acceptance", "flavor", and "aftertaste" of water higher than that of CES. CES was rated higher by the men than women for "overall acceptance" and "flavor" whereas the "flavor" of water was rated higher by the women compared to the men. Also, women gave higher ratings than the men for "thirst quenching" when consuming water. The men felt significantly more "hot/overheated" and perceived "exercise difficulty" to be significantly higher than did the women during the water trials.

Across all trials, the reproducibility of total fluid intake was very good (intraclass correlation coefficient = 0.75) (Shoukri and Pause, 1999). Figure 3.3 presents the total fluid intake, sweat loss, and net fluid balance during the CES and water trials for men, women, and all subjects. Total fluid intake was significantly higher during the CES trials

(all subjects). Men drank significantly more CES than water, whereas total intake did not differ significantly between the 2 fluids in women. Women were in positive fluid balance (increased BM from baseline) during both the CES ($P<0.05$) and water trials, while men were only in positive fluid balance with CES. When total fluid intake was expressed relative to BM women drank significantly more water than the men.

Figure 3.4 illustrates changes in ΔPV (panel A), $S_{[prot]}$ (panel B), and $S_{[Na+]}$ (panel C) during the CES and water trials for men, women, and all subjects. During the CES trials plasma volume (PV) was at or above baseline from the third rest period to the end of recovery (all subjects). However, during the water trials PV was significantly below baseline at this time (all subjects). PV decreased significantly less in the women compared to the men near the end of the protocol in both fluid trials (panel A). During the second half of the protocol $S_{[prot]}$ was significantly greater during the water trials vs. the CES trials for all subjects (panel B). $S_{[Na+]}$ was significantly below baseline near the end of the protocol in both fluid trials (women and all subjects). Women had a significantly lower $S_{[Na+]}$ than the men during the final exercise bout of the CES trials and from the final exercise bout to the end of the recovery period of the water trials (panel C).

Fluid intake per kg BM, T_{re}, T_{sk}, $S_{[gluc]}$, S_{osmol}, BP, HR, and RPE are presented in Table 3.2. Subjects (all) consumed significantly more CES than water during the first, second, and third rest periods, but not during recovery. Women drank significantly more water than the men during the 1st and 2nd rest periods. There were no differences between fluids in the T_{re} responses; however, during the water trial the women had a significantly lower T_{re} than the men from the second exercise period to the end of recovery. $S_{[gluc]}$ was significantly higher during the CES trials from the second bout of exercise to the end of recovery (all subjects). There were no significant differences between fluids or sexes in T_{sk}, S_{osmol}, BP, HR, RPE, U_{osmol} (CES$_{pre}$ = 655±38, CES$_{post}$ = 608±36, water$_{pre}$ = 604±45, water$_{post}$ = 546±38 mosmol/kg), or U_{sg} (CES$_{pre}$ = 1.018±0.001, CES$_{post}$ = 1.018±0.001, water$_{pre}$ = 1.017±0.001, water$_{post}$ = 1.016±0.001 UG) throughout the experiment. S_{osmol}, U_{osmol}, and U_{sg} did not change from baseline in men, women, or all subjects.

Figure 3.5 presents the $S_{[Na+]}$ of one woman (65 yrs, 45.7 kg) who became hyponatremic ($S_{[Na+]}$ = 126 mmol/L) with symptoms (e.g. headache, extreme fatigue, and gastrointestinal distress) during the water trial. Her $S_{[Na+]}$ was 126 mmol/L after

consuming 2.8 L of water, but she did not experience symptomatic hyponatremia during the CES trial ($S_{[Na+]} = 131$ mmol/L) in which she drank 2.7 L of CES.

Discussion

The major findings from this study were (1) when cool palatable fluids were readily available, active adults aged 54-70 yrs drank enough to match sweating rates and maintain their body weight, (2) their fluid intake behavior was repeatable, (3) CES promoted greater voluntary fluid intake and restored PV losses faster than water, and (4) there were sex differences in the fluid intake behavior of older active adults, with women drinking more water per kg BM than men.

Combined Data from All Subjects. The present investigation indicates that older adults drink enough fluid to replace sweat losses incurred during moderate intensity interval exercise in the heat. Furthermore, this drinking behavior was repeatable. The subjects did not experience voluntary dehydration most likely because they had a cool palatable fluid readily available and had ample opportunity to drink between exercise bouts. The lack of voluntary dehydration might also be explained by the low average sweat rate (370 g/h over the entire protocol). In 1947, Rothstein et al. reported that when sweat losses are low (defined as < 400 g/h) young men working in the heat have little difficulty consuming sufficient fluid to replace periodic losses (Rothstein et al., 1947). The current study suggests that older men and women also drink enough fluid to match sweat losses when sweat rate is < 400 g/h. Although subjects drank enough to maintain BM during both fluid trials CES was more effective at stimulating voluntary fluid intake than water, especially in men. As a result of the significantly greater consumption of CES, PV losses were restored faster during the CES trials compared to the water trials. In addition, $S_{[prot]}$ was lower in the CES vs. the water trials during the second half of the protocol. This indicates a greater hemodilution when CES was consumed which supports the conclusion that CES more effectively restored extracellular fluid volume. These data are consistent with other studies showing that CES is more effective than plain water in promoting fluid intake and restoring PV losses in younger exercising subjects (Carter and Gisolfi, 1989; Gonzalez-Alonso et al., 1992; Nose et al., 1988).

Sex Differences. Both men and women consumed more CES than water (but not significantly more by the women). However, significant sex-related differences were detected for the fluids' subjective values. Men rated CES higher than water for "overall acceptance" and "flavor. Conversely, women rated water higher than CES for these categories. In addition, women perceived water to be more "thirst quenching" compared to the men. These differences in the subjective evaluation of the fluids explain why women consumed more water per kg BM compared to the men. The greater fluid intake by the women resulted in a lower T_{re} than that of men during the final 90 min of the water trials. The sex difference in T_{re} corresponded with a sex difference in the ratings of how "hot/overheated" the subjects felt and their perception of "exercise difficulty", i.e. the women's ratings were lower than that of the men's in both categories during the water trials.

During the water trials the $S_{[Na+]}$ of the women was lower than that of the men during the final 30 min of the protocol because the women drank significantly more water relative to BM compared to the men. However, the average $S_{[Na+]}$ of the women at the end of recovery (138 ± 1 mmol/L) was still within the normal range of 135-145 mmol/L. Hyponatremia is defined as $S_{[Na+]}$ less than 135 mmol/L and can be categorized as asymptomatic (usually 130-134 mmol/L) and symptomatic (usually < 130 mmol/L) hyponatremia (Speedy et al., 1999). Common symptoms include headache, light-headedness, confusion, fatigue, gastrointestinal distress, nausea, and malaise (Speedy et al., 2001). In the present study one woman became clinically hyponatremic ($S_{[Na+]} = 126$ mmol/L) with symptoms by the end of the protocol during the water trial. This subject (65 yrs, 45.7 kg) drank 2.8 L of water and gained 2.4 kg of weight during that experiment. She complained of a headache and extreme tiredness near the end of the water trial. In addition, at the end of the recovery period she rated the following assessments of physical well-being much higher during the water trial in comparison to the CES trial: "how much of an effort is it to do anything?" (51.7 vs. 11.7), "how weary do you feel?" (81.4 vs. 14.5), and "how sleepy do you feel?" (73.1 vs. 15.2). She also rated her degree of "stomach upset" (22.4 vs. 4.2), "stomach fullness" (45.5 vs. 6.3), and "burping" (25.2 vs. 6.3) higher during the water trial, which indicates that she may have experienced gastrointestinal distress at this time.

Anecdotal evidence suggests that hyponatremia most commonly occurs in women, especially those with low body weight and who drink large volumes of sodium free fluids (i.e. water) before, during, and after physical activity (Ayus et al., 2000; Backer et al., 1993; Davis et al., 2001; Speedy et al., 1999). A retrospective analysis of symptomatic hyponatremia in marathon runners by Davis et al. (2001) showed that this condition develops in older as well as younger women. Six out of 23 hyponatremic female marathon runners in the analysis were 50 yrs or older. The present study provides supporting evidence that the over-consumption of water may be a health concern among smaller women in this age range. Furthermore, this woman's data support the notion that a CES is superior to water in limiting reductions in $S_{[Na+]}$ during exercise-heat stress. During the CES trial this female subject consumed 2.7 L and had a final $S_{[Na+]}$ of 131 mmol/L. Therefore, although she consumed similar amounts of CES and water, $S_{[Na+]}$ was maintained above that of symptomatic hyponatremia during the CES trial.

Fluid intake differences between men and women are most likely due to differences in behavior and not in thirst perception. There is no evidence to suggest the existence of a physiological mechanism by which thirst perception differs between older men and women; therefore, it may be that women are more health and safety conscious than men and thus make a greater effort to drink, and in some cases over-drink, the fluid that is available.

Summary. In summary, voluntary dehydration does not occur in older adults when cool palatable fluid (either CES or water) is readily available between repeated bouts of moderate intensity exercise in the heat. In addition, CES promotes greater voluntary fluid intake and restores PV losses faster than water during interval cycling suggesting that CES is the more effective fluid replacement beverage for older active adults. Furthermore, older women drink more CES and water than is lost through sweating. Over-consumption of water may put smaller women at an increased risk for developing hyponatremia.

Table **3.1**: Subject characteristics. Values are means ± SE. BMI, body mass index; HR_{peak}, peak heart rate attained during graded exercise test; VO_{2peak}, peak oxygen consumption. * $P < 0.05$, men vs. women.

	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m²)	Adiposity (%)	HR_{peak} (bpm)	VO_{2peak} (ml/kg/min)
Men ($N = 13$)	63 ± 1	84 ± 4	176 ± 2	27 ± 1	24 ± 1	159 ± 4	31 ± 2
Women ($N = 14$)	61 ± 1	66 ± 3*	157 ± 7*	24 ± 1	30 ± 2*	168 ± 4	24 ± 1*
All Subjects ($N = 27$)	62 ± 1	75 ± 3	167 ± 4	26 ± 1	27 ± 1	164 ± 3	27 ± 1

Table **3.2**: Fluid intake, physiological variables, and RPE. T_{re}, rectal temperature; T_{sk}, skin temperature; S_{osmol}, serum osmolality; S_{gluc}, serum glucose concentration; MAP, mean arterial pressure; HR, heart rate; RPE, rating of perceived exertion. * $P < 0.05$, CES vs. water; † $P < 0.05$, men vs. women.

Table **3.2**

		Fluid Intake (ml/kg)			T_{re} (°C)			T_{sk} (°C)	S_{osmol} (mOsmol/kg)	$S_{[gluc]}$ (mg/dl)	MAP (mmHg)	HR (bpm)	RPE
		All	Men	Women	All	Men	Women	All	All	All	All	All	All
Baseline	CES				37.13 ±0.05	37.08±0.08	37.17±0.05	34.08±0.11	289±2	91±1	89±2	73±2	
	Water				37.13±0.05	37.16±0.05	37.11±0.07	34.05±0.09	290±1	93±1	91±2	73±2	
Exercise 1	CES				37.27±0.05	37.28±0.08	37.27±0.05	34.29±0.11	295±1	91±1	99±2	123±3	11.5±0.3
	Water				37.26±0.06	37.30±0.09	37.24±0.08	34.34±0.11	296±1	89±2	100±2	123±3	11.5±0.3
Rest 1	CES	5.2±0.8*	4.6±0.6	5.8±1.4	37.50±0.05	37.49±0.07	37.51±0.06	34.59±0.10	292±1	96±2	92±2	89±3	
	Water	4.0±0.6	3.1±0.5†	4.8±1.0	37.53±0.06	37.60±0.08	37.47±0.08	34.63±0.11	291±1	89±2	92±2	86±3	
Exercise 2	CES				37.66±0.05	37.68±0.08	37.64±0.07	34.61±0.10	298±2	113±4*	100±2	132±4	12.7±0.2
	Water				37.68±0.07	37.80±0.07†	37.59±0.10	34.64±0.10	294±1	84±2	100±2	131±4	12.6±0.2
Rest 2	CES	4.8±0.6*	4.3±0.6	5.3±1.0	37.84±0.06	37.87±0.09	37.82±0.08	34.77±0.13	292±1	125±4*	93±2	94±3	
	Water	3.9±0.5	3.2±0.4†	4.5±0.9	37.83±0.07	38.03±0.08†	37.71±0.10	34.75±0.12	289±1	87±2	93±2	89±3	
Exercise 3	CES				37.93±0.06	37.95±0.10	37.92±0.07	34.66±0.12	294±1	117±5*	99±2	135±4	13.7±0.3
	Water				37.91±0.07	38.09±0.08†	37.79±0.10	34.55±0.12	292±1	81 ±2	99±2	132±4	13.6±0.3
Rest 3	CES	4.5±0.8*	4.0±0.7	5.0±1.3	38.03±0.06	38.04±0.11	38.02±0.07	34.77±0.15	290±1	117±4*	92±2	95±3	
	Water	3.5±0.5	3.2±0.5	3.7±0.9	37.99±0.07	38.24±0.08†	37.82±0.09	34.77±0.12	288±1	87±2	92±2	90±3	
Exercise 4	CES				38.05±0.06	38.04±0.11	38.05±0.06	34.67±0.10	292±1	105±4*	98±2	136±4	14.4±0.3
	Water				38.02±0.07	38.23±0.08†	37.86±0.10	34.53±0.14	291±1	78±1	100±2	136±4	14.7±0.3
Recovery 1	CES				38.07±0.07	38.07±0.13	38.07±0.07	34.81±0.12	289±1	112±3*	88±2	97±3	
	Water				38.06±0.08	38.32±0.09†	37.90±0.11	34.87±0.11	288±1	87±1	90±2	92±3	
Recovery 2	CES	4.2±0.4	4.5±0.7	3.8±0.6	37.91±0.06	37.90±0.12	37.93±0.07	34.42±0.14	288±1	126±4*	88±2	93±3	
	Water	3.8±0.6	3.3±0.6	4.2±1.0	37.91±0.08	38.13±0.10†	37.76±0.11	34.45±0.13	287±1	87±2	88±2	87±3	

29

Figure **3.1**

Figure **3.1**: Schematic of study protocol for each trial. During exercise periods 1-4, subjects cycled at 65% VO_{2peak}. Subjects sat upright on the bike seat for baseline, rest 1-3, and recovery. Base, baseline; BP, blood pressure; BS, blood sample; BW, body weight; Exer, exercise; HR, heart rate; RPE, rating of perceived exertion; US, urine sample; VO_2, oxygen consumption.

Figure **3.2**

Figure **3.2**: Responses to subjective questionnaires on 9-point (*top panel*) and 100-point (*bottom panel*) scales. * $P < 0.05$, CES vs. water; † $P < 0.05$, men vs. women.

Figure **3.3**

Figure **3.3**: Fluid intake, net fluid balance, and sweat loss expressed in absolute units (*Panels A-C*) and relative to body mass (*Panels D-F*). * P < 0.05, CES vs. water; † P < 0.05, vs. baseline; ‡ P < 0.05, men vs. women.

Figure **3.4**

Figure **3.4**: Hematological variables in men, women, and all subjects. A) percent change in plasma volume from baseline (ΔPV), B) serum protein concentration (S_{prot}), and C) serum sodium concentration (S_{Na}). * $P < 0.05$, CES vs. water; † $P < 0.05$, vs. baseline; ‡ $P < 0.05$, men vs. women. Pre, pre-experiment (supine, thermoneutral); Base, baseline (sitting upright, 30°C, 50% RH); Ex, exercise; Rec, recovery.

Figure **3.5**

Figure **3.5**: Serum sodium concentration in 65 yr old, 45.7 kg woman during the CES and water trials. She was hyponatremic ($S_{[Na+]}$ = 126 mmol/L) with symptoms by the end of the water trial but maintained $S_{[Na+]}$ (131 mmol/L) above symptomatic hyponatremia when consuming CES. The dashed line at 130 mmol/L represents the upper limit for clinical hyponatremia. Volume of total fluid intake for each trial is shown in the legend. Pre, pre-experiment (supine, thermoneutral); Base, baseline (sitting upright, 30°C, 50% RH); Exer, exercise; Recov, recovery.

Chapter 4

QUANTITATIVE ANALYSIS OF SERUM SODIUM CONCENTRATION AFTER PROLONGED RUNNING IN THE HEAT

Introduction

Exercise-associated hyponatremia (EAH) is defined as a serum sodium concentration ($S_{[Na+]}$) less than 135 mmol/L and occurs primarily in endurance events such as marathons, ultramarathons, and triathlons (Montain et al., 2001). Although the condition is rare, severe EAH (typically $S_{[Na+]} < 125$ mmol/L) can result in cerebral/pulmonary edema, coma, and death (Backer et al., 1999; Montain et al., 2001; Sawka et al., 2007). Therefore, research regarding the mechanisms and prevention of EAH has been of utmost importance since it was first described in 1985 (Noakes et al., 1985). Montain et al. (2006) and Weschler (2005) have completed detailed theoretical quantitative analyses on sodium balance and, by extension, the etiology of EAH, using a prediction equation developed by Kurtz and Nguyen (2003) to calculate post-exercise $S_{[Na+]}$ based on changes in the mass balance of water, Na^+, and K^+ (Montain et al., 2006; Weschler, 2005).

The mathematical model predicts that $S_{[Na+]}$ is most sensitive to changes in total body water and thus the primary cause of EAH is an increase in body mass (BM). That is, the dilution of $S_{[Na+]}$ and the symptoms associated with EAH are mediated by the consumption of fluid at a rate that grossly exceeds sweating rate. The model also predicts that $S_{[Na+]}$ is moderately sensitive to changes in the mass electrolyte balance of Na^+ and K^+ (ΔE), such that the consumption of a carbohydrate-electrolyte solution (CES) will attenuate the decline in $S_{[Na+]}$ compared to the consumption of water alone (Montain et al., 2006; Weschler, 2005). This theoretical model provides a practical means for athletes to predict $S_{[Na+]}$ and to decrease their risk of EAH. However, its predictions have not been directly compared to empirical data of post-exercise $S_{[Na+]}$ in athletes with known pre-exercise $S_{[Na+]}$, BM, sweating rates, sweat $[Na^+]$ and $[K^+]$, fluid intake, and Na^+ and K^+ intake in a controlled laboratory setting.

Therefore, the purpose of the present study was to compare measured $S_{[Na+]}$ with that predicted by the Nguyen-Kurtz equation in endurance-trained athletes after prolonged running in the heat to assess the equation's accuracy. A second goal of the study was to measure the effects of beverage $[Na^+]$ (0, 18, and 30 mmol/L) and pre-to post-exercise ΔBM (+2%, 0%, -2%, and -4%) on measured $S_{[Na+]}$. Further, we aimed to measure the effects of a BM deficit (-2% and -4%) vs. fluid consumption to match sweating rate (i.e., 0% ΔBM) on prolonged running performance in the heat. We hypothesized that 1) the Nguyen-Kurtz equation would accurately predict the measured post-exercise $S_{[Na+]}$, 2) increased $[Na^+]$ in a CES would attenuate the decline in $S_{[Na+]}$ compared to a beverage with no Na^+, 3) an increase in BM as a result of overdrinking relative to sweat losses (+2% ΔBM) would be associated with a decrease in $S_{[Na+]}$, and 4) a decrease in BM via underdrinking relative to sweat losses (-2% and -4% ΔBM) would impair endurance performance compared to maintenance of pre-exercise BM (0% ΔBM).

Materials and Methods

Subjects. Eight endurance-trained runners (4 men, 4 women; 22-36 yr) volunteered to participate in this study. Subjects were informed of the experimental procedures and associated risks before providing written informed consent. This study was approved by the Institutional Review Board for the Protection of Human Subjects at the Pennsylvania State University. Preliminary screening included a physical exam and a graded-exercise test on a treadmill to determine maximal oxygen uptake (VO_{2max}). Criteria for the subjects' inclusion were $VO_{2max} \geq 50$ mL·kg^{-1}·min^{-1} for men and ≥ 45 mL·kg^{-1}·min^{-1} for women, running ≥ 32 km per week, and not currently taking medications or oral supplements that could interfere with study results. All women were euhmenorrheic with regular cycles (natural, $N = 2$; oral contraceptive users, $N = 2$) and were tested during the follicular phase of their cycle (within 7 days after the onset of menstruation). No subjects reported having experienced EAH previously. Subject characteristics are presented in Table 4.1.

Pre-experiment control. All subjects had been engaged in ≥ 12 weeks of regular running before participation in the study and maintained a consistent training schedule

until completion of all experimental trials. Subjects were instructed to eat their typical pre-race diet the evening before and to abstain from heavy exercise, alcohol, and caffeine at least 24 h before each trial. Diet logs were kept by the subjects to facilitate consistent food and fluid consumption for 24 h before each trial. Subjects reported to the laboratory at 0700 on the morning of test days after an overnight fast. Immediately upon arrival, a blood sample was obtained to confirm normal baseline hydration and Na^+ status. Subjects were considered euhydrated when serum osmolality was < 290 mOsmol/L (Sawka et al., 2007) and eunatremic when $S_{[Na+]}$ was 135-145 mmol/L.

Experimental procedure. There were 12 experimental trials (3 beverages at 4 different % ΔBM). Subjects drank fluid or no fluid to 1) maintain BM (0%), 2) increase BM by 2%, 3) decrease BM by 2%, or 4) decrease BM by 4% in separate trials. The beverages were 1) a 6% carbohydrate solution without Na^+ (Na^+0), 2) a 6% carbohydrate solution with 18 mmol/L Na^+ (Na^+18), and 3) 6% carbohydrate solution with 30 mmol/L Na^+ (Na^+30). Five of the 8 subjects did not have sweating rates high enough to reach -4% ΔBM. Because these 5 subjects did not consume any fluid during this trial, they only completed one -4% ΔBM trial. Thus, a total of 86 trials were completed. Experimental trials were scheduled at least one week apart and were assigned in random order. Both the subject and investigator were blinded to the beverage consumed during the trials.

The night before each experiment, subjects swallowed an ingestible temperature sensor for the measurement of body core temperature (T_c). On test days, subjects had an 18-gauge Teflon catheter placed in an antecubital vein, voided their bladder, and then entered an environmental chamber set at 30º C and 40% rh. Next, the subject was asked to sit quietly for 30 min before the baseline heart rate (HR), blood pressure (BP), T_c, and blood sample were obtained. Next, the subject's initial BM was measured to the nearest 0.05 kg. All BM measurements during the experiment were taken with the subject wearing lightweight running shorts, sport bra (women), thin socks, and running shoes. Next, the subject ran for seven 15-min bouts (70% VO_{2max}) separated by 2 min of rest (2 h of interval running total). Twelve min into each running bout, HR, BP, T_c, rating of perceived exertion (RPE), and a blood sample were obtained. Urine samples were collected during rest periods as needed. The criterion for terminating a trial before the planned 2 h was a $S_{[Na+]} \leq 132$ mmol/L.

Drink protocol. During each rest period, the subjects were toweled off and then had their BM measured. During the 0% ΔBM trials, subjects drank fluid (either Na^+0, Na^+18, or Na^+30) volumes during the rest periods to maintain their initial BM. During the -2% and -4% ΔBM trials, fluid was restricted until the subjects reached their target BM. If the subjects' BM fell below their target BM, they ingested enough fluid (either Na^+0, Na^+18, or Na^+30) to maintain the desired % ΔBM. During the +2% ΔBM trials, subjects drank the necessary fluid (either Na^+0, Na^+18, or Na^+30) volumes to gain 2% of their BM by the end of the 2-h of interval running. The 2% gain in BM was titrated over the 2 h interval running protocol so that BM gain was achieved gradually (to maximize fluid retention and minimize gastrointestinal discomfort).

Performance run. At the end of the 2-h interval running protocol, subjects voided, and then had their BM measured. Next, subjects drank the appropriate volume of Na+0, Na+18, or Na+30 or drank no fluid to maintain the desired % ΔBM. Next, subjects ran at a speed corresponding to 85% VO_{2max} until exhaustion. Subjects were instructed to run until volitional fatigue and received a monetary incentive. No fluid was consumed and no verbal encouragement or feedback on run time or distance was given to subjects during the performance run. Time to exhaustion was recorded to the nearest second. Seven subjects repeated one trial to determine the repeatability of the performance test. The beverage and % ΔBM of repeat trials were selected at random and the subjects were blinded as to which trial they were repeating.

Recovery. During the first 10 min of recovery, subjects walked at 2.5 mph for a gradual cool down. Next, subjects sat quietly for a 50-min recovery period to allow fluid compartments to stabilize and capillary filtration pressure to return to resting values. This was important because the Nguyen-Kurtz equation was derived from measurements made at rest. The subjects' BM was measured at the beginning, 30 min, and end of the recovery period. Subjects drank fluid (either Na^+0, Na^+18, or Na^+30) or no fluid during recovery to maintain the desired % ΔBM. Urine samples were collected at the beginning (as needed) and at the end of the 50-min recovery period. During exercise and recovery, fans were placed around the subject to promote evaporation of sweat and minimize the amount of sweat trapped in their clothing and shoes.

Sweat collection. Sterile sweat patches (PharmChem, Inc.) were placed on the forehead, forearm, scapula, upper chest, and anterior thigh of subjects during the 2nd rest period. The subjects' skin was cleaned with an alcohol swab and dried before the sweat patches were applied. The patches were removed when an adequate sample was obtained. The patches were then placed in air-tight plastic tubes (Sarstedt Salivette, Germany) and then centrifuged at 4°C for 15 min. The sweat samples were aliquoted into cryovials and refrigerated until analysis.

Blood, urine, and sweat analysis. Venous blood samples (9 mL each) were drawn without stasis. A 2-mL aliquot was transferred into an EDTA-treated test tube and immediately analyzed for hematocrit (microhematocrit centrifugation) and hemoglobin (Hemacue Hb 201$^+$). The remaining 7-mL aliquot was transferred into a serum separator tube, allowed 30-60 min to clot, and then centrifuged at 4°C for 15 min. All of the following measurements were made in triplicate. Serum was analyzed for Na$^+$ concentration, K$^+$ concentration ($S_{[K+]}$), and osmolality (S_{osm}; freezing point depression, Advanced DigiMatic Osmometer Model 3D2). Urine samples were analyzed for volume (U_{vol}), Na$^+$ concentration ($U_{[Na+]}$), and K$^+$ concentration ($U_{[K+]}$). Sweat was analyzed for Na$^+$ concentration ($Sw_{[Na+]}$), and K$^+$ concentration ($Sw_{[K+]}$). Serum [Na$^+$] and [K$^+$] were measured using the ion specific electrode method (Diamond Diagnostics, Ciba Corning 614), while urine and sweat [Na$^+$] and [K$^+$] were measured via flame photometry (Instrumentation Laboratory Model IL943). The two methods for [Na$^+$] and [K$^+$] analysis were compared in a subset of serum samples and the coefficient of variation between methods was 0.8% and 2.5% for [Na$^+$] and [K$^+$], respectively.

Measurements. HR was measured using a Polar$^®$ monitor, and BP was measured by brachial auscultation (sphygmomanometry). RPE was assessed using the Borg scale (Borg, 1970). BM was measured to the nearest 0.05 kg using a Seca 770 scale. A CorTemp™ Disposable Temperature Sensor (COR-100) and CorTemp™ Recorder (CT-2000) were used to measure T_c.

Calculations. Mean arterial pressure (MAP) was calculated as MAP = [1/3] pulse pressure + diastolic BP. The percent change in plasma volume from baseline (ΔPV) was calculated from hematocrit and hemoglobin (Dill, 1974). Volume of sweat loss (Sw_{vol}) was calculated from ΔBM corrected for fluid consumed and urine excreted.

Total sweat Na^+ and K^+ loss were calculated from $Sw_{[Na+]}$ and $Sw_{[K+]}$ and Sw_{vol}. Total urine Na^+ and K^+ loss were calculated from $U_{[Na+]}$ and $U_{[K+]}$ and U_{vol}. The net change in total Na^+ and K^+ (ΔE) was calculated as:

$$[Na^+ + K^+ \text{ intake}] - [(Na^+ \text{ and } K^+ \text{ sweat loss}) + (Na^+ \text{ and } K^+ \text{ urine loss})].$$

Predicted post-experiment $S_{[Na+]}$ was calculated according to Kurtz and Nguyen (2003):

$$S_{[Na+]} = \{[S_{[Na+]i} + 23.8) \, TBW_i + 1.03 \, \Delta E] / (TBW_i + \Delta TBW)\} - 23.8$$

where $S_{[Na+]i}$ was initial serum Na^+ concentration, TBW_i was initial total body water (assume = 0.73 of fat free mass, Pace and Rathbun, 1945), and ΔTBW was the pre- to post-experiment change in TBW (assume = ΔBM).

Sweat $[Na^+]$ and $[K^+]$ were measured in samples collected from all 5 sites. However, the $[Na^+]$ and $[K^+]$ reported and used for all calculations in the present study were from the forearm and chest respectively, as these regional sites have been reported to be the most highly correlated with whole body sweat $[Na^+]$ and $[K^+]$ (Patterson et al., 2000).

Subjective ratings. The survey was administered immediately after the 2-h interval running period and at the end of recovery. The survey consisted of 100-point visual analog rating scales (ranging from "none" (0) to "very" or "severe" (100)), which assessed lightheadedness, windedness, stomach bloating, sloshing of stomach contents, stomach upset, side stitch/ache, total body fatigue, and muscle cramping.

Statistical analysis. Fluid and sodium balance data, physiological variables, time to exhaustion, and subjective ratings were analyzed using two-way analysis of variance (beverage vs. % ΔBM) with repeated measures. The Tukey post hoc test was performed when main effects were found. An intraclass correlation coefficient was used to test the reliability of performance between repeated trials and the reliability between predicted and measured post-experiment $S_{[Na+]}$ (Shoukri and Pause, 1999). The slope and intercept of the regression line and line of identity of the scatter plot for predicted vs. measured $S_{[Na+]}$ were compared to determine the accuracy of the Nguyen-Kurtz equation in predicting post-experiment $S_{[Na+]}$. A paired t-test was also used to determine whether there was a significant difference between predicted and measured post-experiment $S_{[Na+]}$. The significance level for all statistical tests was set to alpha = 0.05. All data are presented as means ± SE.

<center>**Results**</center>

Fluid and sodium balance. The net % ΔBM and net ΔE for each trial are presented in Table 4.2. Fluid, Na^+, K^+, and carbohydrate intake during each trial are presented in Table 4.3. As predicted, subjects consumed significantly more fluid and carbohydrate during +2% vs. 0% vs. -2% vs. -4% ΔBM, respectively. Na^+ intake was significantly higher during Na^+30 vs. Na^+18 during the +2% and 0% ΔBM trials.

Fluid, Na^+, and K^+ loss during each trial are presented in Table 4.4. There were no significant differences among beverages within each % ΔBM. U_{vol} was significantly higher during +2% vs. 0%, -2%, and -4% ΔBM trials. There were no differences in urine Na^+ loss, sweat volume, or sweat Na^+ loss among trials. No subject became hyponatremic during the trials (the lowest measured $S_{[Na+]}$ was 136 mmol/L).

Predicted vs. measured $S_{[Na+]}$. The relation between predicted and measured post-experiment $S_{[Na+]}$ are presented in Figure 4.1. When +2%, 0%, -2%, and -4% ΔBM were all included in the analysis (left panel), the slope (0.71) of the regression line was significantly different from one and the intercept (42) was significantly different from zero. Additionally, the paired t-test results showed a significant difference between predicted and measured $S_{[Na+]}$ (P = 0.00) when all trials were included in the analysis. However, when the +2% ΔBM trials were excluded from the analysis (right panel), the slope (0.84) and intercept (23) of the regression line were not significantly different from one and zero, respectively (additionally, paired t-test P = 0.42). Furthermore, the intraclass correlation coefficient between predicted and measured $S_{[Na+]}$ was 0.90. The mean difference between predicted and measured post-experiment $S_{[Na+]}$ was -0.8 ± 0.2 for all trials and -0.2 ± 0.2 when the +2% ΔBM trials were excluded.

Physiological data. Figure 4.2 presents the relation between the ΔBM and the post-experiment $S_{[Na+]}$ for each beverage. The ΔBM was significantly correlated with post-experiment $S_{[Na+]}$ for Na^+0 (r = 0.82), Na^+18 (r = 0.84), and Na^+30 (r = 0.76). The regression equations were $S_{[Na+]}$ = -1.73 ΔBM + 141, $S_{[Na+]}$ = -1.49 ΔBM + 142, and $S_{[Na+]}$ = -1.24 ΔBM + 143 for Na^+0, Na^+18, and Na^+30, respectively.

There were no differences in baseline $S_{[Na+]}$ and S_{osm} among trials. The mean $S_{[Na+]}$ and S_{osm} at baseline were 142.4 ± 0.2 and 285.5 ± 0.3, respectively. The Δ $S_{[Na+]}$, ΔS_{osm}, and % ΔPV from pre- to post-experiment are presented in Figure 4.3. During the

<div align="right">41</div>

+2% ΔBM trials, the decrease in S_{osm} was significantly smaller with Na^+18 and Na^+30 vs. Na^+0.

Table 4.5 presents T_c, HR, MAP, and RPE results at the end of 2-h interval running and recovery for each trial. There were no significant differences among trials at baseline. Pre-experiment resting T_c, HR, and MAP were 37.09 ± 0.03, 58 ± 1, and 84 ± 1, respectively. RPE was 8.9 ± 0.2 at the end of the first running bout. Significant main effects of % ΔBM at the end of exercise and recovery are shown in Table 4.5. There were no significant differences in T_c, HR, MAP, or RPE among beverages at the end of exercise or recovery.

Performance. Time to exhaustion and repeatability of the performance run are presented in Figure 4.4. There were no significant differences among beverages (i.e., no effect of beverage $[Na^+]$) so the average performance times of Na^+0, Na^+18, and Na^+30 were calculated and presented. Time to exhaustion was significantly shorter during -4% and -2% vs. 0% and +2% ΔBM trials. There was no performance difference between -2% and -4% ΔBM or between 0% and +2% ΔBM trials. The intraclass correlation coefficient between repeated trials was 0.96 and the coefficient of variation (CV) between repeated trials was $10 \pm 2\%$.

Subjective ratings. At the end of the 2-h interval running period, subjects felt significantly more lightheaded, winded, and total body fatigue during the -2% and -4% ΔBM trials vs. the +2% and 0% ΔBM trials. There was also a significant difference between the -2% and -4% ΔBM trials for ratings of lightheadedness and windedness. Additionally, subjects rated their muscle cramping higher during the -4% vs. the +2%, and 0% ΔBM trials. At the end of recovery, subjects felt significantly more lightheaded and total body fatigue during the -4% vs. the 0% and +2% ΔBM trials. After 2-h of interval running, subjects rated their lightheadedness 10 ± 4, 8 ± 3, 27 ± 5, 50 ± 8, windedness 14 ± 4, 12 ± 3, 27 ± 6, 60 ± 8, total body fatigue 23 ± 5, 25 ± 4, 38 ± 6, 53 ± 7, and muscle cramping 6 ± 2, 6 ± 3, 12 ± 3, 26 ± 5 during the +2%, 0%, -2%, and -4% ΔBM trials, respectively. At the end of recovery, subjects rated their lightheadedness 8 ± 3, 8 ± 3, 16 ± 4, 26 ± 7, and total body fatigue 27 ± 5, 26 ± 4, 32 ± 5, 42 ± 6 during the +2%, 0%, -2%, and -4% ΔBM trials, respectively.

At the end of the 2-h interval running and recovery period, subjects rated their level of stomach bloating and sloshing of stomach contents higher during the +2% ΔBM vs. the 0%, -2%, and -4%, ΔBM trials. Subjective ratings at the end of interval running were: stomach bloating 57 ± 6, 12 ± 4, 8 ± 3, 5 ± 1 and sloshing of stomach contents 33 ± 7, 11 ± 3, 7 ± 3, 4 ± 2 during the +2%, 0%, -2%, and -4% ΔBM trials, respectively. At the end of recovery, subjects rated their stomach bloating 38 ± 7, 8 ± 2, 4 ± 2, 4 ± 2 and sloshing of stomach contents 12 ± 5, 3 ± 1, 4 ± 2, 3 ± 1, during the +2%, 0%, -2%, and -4% ΔBM trials, respectively.

There were no statistically significant differences among beverages (i.e., no effect of beverage [Na^+]) within % ΔBM levels for any of the subjective ratings at the end of 2-h interval running or recovery.

Discussion

The main findings from this study were 1) the Nguyen-Kurtz equation accurately predicted the measured post-experiment $S_{[Na+]}$ during the 0%, -2%, and -4% ΔBM trials, but not the +2% ΔBM trials, 3) Na^+ consumption attenuated the decline in $S_{[Na+]}$ from pre- to post-experiment during the 0% and +2% ΔBM trials, but the differences among beverages Na^+0, Na^+18, and Na^+30 were not statistically significant, and 4) prolonged running performance was impaired when subjects incurred a 2 and 4% BM deficit due to fluid restriction.

Predicted vs. Measured $S_{[Na+]}$. The results confirm the predictions of the Nguyen-Kurtz equation (2003) when subjects drink to match sweating rate (0% ΔBM) or restrict fluid consumption and lose BM (-2% or -4%) during endurance exercise. These results support the notion that changes in $S_{[Na+]}$ can be predicted by changes in the net mass balance of fluid, Na^+, and K^+ from pre- to post-exercise. As indicated in Fig. 4.2 and 4.3, the pre- to post-exercise $\Delta S_{[Na+]}$ in the present study was most sensitive to the ΔBM (i.e., fluid balance). As predicted, drinking any of the fluids in the current study at a rate greater than sweating rate (+2% ΔBM trials) leads to dilution of $S_{[Na+]}$ and restricting fluid intake (a decrease in BM) leads to an increase in $S_{[Na+]}$. Moreover, Na^+

consumption influences the relation between ΔBM and the $\Delta S_{[Na+]}$. As indicated by the slopes of the regression lines of Na^+0, Na^+18, and Na^+30 in Fig. 4.2, Na^+ consumption (i.e., 0% and +2% ΔBM trials) attenuated the decline in $S_{[Na+]}$, and the higher the $[Na^+]$ in the beverage, the greater the attenuation. Fig. 4.2 also shows that beverage $[Na^+]$ has no effect on $S_{[Na+]}$ when subjects lose BM; however, it is important to note that little or no fluid was consumed during the -2% and -4% BM trials.

Because the accuracy of the Nguyen-Kurtz equation was confirmed in the present study, it is logical to conclude that the assumption made in calculating predicted $S_{[Na+]}$, i.e., that $\Delta TBW = \Delta BM$, is a valid one. However, Noakes et al. (2005) contend that, due to 0.7 kg loss from fuel oxidation, 0.4 kg gain from metabolic water production, and 1.5 kg gain from water released with glycogen utilization, 70-kg endurance athletes can lose $\geq 3\%$ of their BM over the course of a marathon without experiencing a change in TBW. If this assessment was correct, then using ΔBM as a surrogate for ΔTBW in the Nguyen-Kurtz equation would cause the measured decrease in $S_{[Na+]}$ to be larger (more negative) than that predicted for the 0%, -2% and -4% ΔBM trials. Noakes et al.'s theory was not supported in the current study, as there was no significant difference between predicted and measured $S_{[Na+]}$ during the 0%, -2% and -4% ΔBM trials. While it is possible that pre- to post-exercise ΔBM overestimates sweat losses to some extent (due to endogenous water production and/or weight loss from the oxidation of glycogen and fatty acids), it is apparently not enough to affect sodium balance. Similar to Noakes et al.'s view, Hew (2005) suggested that runners who finished a marathon in a 3 kg BM deficit were in a state of euhydration, not dehydration (deficit in body water). Hew came to this conclusion after conducting a retrospective analysis of pre- and post-race measurements of BM and $S_{[Na+]}$ in runners who participated in the Houston Marathon. In Hew's analysis, a scatter plot of ΔBM vs. $\Delta S_{[Na+]}$ illustrates that a 3 kg loss in BM corresponded to a 0 $\Delta S_{[Na+]}$ from pre-to post-race and that a 0 kg ΔBM corresponded to a 6 mmol/L decrease in $S_{[Na+]}$. Hew interpreted these data as evidence that 3 kg of endogenous water production caused the 6 mmol/L decrease in $S_{[Na+]}$ despite a 0 ΔBM. However, Hew did not consider the impact of Na^+ intake and loss on post-race $S_{[Na+]}$. In the present study, a 0% ΔBM also corresponded to a decrease in $S_{[Na+]}$ (-2 mmol/L); however, the decrease in $S_{[Na+]}$ is clearly due to a Na^+ deficit ($\Delta E = -140$ mmol/L, Table 4.2) as runners consumed

a Na^+-free beverage to replace sweat losses. It is possible that the runners in Hew's analysis also incurred a Na^+ deficit during the marathon, which would account, at least in part, for the 6 mmol/L decrease in $S_{[Na+]}$. However, it is difficult to draw any conclusions from Hew's analysis because the runners' sweating rate, sweat $[Na^+]$, Na^+ intake, and actual fluid intake were not measured.

When subjects overdrank relative to their sweat losses (+2% ΔBM trials), the Nguyen-Kurtz equation was not accurate. During the +2% ΔBM trials, the $S_{[Na+]}$ predicted by the Nguyen-Kurtz equation was significantly less than the measured $S_{[Na+]}$. The mean difference between predicted and measured $S_{[Na+]}$ was -2.6 ± 0.05 mmol/L. The subjects' renal systems' were effective in excreting excess fluid, as indicated by the significantly larger urine volumes collected during the +2% ΔBM trials (Table 4.4). Because of the high rate of urine excretion during the +2% ΔBM trials, subjects drank a substantial volume of fluid (~900 mL) during the 50-min recovery period to compensate for urine losses and maintain +2% ΔBM. It is possible that fluid absorption was not complete and a portion of the ingested fluid volume remained in the subjects' stomachs at the time of post-experiment BM measurements. Accordingly, at the end of the recovery period, subjects rated their level of stomach bloating and sloshing of stomach contents significantly higher during the +2% ΔBM vs. the 0%, -2%, and -4%, ΔBM trials. Consequently, measured $S_{[Na+]}$ was not as diluted as would be predicted by the ΔTBW in the Nguyen-Kurtz equation.

It is interesting to compare the measured $S_{[Na+]}$ results of the current study with that predicted by Montain et al. (2006), who used a mathematical model and theoretical conditions to predict $S_{[Na+]}$. In Montain et al.'s model, the environmental conditions, running speeding, body composition, and sweat $S_{[Na+]}$ were systematically varied. The scenario that is most comparable with the experimental conditions and subjects' physical characteristics of the present study is illustrated in Fig. 1 of Montain et al. (2006). In this figure, the theoretical ambient temperature was 28ºC and the athlete weighed 70 kg (63% of which was water), had a sweat $[Na^+]$ of 50 mmol/L, and was running at 10 km/h. By comparison, in the present study the ambient temperature was 30ºC and, on average, the subjects weighed 66 kg, had a sweat $[Na^+]$ of 56 mmol/L, and ran at 10.3 km/h. In Montain et al.'s theoretical example, the athlete consumed 800 mL of water per h to

maintain BM. After 2 h of running, the athlete's $S_{[Na+]}$ decreased from 140 to ~139 mmol/L. In the present study, when subjects drank 870 mL of the Na^+-free beverage to maintain BM, $S_{[Na+]}$ decreased from 141.6 to 139.6 mmol/L. Thus, the $\Delta S_{[Na+]}$ predicted by the mathematical model was similar to the $\Delta S_{[Na+]}$ measured in the current study. In Fig. 4 of Montain et al. (2006), the authors illustrated the theoretical effect of CES (17 mmol/L Na^+ and 5 mmol/L K^+) consumption on $S_{[Na+]}$ under the same conditions described previously. Montain et al.'s model demonstrated that fluid replacement with CES attenuated the dilution of $S_{[Na+]}$. However, the difference in $S_{[Na+]}$ between CES and water after 2 h of exercise was only ~1 mmol/L. Similarly, the difference between Na^+18 and Na^+0 during the 0% BM trials was 1.2 mmol/L in the present study.

Although Na^+ ingestion attenuated the decrease in $S_{[Na+]}$ during the 0% and +2% ΔBM trials (Fig. 4.2 and 4.3), the difference in the decrease in $S_{[Na+]}$ among beverages did not reach statistical significance. Two studies have been able to demonstrate a significant effect of beverage Na^+ on the $\Delta S_{[Na+]}$ during exercise. In a study by Vrijens and Rehrer (1999), the measured rate of $\Delta S_{[Na+]}$ was significantly greater (more negative) with water than with a CES containing 18 mmol/L Na^+ (-2.5 vs. -0.9 mmol/L/h) during 3 h of continuous cycling. Twerenbold et al. (2003) measured the $\Delta S_{[Na+]}$ after 4 h of running in women who consumed equal volumes of either water, CES with 410 mg/L Na^+ (~17 mmol/L Na^+), or CES with 680 mg/L Na^+ (~28 mmol/L Na^+), in separate trials. The subjects finished the trials in positive fluid balance (+2% ΔBM), but the decrease in $S_{[Na+]}$ was significantly greater when runners drank water compared to the CES with 680 mg/L (-6.2 vs. -2.5 mmol/L). The difference in the results of these two studies and the present study may, in part, be explained by the longer exercise times in the Vrijens and Rehrer (1999) and Twerenbold et al. (2003) studies (3-4 h vs. ~2 h in the present study). Their findings support the notion that Na^+ ingestion becomes even more critical as the duration of exercise increases.

During the 0% ΔBM trials, the measured $S_{[Na+]}$ was similar to that predicted by the Nguyen-Kurtz equation when subjects consumed Na^+0 (-2.0 vs. -2.7 mmol/L), Na^+18 (-0.8 vs. -1.6 mmol/L), and Na^+30 (-0.5 vs. -0.6 mmol/L). In the current study, to maintain pre-experiment $S_{[Na+]}$ while also consuming enough fluid to replace sweat losses and maintain BM, a higher [Na^+] in the CES would have been required. According to the

mass balance calculations, the subjects' average ΔE due to sweat Na^+ and K^+ losses was -70 mmol/L/h and sweating rate was 1.2 L/h. Thus, a CES with a combined $[Na^+]$ and $[K^+]$ of 58 mmol /L (i.e., 70 mmol/L divided by 1.2 L/h) would be required to maintain pre-experiment $S_{[Na+]}$, when consumed at a rate equal to sweating rate. That is, to maintain Na^+ balance, the CES should be similar in composition to that of the athlete's sweat (the mean sweat $[Na^+]$ in the current study was 56 mmol/L). This point illustrates the influence of sweat Na^+ losses on the $\Delta S_{[Na+]}$ and the importance of individualized fluid and electrolyte replacement programs for endurance athletes.

Because only four men and four women were tested in the present study, it would be difficult to make any firm conclusions regarding sex differences. However, it is important to note that there were no indications of sex-related differences in the baseline $S_{[Na+]}$, pre- to post-experiment $\Delta S_{[Na+]}$, fluid retention, nor in the relation between predicted and measured $S_{[Na+]}$. Symptomatic hyponatremia occurs more commonly in women than men (Almond et al., 2005; Backer et al., 2005; Eichner, 2002) and it is thought that the smaller BM and TBW of women may contribute to their increased risk for hyponatremia (Almond et al., 2005; Sawka et al., 2007). Because the women in the present study had a smaller TBW (as indicated by the higher adiposity) than the men one would expect the women's regression line to have a steeper (negative) slope, such that at any given % ΔBM the women would have a larger decline in $S_{[Na+]}$. However, this was not the case. In a scatter plot of the % ΔBM vs. the post-experiment $S_{[Na+]}$, the regression lines of the men's and women's data points overlapped (data not shown). Perhaps the lower sweat $[Na^+]$ in the women vs. the men (Table 4.1) compensated for their lower TBW and accounted for the a lack of sex-difference in post-experiment $\Delta S_{[Na+]}$. There is also evidence to suggest that women tend to drink more fluid per kg BM than men and women are more likely to overdrink relative to their sweat losses (Almond et al., 2005; Baker et al., 2005; Hew, 2005; Twerenbold et al., 2003). Fluid intake volumes were fixed in the current study, precluding a comparison of sex-differences in voluntary fluid intake behavior. Nonetheless, it is interesting to note that the women seemed to tolerate the +2% ΔBM trials better than the men, suggesting that the women may have been accustomed to drinking more fluid than they lose through sweating. The syndrome of inappropriate anti-diuretic hormone secretion has also been implicated in the etiology of

EAH in women. However, free water clearance was not significantly different between men (0.8 ± 0.4 mL/min) and women (0.9 ± 0.5 mL/min) during the $+2\%$ ΔBM trials. Thus, there was no indication of a sex-difference in the renal handling of excess fluid, which may be another explanation for the lack of a difference in Na^+ balance between the men and women tested in the current study.

Performance. While ΔBM clearly made a difference, there was no effect of beverage $[Na^+]$ on the time to exhaustion during the performance run in the current study (data not shown). There have been mixed results in the literature regarding the effect Na^+ intake and $S_{[Na+]}$ on endurance performance. For example, performance was not affected by plasma $[Na^+]$ or the rate of change in plasma $[Na^+]$ in female endurance athletes running for a period of 4 h in various environmental conditions (Twerenbold et al., 2003). Conversely, Vrijens and Rehrer (1999) found that a high rate of change (decrease) in plasma $[Na^+]$ was correlated with a decreased time to exhaustion during 3 h of cycling. Further, pre-exercise Na^+ loading (with 164 mmol Na^+/L beverage) is associated with improved cycling time trial performance compared to pre-exercise consumption of an equal volume of a no or low-Na^+ (10 mmol/L) beverage (Coles and Luetkemeier, 2004; Sims et al., 2007). One factor that probably contributed to the improved performance with Na^+ ingestion in the pre-exercise loading studies was the concomitant improved maintenance of PV (Coles and Luetkemeier, 2004; Sims et al., 2007). In these studies, increased performance is likely mediated by improved cardiovascular and/or thermoregulatory function conferred by the maintenance of PV (Fortney et al., 1981; Fortney et al., 1984; Nadel et al., 1980; Nadel, 1981). In the present study, there were no significant differences in the $\%$ ΔPV among beverages (Fig. 4.3), which may explain the lack of a beverage effect on endurance running performance.

A 2% and 4% BM deficit was associated with a significantly decreased time to exhaustion in the current study. The impaired performance could be attributed to the significantly higher T_c and greater cardiovascular strain (as indicated by higher HR) after the 2 h interval running period when subjects lost BM vs. when they consumed enough fluid to either maintain or increase BM by 2%. Likewise, after the 2 h interval running period, subjects rated their perceived exertion, lightheadedness, windedness, and total body fatigue significantly higher during the -2% and -4% ΔBM trials vs. the +2% and 0%

ΔBM trials. It is also possible that the performance differences among levels of % ΔBM could be caused by differences in the amount of carbohydrate consumed. Although each beverage consisted of the same carbohydrate concentration (6%), subjects consumed more total carbohydrate as total fluid volume intake increased (i.e., +2% > 0% > -2% > -4% ΔBM trials; Table 4.3). Thus, the subjects' run time to exhaustion was likely influenced by carbohydrate availability. Below et al. (1995) suggest that fluid and carbohydrate ingestion have independent and additive beneficial effects on endurance performance, which may have been the case in the present study.

It is also important to note that there was no difference in time to exhaustion, sweating rate, or T_c between 0% and +2% ΔBM trials in the present study. These results are consistent with Latzka et al. (1997) who demonstrated that hyperhydration via water or glycerol (~1.5 L increase in TBW) provided no performance or thermoregulatory advantage compared to the maintenance of euhydration during 2 h of compensable exercise-heat stress.

EAH tends to occur more commonly in running than cycling endurance events. Therefore, since the primary aim was to determine the effects of Na^+ intake and ΔBM on the development of EAH, treadmill running was the mode of endurance exercise used in the present study. To test the effects of experimental manipulations on endurance performance, one could administer either a time-to-exhaustion or time-trial exercise test. Time-trial tests are thought to be more meaningful performance tests due, in part, to the lower variability associated with time-trial vs. time-to-exhaustion protocols (Jeukendrup et al., 1996; Laursen et al., 2007). A time-trial is practical for a cycling test where the subject can simply control their pace by adjusting their rpm. However, a time-to-exhaustion test was used in the current study because it would have been difficult (and distracting) for subjects to control their own treadmill speed for a time trial. Although the CV for time to exhaustion in the current study (10%) was higher than the typical CV for time trials (2-3%, Jeukendrup et al., 1996; Laursen et al., 2007), the intraclass correlation coefficient (0.96) indicated excellent reliability (Shoukri and Pause, 1999) between repeated time-to-exhaustion tests, supporting its use as a meaningful performance test. Moreover, the 37% and 63% decrease in time to exhaustion (compared to 0% ΔBM

trials) during the -2% ΔBM and -4% ΔBM trials, respectively, far outweighed the 10% variability between repeated tests.

       Summary and Practical Recommendations. In summary, the main findings from this study were 1) the Nguyen-Kurtz equation accurately predicts post-experiment $S_{[Na+]}$ when subjects drink to match sweating rate (0% ΔBM) or restrict fluid consumption and lose BM (-2% or -4%) during endurance exercise, 2) the Nguyen-Kurtz equation does not accurately predict $S_{[Na+]}$ when athletes overdrink relative to their sweat loss and increase their BM (+2%), 3) compared to Na^+-free beverages, consumption of beverages with Na^+ attenuates the decline in $S_{[Na+]}$ from pre-to post-exercise, and 4) prolonged running performance is impaired when subjects incur a 2% and 4% BM deficit due to fluid restriction. It is clear that both the volume and $[Na^+]$ of fluid consumed during exercise has implications for sodium balance and that a $\geq$ 2% BM deficit impairs endurance performance. Therefore, the current study results suggest that the optimal hydration practice for endurance athletes is to consume fluids at a similar rate (to avoid BM gain and $\geq$ 2% BM loss) and composition to that of their sweat losses.

Table **4.1**: Subject characteristics. Values are means ± SE.
* Mean sweat sodium concentration measured during the experimental trials.
VO_{2max}, maximal oxygen consumption.

	Men (N=4)	Women (N=4)	All Subjects (N=8)
Age (yr)	28 ± 2	28 ± 3	28 ± 2
Weight (kg)	74 ± 5	58 ± 3	66 ± 4
Height (cm)	180 ± 4	169 ± 4	175 ± 4
Body Fat (%)	11 ± 2	16 ± 1	13 ± 1
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	59 ± 2	49 ± 3	54 ± 2
Training distance (km/wk)	58 ± 10	54 ± 8	56 ± 6
Sweat [Na^+] (mmol/L)*	64 ± 8	47 ± 14	56 ± 8

Table **4.2**: Beverage composition, mean net % change in body mass, and net change in E (Na$^+$ and K$^+$) for each trial. Values are means ± SE. All fluids contained 6% carbohydrate. * Only 3 of 8 subjects had sweating rates high enough to reach -4% ΔBM. † $P < 0.05$, vs. Na$^+$0 within the same % ΔBM. ‡ $P < 0.05$, vs. Na$^+$18 within the same % ΔBM. Target ΔBM, target net change in body mass from pre- to post-experiment; ΔE, net change in Na$^+$ and K$^+$ from pre- to post-experiment.

Target ΔBM	Na$^+$0 Na$^+$: 0 mmol/L K$^+$: 0 mmol/L	Na$^+$18 Na$^+$: 18 mmol/L K$^+$: 3 mmol/L	Na$^+$30 Na$^+$: 30 mmol/L K$^+$: 11 mmol/L
	Measured ΔBM (%)		
+2% ΔBM	+1.8 ± 0.2	+1.7 ± 0.1	+1.9 ± 0.1
0% ΔBM	0.0 ± 0.1	-0.1 ± 0.1	-0.2 ± 0.1
-2% ΔBM	-2.0 ± 0.1	-2.1 ± 0.1	-2.1 ± 0.1
-4% ΔBM*	-3.3 ± 0.1	-3.4 ± 0.2	-3.4 ± 0.3
	Net ΔE (mmol)		
+2% ΔBM	-139 ± 14	-65 ± 21†	+14 ± 22†‡
0% ΔBM	-140 ± 16	-98 ± 21	-54 ± 20†
-2% ΔBM	-147 ± 25	-131 ± 23	-118 ± 19
-4% ΔBM	-175 ± 19	-168 ± 28	-160 ± 27

Table **4.3**: Total fluid, sodium, potassium, and carbohydrate intake during each trial. Values are means ± SE. * P < 0.05, among each % ΔBM. † P < 0.05, vs. Na$^+$0. ‡ P < 0.05 vs. Na$^+$18.

Target ΔBM	Beverage	Fluid Volume* (L)	Sodium (mmol)	Potassium (mmol)	Carbohydrate* (g)
	Na$^+$0 (n=8)	4.2 ± 0.3	0	0	233 ± 15
+2%	Na$^+$18 (n=8)	3.9 ± 0.3	71 ± 6†	12 ± 1†	220 ± 17
	Na$^+$30 (n=8)	3.7 ± 0.3	113 ± 9†‡	41 ± 3†‡	211 ± 18
	Na$^+$0 (n=8)	2.6 ± 0.2	0	0	145 ± 10
0%	Na$^+$18 (n=8)	2.7 ± 0.2	49 ± 4†	8 ± 1†	153 ± 11
	Na$^+$30 (n=8)	2.6 ± 0.2	78 ± 5†‡	29 ± 2†‡	145 ± 9
	Na$^+$0 (n=8)	1.1 ± 0.1	0	0	62 ± 8
-2%	Na$^+$18 (n=8)	1.0 ± 0.2	18 ± 3†	3 ± 1	55 ± 9
	Na$^+$30 (n=8)	1.0 ± 0.1	28 ± 4†	11 ± 1†‡	54 ± 7
	Na$^+$0 (n=4)	0.1 ± 0.1	0	0	7 ± 2
-4%	Na$^+$18 (n=5)	0.2 ± 0.1	4 ± 1	1 ± 1	12 ± 2
	Na$^+$30 (n=5)	0.1 ± 0.1	4 ± 2	2 ± 1	8 ± 4

Table **4.4**: Total fluid, sodium, and potassium loss during each trial. Values are means ± SE. * P < 0.05, vs. 0%, -2%, and -4% ΔBM trials. † Urine volume is the total sample volume collected, including 2 scheduled voids (at the end of the 2-h interval running protocol and post-recovery) and as needed throughout the experiment.

Target ΔBM	Beverage	Urine Volume (L)†	Urine Na$^+$ loss (mmol)	Urine K$^+$ loss (mmol)	Sweat Volume (L)	Sweat Na$^+$ loss (mmol)	Sweat K$^+$ loss (mmol)
	Na$^+$0 (n=8)	0.54 ± 0.10*	6 ± 1	7 ± 1	2.6 ± 0.3	116 ± 12	15 ± 3
+2%	Na$^+$18 (n=8)	0.51 ± 0.10*	8 ± 2	11 ± 2	2.4 ± 0.2	117 ± 21	13 ± 1
	Na$^+$30 (n=8)	0.45 ± 0.08*	8 ± 3	10 ± 2	2.2 ± 0.2	112 ± 15	11 ± 1
	Na$^+$0 (n=8)	0.19 ± 0.04	6 ± 1	8 ± 2	2.4 ± 0.2	114 ± 14	12 ± 1
0%	Na$^+$18 (n=8)	0.25 ± 0.07	6 ± 2	6 ± 1	2.5 ± 0.2	131 ± 21	13 ± 1
	Na$^+$30 (n=8)	0.21 ± 0.05	5 ± 1	6 ± 1	2.6 ± 0.2	135 ± 20	13 ± 1
	Na$^+$0 (n=8)	0.12 ± 0.04	9 ± 3	6 ± 1	2.3 ± 0.2	129 ± 27	11 ± 1
-2%	Na$^+$18 (n=8)	0.09 ± 0.02	6 ± 1	8 ± 3	2.3 ± 0.2	125 ± 23	15 ± 2
	Na$^+$30 (n=8)	0.09 ± 0.02	7 ± 2	7 ± 2	2.3 ± 0.2	129 ± 20	12 ± 1
	Na$^+$0 (n=4)	0.10 ± 0.01	7 ± 2	8 ± 2	2.2 ± 0.3	150 ± 21	10 ± 1
-4%	Na$^+$18 (n=5)	0.09 ± 0.03	7 ± 2	7 ± 2	2.6 ± 0.2	140 ± 28	14 ± 1
	Na$^+$30 (n=5)	0.07 ± 0.02	4 ± 2	6 ± 2	2.5 ± 0.2	140 ± 29	13 ± 2

Table **4.5**: Physiological and RPE variables at the end of exercise and recovery for each trial. Values are means ± SE. There were no significant differences among trials at baseline. Pre-experiment resting T_c, HR, and MAP were $37.09 ± 0.03$, $58 ± 1$, and $84 ± 1$, respectively. RPE during the first running bout was $8.9 ± 0.2$. * P< 0.05, vs. 0% and +2% ΔBM. † P<0.05, vs. -2% ΔBM. ‡ P<0.05, vs. +2% ΔBM. Exercise values were obtained during the last bout of the 2-h interval running protocol. HR, heart rate; MAP, mean arterial pressure; RPE, rating of perceived exertion; T_c, body core temperature.

	Target ΔBM	Beverage	T_c (°C)	HR (bpm)	MAP (mmHg)	RPE
Exercise	+2%	Na⁺0 (n=8)	38.05 ± 0.08	152 ± 6	94 ± 2	13 ± 1
		Na⁺18 (n=8)	38.06 ± 0.14	154 ± 5	92 ± 2	13 ± 1
		Na⁺30 (n=8)	37.95 ± 0.13	156 ± 3	95 ± 1	13 ± 1
	0%	Na⁺0 (n=8)	38.28 ± 0.11	158 ± 4	94 ± 2	12 ± 1
		Na⁺18 (n=8)	38.08 ± 0.11	160 ± 3	91 ± 2	13 ± 1
		Na⁺30 (n=8)	38.23 ± 0.17	158 ± 4	92 ± 1	13 ± 1
	-2%	Na⁺0 (n=8)	38.38 ± 0.13*	165 ± 3*	92 ± 1	14 ± 1*
		Na⁺18 (n=8)	38.51 ± 0.10*	168 ± 4*	92 ± 2	15 ± 1*
		Na⁺30 (n=8)	38.48 ± 0.10*	164 ± 4*	89 ± 1	14 ± 1*
	-4%	Na⁺0 (n=4)	38.69 ± 0.04*	171 ± 4*	93 ± 1	16 ± 1*†
		Na⁺18 (n=5)	38.71 ± 0.08*	171 ± 2*	91 ± 1	16 ± 1*†
		Na⁺30 (n=5)	38.83 ± 0.05*	172 ± 1*	89 ± 1	16 ± 1*†
50 min Recovery	+2%	Na⁺0 (n=8)	37.18 ± 0.09	80 ± 5	80 ± 3	----
		Na⁺18 (n=8)	37.16 ± 0.12	81 ± 4	80 ± 2	----
		Na⁺30 (n=8)	37.24 ± 0.06	84 ± 4	80 ± 2	----
	0%	Na⁺0 (n=8)	37.20 ± 0.07	80 ± 4	78 ± 2	----
		Na⁺18 (n=8)	37.21 ± 0.10	85 ± 3	79 ± 2	----
		Na⁺30 (n=8)	37.28 ± 0.08	86 ± 3	80 ± 2	----
	-2%	Na⁺0 (n=8)	37.38 ± 0.05*	89 ± 3‡	74 ± 1*	----
		Na⁺18 (n=8)	37.43 ± 0.09*	91 ± 4‡	77 ± 2*	----
		Na⁺30 (n=8)	37.46 ± 0.07*	85 ± 3‡	75 ± 1*	----
	-4%	Na⁺0 (n=4)	37.66 ± 0.05*†	92 ± 1*	77 ± 3	----
		Na⁺18 (n=5)	37.69 ± 0.08*†	91 ± 5*	80 ± 2	----
		Na⁺30 (n=5)	37.74 ± 0.09*†	89 ± 2*	78 ± 2	----

Figure **4.1**

Figure **4.1**: Relation between predicted (according to Kurtz and Nguyen, 2003) and measured post-experiment serum sodium concentration ($S_{[Na+]}$). The *solid line* is the regression between predicted and measured $S_{[Na+]}$. The *dashed line* is the line of identity. The *left panel* includes data from all trials (*N*=86). The *right panel* includes data from the -4%, -2%, and 0% ΔBM trials only (*N*=62, excludes +2% ΔBM trials). When all trials are included in the analysis, the intercept of the regression line is significantly different from zero and the slope is significantly different from one. When the +2% ΔBM trials are excluded from analysis, the slope and intercept of the regression line are not significantly different from 1 and 0, respectively (i.e., there is agreement between predicted and measured $S_{[Na+]}$). It is likely that fluid absorption was not complete at the time of post-experiment BM measurements, thus measured $S_{[Na+]}$ was not as diluted as would be predicted by the ΔTBW in the Nguyen-Kurtz equation.

Figure **4.2**

Figure **4.2**: Relation between the change in body mass and the measured post-experiment serum sodium concentration ($S_{[Na+]}$). Each beverage is represented by a different symbol. The slope of the regression line becomes more negative (and $S_{[Na+]}$ decreases) as the beverage [Na$^+$] decreases. N = 8 for all trials except -4% ΔBM (Na$^+$0, N = 4; Na$^+$18, N = 5; Na$^+$30, N = 5).

Figure **4.3**

Figure **4.3**: Change in serum sodium concentration ($\Delta S_{[Na+]}$, *panel A*), change in serum osmolality (ΔS_{osm}, *panel B*), and % change in plasma volume (ΔPV, *panel C*) from pre- to post-experiment. Values are means ± SE. * $P < 0.05$, vs. Na^+0 within the same % ΔBM. † $P < 0.05$, among each % ΔBM. N = 8 for all trials except -4% ΔBM (Na^+0, N = 4; Na^+18, N = 5; Na^+30, N = 5).

Figure **4.4**

Figure **4.4**: Time to exhaustion during the performance run at 85% VO_{2max}. There were no differences among fluids (i.e., no effect of beverage $[Na^+]$) so the average performance times of Na^+0, Na^+18, and Na^+30 within each % ΔBM are reported. The inset shows the repeatability of the performance run (seven subjects repeated one randomly-selected trial). The *solid line* is the regression line and the *dashed line* is the line of identity. Values are means ± SE, * $P < 0.05$, vs. 0% and +2% ΔBM trials. N = 8 for all trials except -4% ΔBM (Na^+0, N = 4; Na^+18, N = 5; Na^+30, N = 5).

Chapter 5

**CHANGE IN BODY MASS ACCURATELY AND RELIABLY PREDICTS
CHANGE IN BODY WATER**

Introduction

Body water is lost as a consequence of sweating during prolonged exercise in the
heat. Replacing sweat losses with an appropriate volume of fluid during and after
exercise is critical for athletes to avoid the deleterious effects of fluid and electrolyte
imbalances. For instance, underdrinking (body mass deficit) can impair performance and
physiological and thermoregulatory function (Sawka, 1992; Sawka et al., 2007), while
overdrinking (body mass gain) can lead to dilution of blood sodium (Montain et al., 2006;
Weschler, 2005).

Sweating rates can vary from less than 0.5 to over 2.5 L per hour depending upon
exercise intensity, fitness level, and acclimation state (Sawka, 1992; Sawka et al., 2007).
Because of this considerable variation, it is important that each athlete knows their
individual sweating rate to avoid the consequences of drinking too little or too much
fluid. Measuring pre- to post-exercise body mass change (ΔBM) is often used as a simple
method to assess sweat loss and presumably hydration status. The ACSM (Sawka et al.,
2007) recommends this practice to determine athletes' individual fluid replacement
needs.

However, debate has arisen regarding the relation between BM loss and actual
fluid deficit, i.e., dehydration level. Noakes and colleagues (2005) suggest that ΔBM
overestimates sweat losses and that athletes can lose ≥ 3% of their BM during endurance
events without experiencing a net loss in body water. According to Noakes et al. (2005),
a 70-kg athlete gains 1.9 kg via endogenous water production (via metabolism and
release from glycogen) and loses 0.6 to 0.8 kg via non-sweat sources (oxidation of
glycogen and fatty acids) during a marathon. Thus, Noakes et al. (2005) proposes that a
total BM loss of ≥ 2.2 kg could occur in a 70-kg athlete without a change in TBW, i.e., in
a state of euhydration. While it is possible that ΔBM overestimates sweat losses, Sawka
et al. (2007) and Cheuvront et al. (2007) contend that the actual difference between ΔBM

and ΔTBW is minimal ($\leq$ 1%) and that ΔBM provides a reasonable estimate of sweat losses and hydration status.

The purpose of the present study was to determine the relation between the ΔBM and the ΔTBW in male and female endurance-trained athletes after prolonged running. Additionally, the validity of other commonly used markers of hydration status, including serum osmolality (S_{osm}), urine osmolality (U_{osm}), and urine specific gravity (U_{sg}) were assessed by comparing them with ΔTBW.

Materials and Methods

Subjects. Eight endurance-trained runners (4 men, 4 women; 22-36 yr) volunteered to participate in this study. Subjects were informed of the experimental procedures and associated risks before providing written informed consent. This study was approved by the Institutional Review Board for the Protection of Human Subjects of the Pennsylvania State University. Preliminary screening included a graded-exercise test on a treadmill to determine maximal oxygen uptake (VO_{2max}) and a physical exam. Criteria for inclusion were $VO_{2max} \geq 50$ mL·kg^{-1}·min^{-1} for men and ≥ 45 mL·kg^{-1}·min^{-1} for women, running ≥ 20 miles per week, and not currently taking medications or oral supplements that could interfere with study results. All women were euhmenorrheic with regular cycles (natural, $N = 2$; oral contraceptive users, $N = 2$) and were tested during the follicular phase of their cycle (within 7 days after the onset of menstruation). Subject characteristics are presented in Table 5.1.

Pre-experiment control. Subjects were instructed to abstain from heavy exercise, alcohol, and caffeine the 24 h before each trial. Diet logs were kept by the subjects to facilitate consistent food and fluid consumption for 24 h before each trial. Subjects reported to the laboratory at 0700 on the morning of test days after an overnight fast. Immediately upon arrival, a blood sample was obtained to confirm normal hydration status. Subjects were considered euhydrated when serum osmolality was < 290 mOsmol (Sawka et al., 2007).

Experimental procedure. Subjects drank fluid or no fluid to 1) maintain BM (0%), 2) increase BM by 2%, 3) decrease BM by 2%, or 4) decrease BM by 4% in

separate trials. Because this is a companion paper to a study conducted to determine the effect of Na^+ intake on serum Na^+ concentration (Chapter 4), subjects drank a 6% carbohydrate solution with 0, 18, or 30 mmol/L of Na^+ for each level of ΔBM. Experimental trials were assigned in random order. Data from trials that were scheduled at least three weeks apart were used in this study (to allow sufficient time for clearance of deuterium oxide dose). Data from a total of 73 trials were analyzed for this paper (6-10 trials per subject).

Subjects had an 18-gauge Teflon catheter placed in an antecubital vein, voided their bladder, and then entered an environmental chamber set at 30º C and 40% rh. Next, the subject was asked to sit quietly for 30 min before the baseline blood sample was obtained. Next, the subject's initial BM was measured to the nearest 0.05 kg using a Seca 770 scale. All BM measurements during the experiment were taken with the subject wearing lightweight running shorts, sport bra (women), thin socks, and running shoes. Next, the subject ran for seven 15-min bouts (70% VO_{2max}) separated by 2 min of rest (2 h of interval running total). Twelve min into each running bout a venous blood sample was obtained. After the 2-h interval running protocol, subjects rested for 2 min, and then were asked to run (85% VO_{2max}) until volitional fatigue.

Drink protocol. During each rest period, the subject was toweled off and then had their BM measured. During the 0% ΔBM trials, subjects drank fluid volumes during the rest periods to maintain their initial BM. During the -2% and -4% ΔBM trials, fluid was restricted until the subjects reached their target BM. If the subjects' BM fell below their target BM, they ingested enough fluid to maintain the desired % ΔBM. During the +2% ΔBM trials, subjects drank the necessary fluid volumes to gain 2% of their BM by the end of the 2 h of interval running. The 2% gain in BM was titrated over the 2-h interval running protocol so that BM gain was achieved gradually (to maximize retention and minimize gastrointestinal discomfort).

Recovery. After completing the running protocol, subjects sat quietly for a 1 h recovery period to allow fluid compartments to stabilize. The subjects' BM was measured at the beginning, 30 min, and end of the recovery period. Subjects drank fluid or no fluid during recovery to maintain the desired % ΔBM. Urine samples were collected at the beginning and at the end of recovery. During exercise and recovery, fans

were placed around the subject to promote evaporation of sweat and minimize the amount of sweat trapped in their clothing and shoes.

Sweat collection. Sweat patches (PharmChem, Inc.) were placed on the forearm, chest, back, forehead, and thigh of subjects during the 2nd rest period. The patches were removed when an adequate sample was obtained. The patches were then placed in an air-tight plastic tube (Sarstedt Salivette, Germany) and then centrifuged at 4°C for 15 min. The sweat was pooled from all 5 sites on each subject, aliquoted into a cryovial, and refrigerated until analysis.

TBW assessment. TBW was quantified using the deuterium oxide (D_2O) dilution technique. The subjects' natural background D_2O concentration was determined from the blood sample drawn at 0700 (i.e., pre-dose serum D_2O concentration). After voiding their bladder and having their BM measured, the subjects consumed a 30 gram dose (measured to the nearest 0.01 gram) of 99.9% D_2O (Cambridge Isotope Laboratories, Inc.). Next, 100 mL of distilled water was then poured into the same cup and given to the subject to ensure that all D_2O was ingested. After D_2O dosing, subjects rested for 3 h to allow for equilibration of D_2O with body fluids (Schoeller, 1996). The subjects were not allowed to consume any food or fluids and were instructed to collect all urine voided during this equilibration period.

At the end of the 3-h equilibration period, subjects were allowed to eat a dry, low-sodium snack of their choice (content and calories were consistent among trials within subjects). Then, a post-equilibration blood sample was collected to determine pre-experiment TBW. Post-experiment TBW was determined from the blood sample drawn after recovery. When calculating pre- and post-experiment TBW, corrections were made for lost dosage of D_2O in urine, blood, sweat (each fluid collected and measured directly via FTIR), and breath vapor (estimated according to Mitchell et al., 1972; Wong et al., 1988), as well as nonaqueous hydrogen exchange (Schoeller et al., 1985).

Calculations. Δ*BM (in kg)* was calculated as:

ΔBM = pre-experiment BM – (post-experiment BM – post-experiment urine mass)

Pre-experiment TBW (in kg) was calculated as:

N_{pre} = corrected dose / (post-equilibration serum [D_2O] – pre-dose serum [D_2O]);

where corrected dose = dose – (water vapor D_2O + urine D_2O);

where water vapor D_2O loss during the equilibration period was calculated according to Mitchell et al. (1972) and corrected for isotope fractionation (multiplied by 0.944) according to Wong et al. (1988); and where urine D_2O loss was measured from the pooled urine sample collected during the equilibration period.

Then, N_{pre} was corrected for nonaqueous hydrogen exchange according to Schoeller et al. (1985):

$$TBW_{pre} = N_{pre} / 1.041$$

Post-experiment TBW (in kg) was calculated as:

N_{post} = corrected dose / (post-experiment serum $[D_2O]$ – pre-dose serum $[D_2O]$);

where corrected dose = dose – (water vapor D_2O + urine D_2O + blood D_2O + sweat D_2O);

where water vapor D_2O loss during the experiment protocol was calculated according to Mitchell et al. (1972) and corrected for isotope fractionation (multiplied by 0.944) according to Wong et al. (1988); where urine and blood D_2O loss was measured from the urine and blood samples collected during the experiment; and where sweat D_2O loss was measured from the pooled sweat sample collected during the experiment and total sweat loss, which was calculated as:

Total sweat loss = BM loss – (evaporative water loss + blood loss);

where BM loss was the total BM loss measured during rest periods; where evaporative water loss was calculated according to Mitchell et al. (1972); and where blood loss was the total volume of blood samples collected for analyses (blood volume was converted to mass using a blood specific gravity of 1.0506 (Trudnowski and Rico, 1974)).

Then, N_{post} was corrected for nonaqueous hydrogen exchange according to Schoeller et al. (1985):

$$TBW_{post} = N_{post} / 1.041$$

Finally, *ΔTBW (in kg)* was calculated as:

$$\Delta TBW = TBW_{post} - TBW_{pre}$$

D₂O analysis. D_2O was extracted from serum, urine, and sweat samples according to an equilibration procedure developed and validated by Davis et al. (1987). First, 1.5 mL of sample was placed into the center well and 1.5 mL of distilled water into the surrounding moat of a covered Conway diffusion dish (Bel-Art Products; Pequannock, NJ). Then, an airtight top was placed on the diffusion dish and secured by parafilm and tape. Diffusion of H_2O and D_2O occurred through the vapor phase during incubation of the samples for 48 h at 37 ºC. The dishes were then cooled to room temperature and the fluid in the outer moat (1.5 mL of the original water phase) was aliquoted into an airtight vial with minimal dead space for subsequent analysis. The D_2O concentration in the extracted samples was doubled to calculate the amount contained in the original sample before the equilibration procedure. Periodically, standards with a known D_2O concentration were equilibrated and analyzed to determine the D_2O recovery rate. The D_2O concentration of the extracted sample was 48-52% of the original sample, confirming that the equilibration process was complete.

The D_2O concentration in serum, urine, and sweat was measured in duplicate using a PC-controlled Fourier Transform Infrared Spectrometer (Bruker IFS 66/s, OPUS 6.0) equipped with an MCT-A detector and a sealed liquid cell (100 μm path length, CaF_2 windows, Specac Model #20502, Woodstock, GA). Temperature regulation of the liquid cell was accomplished using a water heating jacket (Specac Model #20710) coupled to a water bath (Neslab RTE-111 with Digital Plus controller). Cell temperature was monitored using a T-type thermocouple placed in contact with the cell window. Sample introduction and evacuation was accomplished via custom fitted Luer lock terminated Teflon tubing (Hamilton #90619, Reno, NV). This technique permitted rapid sample changes without removal of the cell and as such eliminated errors associated with cell position. A 4mm internal aperture was used to minimize MCT nonlinearities associated with a high photon flux.

The following acquisition and processing parameters were used: 1000 scans, 32 cm⁻¹ resolution, Norton-Beer medium apodization function, and a zerofilling factor of 16. A cell temperature of 25.0 ± 0.10 º C was selected to be near the laboratory ambient temperature for optimal control and to facilitate analysis of room temperature samples. Temperature equilibration of the cell started 20 minutes before measurements. The cell

was rinsed (3 mL distilled water), purged, and allowed time for temperature equilibration before injection of the next sample. A temperature-controlled cell was used because minor variations in cell temperature produce variations in absorbance (Byers, 1979; Lukaski and Johnson, 1985).

Quantification of the D-O stretching region was done by applying a linear baseline function from 2715-2400 cm^{-1} and then integrating from the peak maximum to 2715 cm^{-1} (OPUS 6.0, H-Type Method). This method was used to avoid integration errors associated with a variation in ambient CO_2 levels (peak at 2350 cm^{-1}).

D_2O concentrations were calculated from a linear standard curve. Standards were prepared on the morning of each day of analysis by gravimetric dilution of known quantities of D_2O (99.9%, Cambridge Isotope Laboratories, Inc.) in distilled water and ranged from 50 to 700 ppm. During each day of analysis the D_2O standard solutions were analyzed twice at random intervals to monitor and confirm the integrity of the system. Standards were periodically sent out for isotope ratio mass spectrometry analysis (Isotech Laboratories, Inc.) to confirm D_2O concentration. Distilled water was also used as the spectral reference for all measurements. The coefficient of variation (CV) for duplicate measurements in the same assay of sample aliquots was 0.5% (the CV was the same when a subset of samples were run in triplicate).

Blood and urine analysis. Venous blood samples (9 mL each) were drawn without stasis. A 2-mL aliquot was transferred into an EDTA-treated test tube and immediately analyzed for hematocrit (microhematocrit centrifugation) and hemoglobin (Hemacue Hb 201[+]) in triplicate. The remaining 7-mL aliquot was transferred into a serum separator tube, allowed 30-60 min to clot, and then centrifuged at 4°C for 15 min. The percent change in plasma volume from baseline (ΔPV) was calculated from hematocrit and hemoglobin (Dill and Costil, 1974). Serum and urine were analyzed for osmolality (freezing point depression, Advanced DigiMatic Osmometer Model 3D2) in triplicate. Urine samples were also analyzed for specific gravity (Refractometer, Atago A300CL).

Statistical analysis. A Student's unpaired t-test was used to determine significant differences between sexes in the subjects' physical characteristics. An intraclass correlation coefficient was used to determine the reliability of ΔBM as a predictor of

ΔTBW (as described in ref. Shoukri and Pause, 2005). A Bland-Altman plot (Bland and Altman, 1986) was used to assess the agreement between ΔBM and ΔTBW. The slope and intercept of the regression line and line of identity of the scatter plot for ΔBM vs. ΔTBW were compared to determine the accuracy of ΔBM as a predictor of ΔTBW. A paired t-test was also used to determine whether there was a significant difference between ΔBM and ΔTBW. Regression analyses and Pearson correlations were used to describe the relations between ΔTBW and each of the predictors of ΔTBW (i.e., ΔBM, $S_{osm,}$ U_{osm}, and U_{sg}). A one-way analysis of variance with repeated measures was used to determine significant differences in the pre- to post-experiment % ΔPV among 0%, +2%, -2%, and -4% ΔBM. The significance level for all statistical tests were set to alpha = 0.05. All data are presented as means ± SD.

Results

Of the baseline subject characteristics (Table 1), men and women differed (P < 0.05) only in VO_{2max} and pre-experiment TBW. There were no statistically significant sex differences in the relation between ΔBM and ΔTBW or the relations among S_{osm}, U_{osm}, and U_{sg} vs. ΔTBW. Therefore, all data reported in Figures 5.1-5.3 and Table 5.2 include the results for men and women combined.

The Bland-Altman plot for comparing the two methods of measuring sweat loss (ΔBM vs. ΔTBW) is shown in Figure 5.1. The average difference between ΔBM and ΔTBW was 0.09 ± 1.09 kg. Seventy out of 73 data points were within ±2 SD of the mean difference between the methods. There was no significant correlation between the mean of ΔBM and ΔTBW vs. the difference between ΔBM and ΔTBW (r = -0.22, P = 0.07).

Figure 5.2 presents ΔBM plotted against ΔTBW. The slope (0.9233) and intercept (0.0991) of the relation between ΔBM and ΔTBW were not significantly different from 1 and 0, respectively. The paired t-test results showed no significant difference between ΔBM and ΔTBW (P=0.58). The intraclass correlation coefficient between ΔBM and ΔTBW was 0.76.

Figure 5.3 presents post-experiment S_{osm} (Fig. 5.3A), U_{osm} (Fig. 5.3B), and U_{sg} (Fig. 5.3C) plotted against ΔTBW. The correlations between ΔTBW and post-experiment

S_{osm} (r = 0.61), U_{osm} (r = 0.60), and U_{sg} (r = 0.58) were each statistically significant. Table 2 describes the predicted changes in BM, S_{osm}, U_{osm}, and U_{sg} at 0%, +3%, -3%, and -6% ΔTBW.

The ΔPV from pre-to post experiment was +4.7 ± 3.6%, +2.8 ± 3.3, -3.3 ± 3.0, and -7.5 ± 3.1% in the +2%, 0%, -2%, and -4% ΔBM trials, respectively. There was a statistically significant difference in ΔPV among all trials except 0% vs. +2% ΔBM.

There were no statistically significant effects of beverage Na^+ concentration on ΔPV, the relation between ΔBM and ΔTBW, or the relations among S_{osm}, U_{osm}, and U_{sg} vs. ΔTBW.

The run times to volitional fatigue were 26 ± 17, 22 ± 13, 14 ± 8, and 8 ± 7 min during the +2%, 0%, -2%, -4% ΔBM trials, respectively. Because performance was not the research question of interest in this manuscript these results will not be mentioned any further in this paper; however, they are discussed in further detail elsewhere (Chapter 4).

Discussion

The major findings from this study were: 1) pre- to post-exercise ΔBM is an accurate and reliable method to assess the ΔTBW in endurance-trained men and women after prolonged running in the heat and 2) ΔBM is a better method to predict ΔTBW than S_{osm}, U_{osm}, and U_{sg}.

ΔBM vs. ΔTBW. The ACSM recommends that athletes estimate their sweating rates by measuring their BM before and after exercise (Sawka et al., 2007) because ΔBM provides a reasonably accurate estimate (within 1%) of the acute ΔTBW during exercise (Cheuvront et al., 2007; Sawka et al., 2007). However, Noakes and colleagues (2005) suggest that a 70-kg athlete can lose ≥ 2.2 kg (≥ 3% of BM) during endurance events without experiencing a net loss in body water. Noakes et al. (2005) contend that this calculation is based on evidence that not all of the weight lost during exercise is due to fluid loss that needs to be replaced to maintain pre-exercise TBW. According to Noakes et al. (2005), during a 42-km marathon, a 70-kg athlete will lose approximately 0.6 to 0.8 kg from fuel oxidation. In addition, the athlete will gain 0.4 kg via metabolic water production and 1.5 kg via release of water as glycogen is utilized for fuel. Further,

Noakes et al. (2005) assume that this water bound to glycogen represents a store of water that can be lost as sweat but which does not contribute to a reduction in the exchangeable TBW pool. Thus, according to Noakes et al. (2005), a total BM loss of $\geq$ 2.2 kg ($\geq$ 3% of BM) could occur in a 70-kg athlete without a ΔTBW.

This debate centers on whether endogenous water production plays a significant role in the overall ΔTBW during prolonged exercise. Sources of endogenous water gain include the production of metabolic water via cellular metabolism and the release of water from glycogen, as this substrate is utilized during exercise. However, metabolic water production (~0.13 g/kcal) is offset by respiratory water loss (~0.12 g/kcal) during exercise (Cheuvront et al., 2007,Consolazio et al., 1963; Mitchell et al., 1972), resulting in water turnover with no net ΔTBW (via these factors). Studies attempting to determine the amount of water stored with glycogen have been inconclusive, so it is unknown exactly how much water is released as a result of glycogen utilization during prolonged exercise (Sherman et al., 1982). Further, there is no reason to suspect that the water stored with glycogen is not already part of the TBW pool (Cheuvront et al., 2007). Loss of BM due to oxidation of muscle and liver glycogen and fatty acids stored in adipocytes does represent non-sweat sources of BM loss during exercise, i.e., would cause a decrease in BM without effecting TBW. However, even according to Noakes et al.'s calculations, this would only amount to about 0.7 kg in a 70-kg athlete (1% of BM) over the course of a 42-km marathon. Thus, the pre- to post-exercise ΔBM would be expected to be within 1% of ΔTBW.

The results of the present study suggest that the pre- to post-exercise ΔBM is an accurate and reliable predictor of an acute ΔTBW during prolonged exercise in the heat. The slope and intercept of the relation between the ΔBM and the ΔTBW (Fig. 5.2) were not significantly different from 1 and 0, respectively, indicating no difference between methods. Further, the intraclass correlation coefficient (0.76) indicates that there is excellent reliability (Shoukri and Pause, 1999) between the ΔBM and the ΔTBW.

Despite the comparability of the average changes in BM and TBW (-0.49 vs. -0.35 kg), it is important to consider the potential sources of variability that may account for the $\pm$ 1.1 kg standard deviation between ΔBM and ΔTBW. It is possible that some of the difference between these methods is caused by endogenous water production and/or

non-sweat sources of weight loss (from the oxidation of glycogen and fatty acids). However, these factors would cause ΔBM to overestimate sweat losses, i.e., the decrease in BM would be consistently and significantly greater than the decrease in TBW. This systematic overestimation was not observed in the present study. The data points were evenly distributed within $\pm$ 2 SD (Fig. 5.1) and the average difference between ΔBM and ΔTBW was 0.09 kg, suggesting only a slight tendency for ΔBM to overestimate ΔTBW. Moreover, it is probable that most of the overall $\pm$ 1.1 kg standard deviation between methods can be simply accounted for by the variability associated with the D_2O measurement. While the CV for each individual D_2O measurement was low (0.5%), the CV for the overall calculation of ΔTBW would be higher (i.e. there would be an additive effect). Considering all of the steps involved in the TBW calculations, the overall CV is approximately 2.5% which would be equivalent to approximately $\pm$ 1 kg of body water (in an individual with 42 kg of TBW).

The current study does not support the view that athletes who lose $\geq$ 3% of BM after endurance exercise are actually in a state of euhydration (i.e., 0% ΔTBW). Another line of evidence in opposition to Noakes et al.'s view (2005) is that an acute decrease in BM is associated with impaired physiological function and endurance performance. Noakes et al.'s conclusions (2005) contradict the preponderance of hydration-related literature which suggests that as little as a 2-3% acute decrease in BM is associated with impaired cardiovascular and thermoregulatory function (Sawka, 1992; Sawka et al., 2007). Several studies have shown that there is a significantly greater increase in heart rate and body core temperature when athletes lose 3% BM compared to 0% ΔBM during exercise (Baker et al., 2007; Montain and Coyle, 1992; Sawka, 1992; Sawka et al., 1985). The driving factor of impaired physiological function is likely a decrease in plasma volume (hypovolemia) associated with a 3% decrease in BM (Baker et al., 2007, Montain and Coyle, 1992, Sawka et al., 1985). When athletes fail to replace sweat losses during exercise the consequence is hypovolemia, which mediates a decline in venous return, cardiac filling pressure, stroke volume, cardiac output, and a compensatory augmentation in heart rate compared to 0% ΔBM (Montain and Coyle, 1992; Nadel, 1981; Nadel et al., 1980). Additionally, hypovolemia and the concomitant increase in plasma osmolality leads to impairment of heat-dissipating mechanisms (i.e., skin blood flow and sweating

rate), which accelerates the rise in body core temperature above that of 0% ΔBM (Fortney et al., 1981; Fortney et al., 1984; Nadel et al., 1980; Sawka, 1992). Further, an impairment of cardiovascular and thermoregulatory function contributes to an increased perceived effort and a decline in endurance performance when athletes lose 3% of BM during exercise in temperature and warm environments (Montain and Coyle, 1992; Sawka, 1992; Sawka et al., 2007). Clearly, a body water deficit is mediating the hypovolemia and the associated decrement in physiological function and performance that athletes experience when they lose ≥ 3% of their BM. Accordingly, in the current study, the ΔPV from pre-experiment to post-recovery was -7.5 ± 3.1% when subjects lost 3.4% of their BM during the trials.

   Biological Markers of ΔTBW. The isotope dilution technique is considered the most accurate method to measure ΔTBW (Armstrong, 2007; Schoeller, 1996). However, the expensive and time-consuming nature of this technique limits isotope dilution as a practical means to assess ΔTBW in most field and laboratory situations. Given the importance of fluid balance in exercise performance and health, athletes need a practical, yet accurate and reliable method to assess their hydration status in the field. In addition to ΔBM, other techniques often used to assess hydration status include S_{osm}, U_{osm}, and U_{sg} (Sawka et al., 2007). Table 5.2 presents the regression and correlation analyses of each predictor vs. ΔTBW. ΔBM is most highly correlated with ΔTBW (0.76, $P < 0.05$), and thus is the best predictor of the change in hydration status during exercise. Although the correlations are not as high as with ΔBM, post-experiment S_{osm} (0.61), U_{osm} (0.60), and U_{sg} (0.58) are also significantly correlated with ΔTBW.

   Table 5.2 describes the predicted changes in BM, S_{osm}, U_{osm}, and U_{sg} at various levels of % ΔTBW. The regression analyses predict that for a 66 kg athlete with 42 kg of TBW (mean values for subjects in the present study), a 2% decrease in BM (or 3% decrease in TBW) from pre- to post-exercise would have a S_{osm} of 291 mOsmol/kg, a U_{osm} of 696 mOsmol/kg, and a U_{sg} of 1.022. These values are consistent with the indices of hydration status provided in the ACSM position stand on exercise and fluid replacement (Sawka et al., 2007); that is, $S_{osm} ≥ 290$ mOsmol/kg, $U_{osm} ≥ 700$ mOsmol/kg, and $U_{sg} ≥ 1.020$ are indicative of dehydration. These biomarkers can also be useful for detecting when an athlete is overdrinking relative to their sweat losses. According to

Table 5.2, the indicators of a 2% gain in BM due to overdrinking include a S_{osm} of 275 mOsmol/kg, a U_{osm} of 96 mOsmol/kg, and a U_{sg} of 1.002.

Summary and Practical Recommendations. In summary, measuring the pre- to post-exercise ΔBM in male and female endurance athletes is an accurate and reliable method to assess the ΔTBW after prolonged running in the heat. Athletes can use pre- to post-exercise ΔBM to obtain a reasonable estimate of their sweat loss and their hydration status. Therefore, this practice can confidently be used to assess athletes' fluid replacement needs during and after exercise. While measures of serum and urine concentration (e.g., S_{osm}, U_{sg} and U_{osm}) are not as highly correlated with ΔTBW as ΔBM, they are still effective and can be used to gauge hydration status when pre- and post-BM measurements are not available.

Table **5.1**: Subject characteristics. Values are mean ± SD. Percent body fat calculated from baseline TBW. TBW, total body water at baseline; VO_{2max}, maximal oxygen consumption. * P < 0.05, men vs. women.

	Men (*N*=4)	Women (*N*=4)	All Subjects (*N*=8)
Age (yr)	28 ± 4	28 ± 6	28 ± 5
Weight (kg)	74 ± 10	58 ± 6	66 ± 11
Height (cm)	180 ± 9	169 ± 8	175 ± 10
TBW (kg)	48 ± 2*	36 ± 2	42 ± 2
Body Fat (%)	11 ± 3	16 ± 3	13 ± 5
VO_{2max} (ml·kg^{-1}·min^{-1})	59 ± 3*	49 ± 4	54 ± 6
Training distance (mi/wk)	36 ± 12	34 ± 10	35 ± 11

Table **5.2**: Relations between ΔTBW and predictors of hydration status. Baseline values represent mean ± SD of subjects' S_{osm}, U_{osm}, and U_{sg} immediately before the 2 h interval running protocol. The subjects' mean baseline BM was 66 ± 11 kg and TBW was 42 ± 2 kg. The best predictor of ΔTBW was ΔBM, followed by post-experiment S_{osm}, U_{osm}, and U_{sg}. BM, body mass; TBW, total body water; S_{osm}, serum osmolality; U_{osm}, urine osmolality; U_{sg}, urine specific gravity.

Predictors	Regression Equation	r	Baseline	Maintain TBW	Decrease TBW by 3%	Decrease TBW by 6%	Increase TBW by 3%
ΔBM (%)	ΔTBW = 0.9233 BM + 0.991	0.76	--------	0	-2	-4	+2
S_{osm} (mosmol/kg)	ΔTBW = -0.1579 S_{osm} + 44.8	0.61	285 ± 2	283	291	299	275
U_{osm} (mosmol/kg)	ΔTBW = -0.0042 U_{osm} + 1.66	0.60	452 ± 207	396	696	996	96
U_{sg}	ΔTBW = -130 U_{sg} + 131	0.58	1.011 ± 0.005	1.012	1.022	1.032	1.002

Figure **5.1**

Figure **5.1**: Bland-Altman plot for ΔBM vs. ΔTBW. The *solid horizontal line* is the mean difference between methods (0.09 kg). The *dashed lines* indicate ± 2 SD (± 2.2 kg) from the mean difference. Seventy out of 73 data points are within ± 2 SD. The *solid diagonal line* is the regression between the mean and the difference of the two methods (r = -0.22, P = 0.07).

Figure **5.2**

Figure **5.2**: Scatter plot for ΔBM vs. ΔTBW. The *solid line* is the regression between the two methods. The *dashed line* is the line of identity. The intercept and slope of the regression line are not significantly different from 0 and 1, respectively. The intraclass correlation coefficient is 0.76, which is indicative of excellent reliability between ΔBM and ΔTBW. Paired t-test, P = 0.58.

Figure **5.3**

A

y = -0.1579x + 44.8

r = 0.61

Post-Experiment Urine Specific Gravity

B

y = -0.0042x + 1.66

r = 0.60

Post-Experiment Urine Osmolality (mosmol/kg)

C

y = -130x + 131

r = 0.58

Post-Experiment Serum Osmolality (mosmol/kg)

Figure **5.3**: Scatter plots for post-experiment serum osmolality (S_{osm}, *panel A*), urine osmolality (U_{osm}, *panel B*), and urine specific gravity (U_{sg}, *panel C*) vs. ΔTBW. Regressions between methods are indicated by solid lines in each panel. The correlations between post-experiment S_{osm}, U_{osm}, and U_{sg} vs. ΔTBW were each statistically significant.

Chapter 6

PROGRESSIVE DEHYDRATION CAUSES A PROGRESSIVE DECLINE IN
BASKETBALL SKILL PERFORMANCE

Introduction

Recent field research with players of the National Basketball Association (NBA) indicates that inadequate hydration practices are common in this group of athletes. In 2004-2005, Osterberg et al. (2004-5) showed that NBA players were inadequately hydrated prior to and during NBA pre-season practices and NBA summer league games. Specific gravity measurements of urine samples collected from NBA players prior to competition indicated that approximately half of the players were $\geq 1\%$ hypohydrated (Casa et al., 2000) before the practice or game commenced. During competition, only about 40% of sweat losses were replaced; thus, players accrued an additional 1-3% dehydration (DEH) throughout the course of practices and games (2004-5). These field study results suggest that a combination of inadequate pre-game and in-game hydration practices can lead to up to 4% DEH by the end of competition. Considering that the outcome of most basketball games is decided in the final minutes of play, the effect of 1-4% DEH on basketball performance merits study in a more controlled setting.

DEH has been implicated in impaired performance in many prolonged aerobic exercise tasks (Armstrong et al., 1985; Cheuvront et al., 2005; McConell et al., 1997; Sawka and Pandolf., 1990). For example, compared with euhydration (EUH, enough fluid consumed to maintain initial body mass), cycling time to exhaustion was significantly shorter (McConell et al., 1997) and total work completed in a 30-min cycling time trial was significantly less (Cheuvront et al., 2005) when subjects were 3.2% and 3.0% DEH, respectively. In addition, compared with EUH, running velocity decreased by 6-7% during 5,000 m and 10,000 m outdoor track races when runners were 1.6% and 2.1% DEH, respectively (Armstrong et al., 1985).

The impact of DEH on performance of various short-duration, high-intensity activities has also been tested previously. University and semi-professional soccer players' performance in a soccer skill test following intermittent high-intensity shuttle

running was significantly impaired during 2.4% DEH trials compared to *ad libitum* fluid intake (which led to 1.4% DEH) trials (McGregor et al., 1999). Conversely, compared to a control condition (0.6-0.7% DEH), 2.2 and 2.5% DEH were not detrimental to competitive sprint (50 m, 200 m, and 400 m) or power (vertical jump) performance in high school and collegiate track athletes (Watson et al., 2005).

The game of basketball is characterized by intermittent bouts of high-intensity activity repeated over a prolonged period of time and requires the execution of complex sport-specific skills. Only two studies have tested the effects of DEH on basketball-specific skills. Dougherty et al. (2006) showed that shooting percentage and on-court sprinting and lateral movement times within a simulated game context were significantly impaired by prior 2% DEH relative to placebo EUH in 12-15 yr old male basketball players. Conversely, Hoffman et al. (1995) found no difference in basketball shooting performance in 17 yr old boys playing a simulated 2-on-2 full-court basketball game when no fluid was given (causing progressive 1.9% DEH) vs. EUH.

The impact of DEH on basketball performance in adult players has not been investigated. Additionally, in most previous DEH-related research, investigators have studied the impact of a single level of DEH (e.g. 2% DEH) vs. EUH on exercise/sport performance. Few investigators have tested progressive levels of DEH in a dose-response manner to determine whether a critical level of water deficit exists at which performance is impaired compared to that of EUH. Therefore, the aim of the present study was to determine the effect of 1 to 4% DEH vs. EUH on performance of basketball-specific shooting and movement drills during a simulated game in highly skilled 17-28 yr old male players. DEH levels of 1, 2, 3 and 4% were tested (on separate days) and compared to EUH control (flavored water with 0% carbohydrate and 18.0 mmol/L sodium) to determine whether there is a DEH threshold at which performance is significantly impaired. Additionally, EUH with a 6% carbohydrate-electrolyte solution (CES) was compared to EUH control to determine whether addition of carbohydrate enhances basketball performance over EUH with a carbohydrate-free solution.

Methods

Subjects. Seventeen highly skilled male basketball players (17-28 yr) volunteered to participate in this study (Table 6.1). The players' highest level of competitive basketball experience ranged from high school ($N = 9$) to college (Division III, $N = 4$; Division I, $N = 4$). Each was a first-team member and standout player (assessed by self-reported basketball game statistics) for their respective teams. Participants were informed of the experimental procedures and associated risks before providing written informed consent. This study was approved by the Institutional Review Board for the Protection of Human Subjects of The Pennsylvania State University. Preliminary screening included a resting 12-lead electrocardiogram, skinfold measurement to determine adiposity, blood analysis (CHEM-24), a graded exercise test on a treadmill to determine maximal oxygen uptake (VO_{2max}), a physical exam, and maximal vertical jump measurement.

Experimental procedure. Seventeen subjects completed six experimental trials: (1) EUH with a commercially available lemon-lime flavored CES (6% carbohydrate and 18.0 mmol/L Na), (2) EUH control (lemon-lime flavored water with 0% carbohydrate and 18.0 mmol/L sodium included to enhance palatability), (3) 1% DEH, (4) 2% DEH, (5) 3% DEH, and (6) 4% DEH. Experimental trials were scheduled at least one week apart and assigned in a randomized, counterbalanced order.

Subjects reported to the laboratory on the morning of each test day after having swallowed a disposable temperature sensor the night before and fasted overnight. Immediately upon arrival they voided and then were weighed (all body mass measurements were taken with the subject wearing shorts and heart monitor only). Next, the subject ate a low-carbohydrate standardized breakfast (550 kcal total: 50 g (36%) carbohydrate, 16 g (25%) fat, and 56 g (39%) protein) and drank 5 mL of water per kg body weight. After breakfast the subject had an 18-gauge Teflon catheter placed in an antecubital vein in one arm. After emptying his bladder, the subject entered an environmental chamber set at 40°C and 20% relative humidity. Next, the subject was weighed (initial body mass) and then asked to stand quietly on a treadmill for 10 min before the baseline blood sample, heart rate (HR), blood pressure (BP), and core temperature (T_c) were obtained. Next, the subject walked (in shorts, socks, shoes, and

heart rate monitor only) for nine 15-min bouts (50% VO2max) separated by 5 min of rest. Ten min into each walking bout a BS, T_c, HR, BP, and rating of perceived exertion (RPE) were obtained. At the end of the 3 h interval walking protocol, the subject exited the chamber and was asked to complete the Fatigue Survey, a visual-analog rating scale with questions pertaining to physical well-being (described later) and then empty his bladder.

EUH/DEH protocol. Subjects were weighed during each rest period to determine periodic sweat loss. During the EUH trials, subjects drank enough CES or flavored water with sodium during rest periods to fully replace sweat and urine losses and maintain their initial body mass. During the DEH trials, fluid was restricted until the subjects reached their target body mass (i.e., body mass that corresponds with the desired % DEH). If the subjects' body mass fell below their target body mass, they ingested distilled water to maintain the desired % DEH.

Recovery period. After the catheter was removed from the subject's arm, he sat in a thermoneutral room (ambient temperature = 23°C) for a 70-min recovery period. During this time, body mass, T_c, HR, and BP were measured at 15-min intervals. The subject drank water, CES, or flavored water with sodium as needed to maintain the desired hydration state. A urine sample was collected at the end of recovery.

Basketball drill session. Following recovery, the subject was moved to a nearby gymnasium where he completed an orchestrated sequence of continuous basketball drills designed to simulate a fast-paced basketball game. The drill session commenced 20 min after the recovery period. Basketball drills were 80 min in duration and consisted of four 15-min quarters with 5-min breaks between quarters and a 10-min break at halftime. The drill session was designed in consultation with coaches from NCAA Division I, II, and III basketball programs and incorporated most aspects of the game of basketball including: speed (sprinting drills), agility/lateral movement (defensive slide drills), explosiveness (vertical jump drills), shooting (off the dribble and off the pass), and a combination of two or more of these basketball-specific tasks. The simulated basketball game was designed to include drills that were relatively simple and routine to experienced basketball players. In addition, the subjects were familiarized with the drills prior to their first experimental trial to avoid a learning effect.

The first quarter of the simulated basketball game consisted of seven drills: 1) baseline jump shots: start at half court, sprint to cone at baseline area, receive pass from investigator standing on foul line, shoot 15 ft baseline jump shot, sprint to opposite sideline at half court, repeat (number made in 2 min); 2) layup shooting: start at elbow, dribble in, shoot a layup, then get own rebound, dribble to opposite elbow, dribble in, shoot a layup, repeat (number made in 2 min); 3) ladder suicide sprints: start at baseline, sprint to foul line, sprint back to baseline, sprint to half court, sprint back to baseline, sprint to opposite foul line, sprint back to baseline, sprint to opposite end line, sprint back to baseline, sprint to opposite foul line, sprint back to baseline, sprint to half court, sprint back to baseline, sprint to foul line, sprint back to baseline (time to completion); 4) 30 vertical jumps: subject was asked to repeatedly touch a mark set at 70% of his maximum vertical jump 30 times as quickly as possible (time to completion); 5) zigzags: defensive slides to each cone set in a zigzag pattern from baseline to baseline on one side of the basketball court, performed over four lengths of the court total (time to completion); 6) around the world shooting: continuous 15-foot shooting from seven spots (number made in 2 min); and 7) full court combination: start at corner, sprint forward to half court, defensive slides across midline, sprint forward to opposite corner, defensive slides across opposite baseline, backpedal to half court, defensive slides across midline, backpedal to baseline, defensive slides across baseline (time to completion). The second quarter consisted of seven drills: 8) foul line jump shots: start at corner, zigzag defensive slides to half court, sprint to center court, pick up basketball, dribble to foul line, shoot a foul line jump shot, sprint to opposite corner, repeat (number made in 3 min); 9) 3-point shooting: continuous 3-point shooting from seven spots (number made in 2 min); 10) 20 court-width sprints (time to completion); 11) maximum vertical jump: subject allowed one step and then must jump off two feet (best height of three attempts); 12) 30 lane slides: defensive slides across width of key (time to completion); 13) key combination: start on baseline at corner of key, sprint forward to top corner of key, diagonal defensive slides to opposite corner of key on baseline, sprint forward to top of key corner, diagonal defensive slides to opposite corner of key (time to complete five); and 14) free throw shooting (number made in 20 attempts). The drills performed in the third quarter were

the same as that of the first quarter and the drills performed in the fourth quarter were the same as that of the second quarter.

Performance measures included single and total number of stationary shots made (15-foot, 3-point, and free throw shots), single and total number of shots "on the move" (i.e., subject required to sprint, defensive slide, and/or dribble between shot attempts) made (layups, baseline jump shots, and foul line jump shots), single maximum vertical jump, single repetitive vertical jumps, single and total sprint times (ladder suicide and 20 court widths), single and total defensive slide times (zigzags and 30 lane slides), and single and total times for the sprinting-defensive slide combination drills (full court and key combinations). Additionally, a total score was calculated for all timed drills (total time to complete ladder suicide, 30 vertical jumps, zigzags, full court combination, 20 court widths, 30 lane slides, and key combination) and all shooting drills (total number of baseline jump shots, layups, 15 ft, foul line jump shots, 3-point, and free throw shots made) to represent overall skill performance over the entire course of the simulated basketball game.

The desired hydration state was maintained throughout the basketball drill session by weighing the athlete at the end of each quarter and having him drink the appropriate volume of fluid during the rest periods. The subject's T_c, HR, and RPE were obtained immediately after he completed the full court combination drill in the first and second quarters and after the key combination drill near the end (i.e. before free throw shooting) of the second and fourth quarters. At the end of the second and fourth quarters, the subject was asked to void his bladder and complete the Fatigue Survey.

Measurements. Heart rate was measured using a Polar® heart rate monitor, and blood pressure was measured by brachial auscultation (sphygmomanometry). Rating of perceived exertion was assessed using the Borg scale (Borg, 1970). Body mass was measured to the nearest 0.05 kg using a Seca 770 scale. A CorTemp™ Disposable Temperature Sensor (COR-100) and CorTemp™ Recorder (CT-2000) were used to measure T_c. The vertical jump drills were performed using a Vertec™.

Blood and urine analysis. Venous blood samples (9.5 mL each) were drawn without stasis. A 2-mL aliquot was transferred into an EDTA-treated test tube and immediately analyzed for hematocrit (microhematocrit centrifugation) and hemoglobin

(Hemacue Hb 201+) in triplicate. The remaining 7.5-mL aliquot was transferred into a serum separator tube, allowed 30-60 min to clot, and then centrifuged at 4°C for 15 min. Serum was analyzed for glucose concentration ($S_{[gluc]}$; hexokinase UV method, Olympus Model AU5200), sodium concentration ($S_{[Na+]}$; ion specific electrode method, Olympus Model AU5200), total protein concentration ($S_{[prot]}$; biuret method, Olympus Model AU5200), and osmolality (S_{osmol}; freezing point depression, Advanced DigiMatic Osmometer Model 3D2) in triplicate. Urine samples were analyzed for specific gravity (U_{sg}; Refractometer, Atago A300CL), osmolality (U_{osmol}), volume (U_{vol}), and color (U_{col}). Urine color was determined by holding each specimen container next to a validated color scale (Armstrong et al., 1994) in a well-lit room. The eight-color scale ranges from 1 (very pale yellow) to 8 (brownish green).

Calculations. Mean arterial pressure (MAP) was calculated as MAP = [1/3] pulse pressure + diastolic BP. Sweat loss (SL) was calculated from Δ body mass corrected for fluid consumed and urine excreted. The percent change in plasma volume from baseline (ΔPV) was calculated from hematocrit and hemoglobin (Dill and Costil, 1974).

Subjective ratings. The Fatigue Survey was administered to the subject after the heat chamber exercise (min 180), at halftime (min 305), and at the end of the simulated basketball game (min 350). The survey consisted of a 100-point visual analog rating scales (ranging from "none" (0) to "very" or "severe" (100)) which assessed subjective feelings of lightheadness, windedness, hotness, side stitch/ache, muscle cramping, total body fatigue, upper body fatigue, and leg fatigue.

Statistical analysis. The two distinct hypotheses tested were 1) 1 to 4% DEH will progressively impair basketball performance compared with EUH control, and 2) CES EUH will improve basketball performance measures compared with EUH control. To present the comparison of skill performance results for 1 to 4% DEH vs. EUH control and CES vs. EUH control trials, data from the EUH control trial were subtracted from 1 to 4% DEH and CES trials because the data are paired data from the same subject. Taking the difference from EUH control better reflects each hypothesis directly, removes the subject effect, and provides an effective comparison of the treatments.

To determine the DEH threshold (% DEH at which results are significantly different from EUH control) for basketball performance, subjective ratings,

physiological, and RPE variables, a repeated measures analysis of variance (ANOVA) was conducted. The data were analyzed with a linear mixed model by using PROC MIXED in SAS 9.1. The covariance structure was chosen by the Akaike information criterion (AIC). Treatment groups (hydration status) were treated as fixed effects and subjects were treated as random effects. The P values were adjusted for multiple comparisons between the treatment groups using Dunnett's post hoc test. Similarly, a linear mixed model was used to compare basketball performance, subjective ratings, physiological, and RPE variables between CES EUH and EUH control. The significance level for all statistical tests was set at alpha = 0.05. All data are presented as means ± SD, unless otherwise indicated.

Results

There were no statistically significant differences between CES EUH and EUH control in the basketball performance scores, subjective ratings, RPE, or physiological variables (except that $S_{[gluc]}$ was higher in CES trials at the end of heat chamber exercise); thus, CES EUH was excluded from the presentation for simplification. All subsequent comparisons of 1-4% DEH vs. EUH refer to EUH with the lemon-lime flavored water containing 0% carbohydrate and 18 mmol sodium.

Physiological and RPE variables. HR, T_c, MAP, RPE, sweat loss, U_{vol}, U_{col}, U_{sg}, and U_{osmol} data are presented in Table 6.2. There were no significant differences in the physiological variables among trials at baseline. At the end of the heat chamber exercise, HR was significantly and progressively higher during 1-4% DEH and T_c was significantly and progressively higher during 2-4% DEH compared to the EUH control trial. At the end of the 70-min recovery period, HR and T_c only remained significantly elevated above that of EUH control in the 4% DEH trial. At halftime (end of the second quarter) of the 4% DEH trial the subjects' T_c remained elevated above that of EUH control. During 3-4% DEH the subjects' sweat loss was significantly less than that of EUH control throughout the entire basketball "game" (end of second and fourth quarters).

Blood variables. $S_{[gluc]}$, $S_{[Na+]}$, S_{osmol}, $S_{[prot]}$, and ΔPV at baseline and the end of the heat chamber exercise are presented in Table 6.3. There were no significant

differences in the blood variables among trials at baseline. Compared to the EUH control trial, S_{osmol} and ΔPV (% decrease) were significantly and progressively higher during 1-4% DEH, $S_{[Na+]}$ was significantly higher during 2-4% DEH, and $S_{[prot]}$ was significantly higher during 3-4% DEH at the end of the heat chamber exercise.

Subjective ratings. Responses to the subjective questionnaires are presented in Figure 6.1. At the end of the heat chamber exercise (Figure 6.1A), subjects rated their feelings of lightheadedness, windedness, over-heatedness, muscle cramping, total body fatigue, upper body fatigue, and leg fatigue significantly higher during 3-4% DEH vs. EUH control. Compared to EUH control, subjects reported feeling significantly more lightheaded and having greater leg fatigue during 3-4% DEH while they felt greater upper and total body fatigue during 4% DEH at the end of the basketball "game" (Figure 6.1B).

Fluid intake. During the EUH trials subjects consumed 486 ± 189, 641 ± 123, and 501 ± 152 mL of CES or 443 ± 251, 575 ± 192, and 430 ± 191 mL of flavored water with sodium, at the end of quarters 1, 2, and 3 of the drill session, respectively. During the DEH trials, the volume of distilled water consumed at the end of each quarter (in chronological order from quarter 1 to 3) was 437 ± 171, 597 ±201, and 493 ± 163 mL for 1% DEH; 465 ± 173, 512 ± 223, and 361 ± 167 mL for 2% DEH; 315 ± 208, 408 ± 155, and 320 ± 153 for 3% DEH; and 216 ± 92, 388 ± 180, and 299 ± 173 for 4% DEH. Fluid volumes seemed to be well tolerated by the athletes in terms of gastrointestinal comfort, as their subjective ratings of side stitch or ache were relatively low, even during the EUH trials (e.g. 11 ± 15 on a 100-pt scale, at the end of drills during CES trials).

Basketball performance. Each individual basketball drill was performed twice per drill session (i.e., once per half). Within trials, there were no statistically significant differences in individual drill performance scores between half 1 and half 2; therefore, scores for each individual drill were calculated as the average of the two halves. Then, total performance scores were calculated as the sum of all individual drill performance scores within the same drill category.

There were no statistically significant differences in overall basketball performance between CES and EUH control. The total number of shots made (sum of baseline jump shots, layups, around the world shots, foul line jump shots, 3-point shots,

and free throws single performance scores) was 83 ± 3 shots for CES and 86 ± 3 shots for EUH control trials. The total time to complete basketball-specific movement drills (sum of ladder suicide, 30 vertical jumps, zigzags, full court combination, 20 court-width sprints, 30 lane slides, and key combination single performance scores) was 370 ± 13 sec for CES and 386 ± 11 sec for EUH control trials.

Figure 6.2 illustrates the single drill and total results relative to EUH control for shots "on the move". The threshold for significantly impaired performance for baseline jump shots and layups was 4% and 3% DEH, respectively. Single performance scores for foul line jump shots were not affected by any level of DEH. When the results from all three drills were combined, the total number of shots "on the move" made was significantly impaired by 3-4% DEH. Thus, the DEH threshold for significantly impairing this type of basketball shooting performance was 3% DEH.

The single drill and total sprint times relative to EUH control are presented in Figure 6.3. The threshold for impaired performance for the ladder suicide and 20 court-width sprints was 1% and 3%, respectively. When the results from the two drills were combined, the total sprint time was significantly slower during 2-4% DEH. Thus, the DEH threshold for significantly impairing on-court sprinting performance was 2% DEH.

A summary of the performance results for the remainder of the drills are presented in Table 6.4. The DEH threshold for significantly impaired performance for total defensive slide time was 3% DEH (single drills: zigzags = 3% DEH, 30 lane slides = 4% DEH). The DEH threshold for significantly impaired performance for total sprinting-defensive slide combination time was also 3% (single drills: full court combo = 3% DEH, key combo = 4% DEH). Repeated vertical jump performance was significantly impaired with 4% DEH, while maximum vertical jump was not significantly affected by any level of DEH compared to EUH control. The DEH threshold for significantly impaired performance for total stationary shooting was 4% DEH (single drills: around the world = 2% DEH, 3-point shots = not significantly affected, free throws = not significantly affected). Finally, when basketball shooting results are expressed as a percentage, (shots made/shots attempted) performance is only significantly impaired for layup shooting (threshold = 3% DEH).

The combined results for all timed drills and all shooting drills are illustrated in Figure 6.4. These scores represent overall skill performance during the entire 80-min simulated basketball game. Total time to complete all basketball-specific movement drills (sprinting, defensive slides, sprinting-defensive slide combination, and repeated vertical jumps) progressively increased and total number of shots made (stationary shots and shots "on the move") progressively decreased as DEH progressed from 1 to 4%. The threshold, or % DEH at which the performance decrement reached statistical significance, was 2% for all timed drills combined and all shots combined.

Although the basketball performance results for the DEH trials are presented as differences from the EUH control condition (Figures 6.2-6.4) to better address the specific research hypotheses, it should be noted that the same incidence of statistically significant differences between 1-4% DEH and EUH control was found whether the data were expressed in relative or absolute terms.

Discussion

The main findings from this study were: 1) 17-28 yr old skilled basketball players experienced a progressive deterioration in performance as DEH progressed from 1 to 4%, 2) the threshold, or % DEH at which the overall performance (i.e., combined scores for the entire 80-min simulated game) decrement reached statistical significance, was 2%, and 3) EUH with a CES did not enhance basketball performance over EUH with a carbohydrate-free solution, contrary to previous data in children (Dougherty et al., 2006).

In the current investigation, the DEH threshold for impaired performance of the fourteen individual drills varied from 1% DEH to not significant within the 1 to 4% DEH range tested. Compared to EUH control, an impairment in performance occurred in one drill at 1% DEH (ladder suicide sprints), one drill at 2% DEH (around the world shooting), four drills at 3% DEH (zigzag slides, court-width sprints, layup shooting, and full court combination) and four drills at 4% DEH (repeated vertical jumps, key combination, lane slides, and baseline jump shots). Performance of the remaining four individual drills (maximum vertical jump, 3-point shooting, free throws, and foul line jump shots) was not affected by 1 to 4% DEH compared to EUH control. Because

basketball is a sport characterized by intermittent bouts of sprinting, lateral movement, and jumping interspersed with the execution of complex sport-specific skills, such as dribbling and shooting the basketball, single drill scores were combined to reflect the players' overall performance during the 80-min basketball drill session. These combined scores provide a reasonable representation of how 1 to 4% DEH might affect the players' performance during an actual basketball game. When performance results were collapsed into two major categories (timed drills and shooting drills) the critical water deficit causing a decrease in basketball skill performance was 2% of initial body mass (Figure 6.4).

Only two previous studies have tested the effects of DEH on basketball-specific skills. The current data are in agreement with Dougherty et al. (2006) who found that 2% DEH significantly impaired on-court sprinting performance in 12-15 yr old boys and Hoffman et al. (1995) who found that 1.9% DEH had no detrimental effect on vertical jump height or shooting percentage in 17 yr old boys. Although, in the current study, overall shooting percentage (i.e., shooting accuracy) was not affected by DEH, significantly fewer absolute number of shots were attempted and made when subjects were ≥ 2% DEH compared to EUH control. This is most likely due to slower sprinting and lateral movement between shot attempts during the allotted time period (2 or 3 min, depending on the drill). Successful scoring in basketball is highly dependent on a player's speed and agility to create a good shot opportunity. Thus, as the current study indicates, 1-4% DEH may not impact shooting accuracy in 17-28 yr old male players, but would impair their scoring ability (i.e., total points scored) within the context of a fast-paced, competitive basketball game. Accordingly, in the current study, DEH had a greater deleterious impact on the number of shots made when players were shooting "on the move" (i.e., players were required to sprint, defensive slide, and/or dribble between shot attempts; 2% DEH: $P = 0.05$, 3% DEH: $P < 0.0001$, 4% DEH: $P < 0.0001$, vs. EUH control, Fig. 6.2) than when shooting from a stationary position (minimal movement between shot attempts; 4% DEH: $P = 0.02$, vs. EUH control, Table 6.4).

The current study supports the contention that athletic events do not have to take place in a hot environment for DEH to have a detrimental impact on exercise/sport performance. The basketball drills were conducted indoors (ambient temperature = 23-

24°); thus, the current study supports research showing that DEH impairs performance during various types of physical activity performed in thermoneutral conditions, including high-intensity intermittent running (Maxwell et al., 1999), distance track running (Armstrong et al., 1985), prolonged cycling (Cheuvront et al., 2005; McConell et al., 1997), and maximal aerobic power (Caldwell et al., 1984) in young adult athletes, anaerobic power in collegiate wrestlers (Hickner et al., 1991; Webster et al., 1990), and basketball skills in 12-15 yr old boys (Dougherty et al., 2006).

During the heat chamber exercise (controlled exercise intensity, 50% VO_{2max}), HR and T_c were significantly higher with DEH vs. EUH control and progressively increased from 1 to 4% DEH. In the current study, HR increased 7 bpm and T_c increased 0.28°C above that of EUH control for every 1% increase in DEH. Additionally, S_{osmol} increased by 3.5 mosmol/kg and PV decreased by 2.4% compared to that of EUH control for every 1% increase in DEH. These results are consistent with the extensive literature on the effects of graded DEH on physiological function during exercise in hot environments (Montain and Coyle, 1992; Sawka and Pandolf, 1990). Conversely, during the basketball "game" (self-selected pace to complete drills) HR and T_c were not significantly higher during DEH compared to EUH control trials. Thus, there are no clear physiological mechanisms to account for the DEH-related impairment of basketball performance in the present study. DEH was not associated with higher T_c and HR during the simulated basketball game most likely because subjects' decreased their exercise intensity when they were DEH; as indicated by the significantly slower sprint times (Fig. 6.3) and increased feelings of fatigue (Fig. 6.1B) associated with DEH. Accordingly, the impaired basketball performance in the present study can be partially explained by the subjective measures of physical well being; that is, increased feelings of leg fatigue and lightheadedness associated with DEH (significantly different at 3 and 4% DEH) compared to EUH control. The deleterious effect of fluid restriction on subjective feelings of fatigue and physical well being is consistent with previous investigations. For example, boy basketball players in the study by Dougherty et al. (2006) reported feeling more lightheaded and upper-body fatigue during 2% DEH compared to the placebo EUH condition. Because of the complexity and dynamic nature of the game of basketball, optimal cognitive function is necessary for successful sport performance (Carr, 2003).

DEH has been implicated in impaired performance of various cognitive tasks, including, visuomotor tracking, short-term memory, response time, coordination, and attentional vigilance (Cian et al., 2001; Gopinathan et al., 1988; Sharma et al., 1986). Thus, DEH-induced impairment of cognitive function could also account for some of the basketball performance differences between DEH and EUH control trials in the current study.

In the current study, there were no differences in basketball performance whether EUH was maintained with CES or a carbohydrate-free solution. Interestingly, in a similar study conducted in our lab with 12-15 yr old boy basketball players, performance was significantly enhanced with CES compared to placebo EUH (lemon-lime flavored water with 0% carbohydrate and 18mmol sodium) (Dougherty et al., 2006). The age-related differences in basketball skill performance with CES vs. EUH control may be attributed to cognitive function changes with carbohydrate availability. For example, glucose administration has been shown to improve attention and reaction to frustration in children (Benton et al., 1987), whereas glucose intake has no effect on variables of attention in adults (Flint and Turek, 2003). Thus, we speculate that children may be more apt than adults to experience an improvement in performance of complex tasks, such as basketball drills, when exogenous glucose is provided. The inconsistent results between the current study and that with 12-15 yr old boys may also be explained by age-related differences in substrate utilization during exercise. Timmons et al. (2003) have shown that the oxidation rate of exogenous carbohydrate during 60 min of submaximal cycling is significantly higher in early pre-pubertal boys than in men. A greater metabolic reliance on exogenous carbohydrate in boys than in men may explain why CES consumption improved basketball performance of the 12-15 yr old boys, but had no affect on the performance of the 17-28 yr old men (compared to EUH control).

Limitations. The CES consisted of lemon-lime flavoring, 6% carbohydrate, and 18.0 mmol/L sodium, while the EUH control beverage consisted of lemon-lime flavoring, 0% carbohydrate, and 18.0 mmol/L sodium. During the 1-4% DEH trials, distilled water was consumed to maintain the DEH body mass. Sodium was included in the EUH fluids to enhance palatability and provide an osmotic impetus to retain the ingested fluid in the vascular space. Because the EUH control beverage was carbohydrate-free, this drink served as an adequate control condition to compare to the effect of carbohydrate intake

(via CES) on performance. However, the EUH control drink (flavored water with sodium) was not a true control condition with which to compare 1-4% DEH since a sodium-free drink (distilled water) was consumed during the DEH trials. Due to this study design, it is possible that some of the performance benefits of EUH control over DEH could be attributed to the ingestion of sodium (and its effects on body fluid distribution) during the EUH control condition vs. no sodium intake during the 1-4% DEH trials.

The investigators and subjects were well-blinded to the EUH control vs. the CES trials because the EUH beverages were coded and were of the same color and flavor. In addition, all attempts were made to blind the subjects to their level of DEH. The volume of fluid consumed during the experiment was not obviously different among successive DEH levels ($\leq$ 150 mL difference per quarter). It is possible that the subjects could have sensed this small difference in volume consumption among DEH trials; however, it is not likely because: 1) fluid was given to the subjects in an opaque bottle, 2) at least 1 week elapsed between trials, and 3) the trials were completed in random order. While it was realistic to disguise successive DEH levels, it was difficult to completely blind subjects to EUH control vs. 1-4% DEH since distilled water was consumed during DEH trials to maintain DEH body mass (compared to the flavored drink ingested during the EUH control trials).

Summary. In summary, basketball performance measures of 17-28 yr old highly skilled male basketball players were similar when players maintained EUH with CES vs. a carbohydrate-free solution, contrary to previous data in children. Players experienced a progressive deterioration in performance, compared to EUH control, as DEH progressed from 1 to 4%. The critical level of water deficit at which overall performance (combined scores for the entire 80-min simulated game) was significantly impaired compared to EUH control was 2% DEH. Therefore, players should be advised to implement adequate pre-game and in-game hydration strategies to prevent $\geq$ 2% DEH and its detrimental impact on basketball performance.

Table **6.1**: Subject characteristics. [*] Number of years subject reported playing competitive basketball; VO$_{2max}$, maximal oxygen consumption

Men (N=17)	Means ± SD	Range
Age (yr)	21.1 ± 2.4	17-28
Height (cm)	185 ± 8.6	172-206
Weight (kg)	81.6 ± 12.1	63.6-104.5
Body Fat (%)	10.0 ± 4.0	5-19
Playing Experience (yr)[*]	9.6 ± 2.0	7-13
College	$N = 8$	
High School Starters	$N = 9$	
Max Vertical Jump (cm)	81.4 ± 8.3	59.7-99.1
VO$_{2max}$ (mL $\cdot$kg^{-1} $\cdot$min^{-1})	55.7 ± 4.9	47.0-63.0

Table **6.2**: Physiological and RPE variables. Values are mean ± SD at end of each time period. DEH, 1-4% dehydration trials; EUH control, euhydration control trials; HR, heart rate; T_c, core temperature; MAP, mean arterial pressure; RPE, rating of perceived exertion SL, sweat loss; U_{vol}, urine volume; U_{col}, urine color; U_{sg}, urine specific gravity; U_{osmol}, urine osmolality; * P < 0.05, vs. EUH C.

Table **6.2**

	HR (bpm)	T_c (°C)	MAP (mmHg)	RPE	SL (mL)	U_{vol} (mL)	U_{col}	U_{sg} (UG)	U_{osmol} (mOsmol/L)
Baseline									
EUH C	76 ± 10	36.74 ± 0.16	95 ± 4			85 ± 35	5 ± 1	1.024 ± 0.004	820 ± 210
DEH									
1%	73 ± 10	36.74 ± 0.30	93 ± 5			85 ± 26	5 ± 1	1.022 ± 0.006	774 ± 201
2%	71 ± 9	36.78 ± 0.28	93 ± 7			92 ± 27	5 ± 1	1.023 ± 0.005	795 ± 180
3%	72 ± 7	36.79 ± 0.30	94 ± 7			105 ± 67	5 ± 1	1.025 ± 0.006	826 ± 181
4%	74 ± 7	36.72 ± 0.26	93 ± 8			115 ± 75	5 ± 2	1.021 ± 0.006	741 ± 240
Chamber									
EUH C	128 ± 17	37.36 ± 0.41	97 ± 6	13 ± 2	2985 ± 809	181 ± 136	5 ± 2	1.021 ± 0.008	725 ± 244
DEH									
1%	140 ± 15*	37.65 ± 0.53	95 ± 5	13 ± 2	3237 ± 815	123 ± 51	6 ± 1	1.025 ± 0.004	845 ± 126
2%	146 ± 14*	37.84 ± 0.57*	94 ± 7	14 ± 2	2919 ± 745	162 ± 108	5 ± 1	1.025 ± 0.005	856 ± 177
3%	148 ± 19*	38.29 ± 0.50*	90 ± 7*	15 ± 2*	3014 ± 644	110 ± 50	7 ± 1*	1.026 ± 0.006	831 ± 170
4%	157 ± 16*	38.47 ± 0.33*	90 ± 6*	15 ± 2*	3200 ± 805	119 ± 60	7 ± 1*	1.024 ± 0.005	789 ± 184
Recovery									
EUH C	67 ± 9	36.85 ± 0.33	84 ± 6		382 ± 207	191 ± 152	3 ± 1	1.011 ± 0.008	344 ± 253
DEH									
1%	69 ± 13	36.81 ± 0.32	84 ± 5		379 ± 232	116 ± 64	4 ± 2	1.017 ± 0.010*	535 ± 317
2%	74 ± 13	37.00 ± 0.39	86 ± 6		295 ± 193	62 ± 40*	5 ± 1*	1.024 ± 0.005*	854 ± 104*
3%	71 ± 14	37.16 ± 0.37	80 ± 7		219 ± 139	45 ± 24*	6 ± 1*	1.029 ± 0.006*	969 ± 126*
4%	77 ± 17*	37.28 ± 0.36*	83 ± 6		249 ± 133	55 ± 42*	6 ± 1*	1.029 ± 0.005*	934 ± 104*
Quarter 2									
EUH C	184 ± 11	38.38 ± 0.44		15 ± 2	904 ± 307	115 ± 106	2 ± 1	1.010 ± 0.008	266 ± 236
DEH									
1%	187 ± 7	38.31 ± 0.57		16 ± 2	942 ± 315	92 ± 63	4 ± 2*	1.014 ± 0.010	428 ± 304
2%	188 ± 7	38.22 ± 0.42		16 ± 2	921 ± 334	56 ± 47	4 ± 2*	1.021 ± 0.009*	710 ± 302*
3%	184 ± 11	38.49 ± 0.45		16 ± 2	688 ± 97*	35 ± 31*	6 ± 1*	1.028 ± 0.006*	908 ± 104*
4%	186 ± 8	38.65 ± 0.46*		16 ± 2	580 ± 206*	24 ± 19*	5 ± 1*	1.027 ± 0.005*	878 ± 115*
Quarter 4									
EUH C	180 ± 13	38.22 ± 0.66		17 ± 2	887 ± 378	54 ± 54	5 ± 2	1.016 ± 0.008	413 ± 193
DEH									
1%	185 ± 10	38.14 ± 0.63		17 ± 2	993 ± 300	34 ± 35	6 ± 2*	1.021 ± 0.007	540 ± 184
2%	188 ± 8*	38.08 ± 0.34		17 ± 2	730 ± 239	17 ± 12	6 ± 1*	1.024 ± 0.006*	589 ± 148
3%	181 ± 13	38.47 ± 0.48		17 ± 2	679 ± 240*	17 ± 11	7 ± 1*	1.028 ± 0.005*	679 ± 129*
4%	181 ± 13	38.38 ± 0.51		17 ± 2	674 ± 321*	15 ± 12	7 ± 1*	1.027 ± 0.004*	617 ± 129

Table **6.3**: Blood variables. Values are mean ± SD at baseline and end of the heat chamber exercise. DEH, 1-4% dehydration trials; EUH control, euhydration control trial; $S_{[gluc]}$, serum glucose concentration; $S_{[Na+]}$, serum sodium concentration; S_{osmol}, serum osmolality; $S_{[prot]}$, serum protein concentration; ΔPV, % change in plasma volume; * $P < 0.05$, vs. EUH C

	$S_{[gluc]}$ (g/dl)	$S_{[Na+]}$ (mmol/L)	S_{osmol} (mOsmol/kg)	$S_{[prot]}$ (g/dl)	ΔPV (%)
Baseline					
EUH C	87 ± 6	142 ± 2	287 ± 5	7.3 ± 0.5	
DEH					
1%	89 ± 10	141 ± 1	287 ± 4	7.2 ± 0.4	
2%	90 ± 7	142 ± 1	288 ± 5	7.1 ± 0.4	
3%	89 ± 13	141 ± 2	286 ± 5	7.2 ± 0.3	
4%	82 ± 12	142 ± 2	288 ± 6	7.2 ± 0.3	
Min 170 (end)					
EUH C	75 ± 8	141 ± 3	286 ± 5	7.7 ± 0.4	-2.8 ± 3.5
DEH					
1%	74 ± 10	141 ± 2	289 ± 6*	7.9 ± 0.4	-7.7 ± 4.4*
2%	79 ± 6	144 ± 2*	295 ± 5*	7.9 ± 0.5	-8.9 ± 3.7*
3%	79 ± 8	145 ± 2*	298 ± 5*	8.1 ± 0.5*	-11.5 ± 3.6*
4%	79 ± 12	147 ± 3*	300 ± 7*	8.3 ± 0.4*	-12.3 ± 2.7*

Table **6.4**: Remainder of basketball drills and shooting percentage. This table presents a summary of the performance results for the remainder of the basketball drills (those drills not presented in Fig. 2-4) and shooting performance expressed as field goal percentage. * % DEH at which performance decrement (compared to the EUH control trial) becomes statistically significant ($P < 0.05$); ns, no significant performance differences between EUH control and 1-4% DEH.

	Dehydration Threshold*
Defense	3%
Zigzags	
30 Lane Slides	
Sprinting-Defense Combination	3%
Full Court	
Key	
Maximum Vertical Jump	ns
30 Vertical Jumps	4%
Stationary Shooting	4%
Around the World	
3-Pointers	
Free Throws	
Shooting Percentage	
Lay-ups	3%
All Other Shots	ns

Figure **6.1**

A) end of heat chamber exercise

lightheaded

winded

overheated

muscle cramping

total body fatigue

upper body fatigue

leg fatigue

Figure **6.1**

Figure **6.1**: Responses to subjective 100-point visual analog rating scales (ranging from "none" (0) to "very" or "severe" (100)) at the end of the heat chamber exercise (A) and at the end of the basketball "game" (B). Only scales yielding significant effects at these time points are presented. Data presented as means ± SE. DEH, 1-4% dehydration trials; EUH C, euhydration control trial; * P < 0.05, vs. EUH C.

Figure **6.2**

Figure **6.2:** Box plots of "shots on the move" made during 1-4% DEH relative to EUH control for individual drills (left) and total (sum of baseline jump shots, layups, and foul line jump shots single performance scores) shots made (right). The top, bottom, and line through the middle of the box correspond to the 75th, 25th and 50th (median) percentile, respectively. The whiskers extend from the 10th (bottom) to the 90th percentile (top). The black square near the center of each box represents the mean difference from EUH control for each trial. Mean values for the EUH control trial (the basis for each relative comparison) is shown at the right of each panel.

Figure **6.3**

Figure **6.3**: Box plots of sprint times during 1-4% DEH relative to EUH control for individual drills (left) and total (sum of the ladder suicide and 20 court widths single performance scores) sprint times (right). The top, bottom, and line through the middle of the box correspond to the 75th, 25th and 50th (median) percentile, respectively. The whiskers extend from the 10th (bottom) to the 90th percentile (top). The black square near the center of each box represents the mean difference from EUH control for each trial. Mean values for the EUH control trial (the basis for each relative comparison) is shown at the right of each panel.

Figure **6.4**

Figure **6.4**: Box plots of all timed drills (sum of ladder suicide, 30 vertical jumps, zigzags, full court combination, 20 court-width sprints, 30 lane slides, and key combination single performance scores) and all shots (sum of baseline jump shots, layups, around the world shots, foul line jump shots, 3-point shots, and free throws single performance scores) during 1-4% DEH relative to EUH control. The top, bottom, and line through the middle of the box correspond to the 75th, 25th and 50th (median) percentile, respectively. The whiskers extend from the 10th (bottom) to the 90th percentile (top). The black square near the center of each box represents the mean difference from EUH control for each trial. Mean values for the EUH control trial (the basis for each relative comparison) is shown at the right of each panel.

Chapter 7

**DEHYDRATION IMPAIRS VIGILANCE-RELATED ATTENTION IN MALE
BASKETBALL PLAYERS**

Introduction

Success in skill sports such as basketball requires optimal concentration and
attentional skills to facilitate adaptation to a dynamic performance environment. When
basketball players are in their optimal performance state they are focusing their attention
solely on the cues relevant to the task being performed. For example, a successful point
guard is one that has clear vision of the court and the movements of his teammates (and
defenders) so that he can react quickly and pass the ball to his teammates when they are
in scoring position (Carr, 2003). Conversely, players may perform poorly if they lose
concentration (e.g. player missed the coaches' instructions or play call) or become
distracted (e.g. player missed a free throw because he was disturbed by crowd noise).
These examples illustrate the importance of optimal, selective, and sustained attention to
basketball performance (Abernethy, 1993; Moran, 1996).

Attentional skills influence the efficiency and effectiveness of an athlete's
information processing because they affect the types of information selected for
processing, the amount of information being processed, and the athlete's readiness to
respond to environmental cues (Posner and Boies, 1971). Whereas information
processing capacity is a fixed resource, the processes that athletes use to select cues for
attention (i.e., selectivity) and their readiness to respond to different cues (i.e., alertness)
are more dynamic and subject to change in different situations and in response to
different stressors. The present study focused on vigilance, a particular aspect of
alertness that represents an individual's ability to sustain a high level of alertness over an
extended period of time. Vigilance is a particularly important skill for athletes, such as
basketball players, whose competitive environments impose strong attentional demands
because of their complexity and dynamic nature, last for extended periods of time, and
provide minimal or infrequent rest.

Continuous performance tests are commonly used to assess vigilance-related attentional performance (Kindlon, 1998). These tests require participants to respond selectively to stimuli presented over an extended time period. To ensure that participants are processing stimulus qualities, a number of irrelevant stimuli are mixed into the array of relevant stimuli. The ratio of relevant-to-irrelevant stimuli can also be varied to study the effects of stimulus frequency/rareness on attentional performance. For example, the Test of Variables of Attention (TOVA; Greenberg et al., 1996) is a 21.8-min continuous performance test that uses a 3.5:1 ratio (target-frequent) of relevant-to-irrelevant stimuli for the first half of the test, and a 1:3.5 ratio (target-infrequent) of relevant-to-irrelevant stimuli for the second half of the test.

The results of a TOVA assessment are derived from signal-detection theory (Green and Swets, 1966). Briefly, signal detection theory posits that vigilance represents an individual's ability to consistently and accurately distinguish attentional signals from attentional noise. Every environment creates a certain amount of random neural activity (noise) for information processors that is generated without a relevant cue (signal) being present. When a relevant signal is added to the environment, neural activation increases. Repeated sampling thus yields separate distributions for noise and signal + noise. The displacement between these distributions represents an individual's sensitivity to the signal. When neural activation exceeds an established cut-off point, individuals conclude that a signal is likely to be present.

Signal-detection theory offers several parameters for assessing attentional performance using simple decision tasks. These measures include the mean response time for correctly-identified stimuli, the number of omission errors (false negatives), the number of commission errors (false positives), and sensitivity (i.e., ratio of correct responses to false positives). Longer response times for correctly identifying signals in the environment represent greater information processing demands as individuals attempt to determine whether the neural activation exceeds the cut-off threshold. When a signal is present in the environment, individuals may either detect (true positive) or fail to detect (i.e., omission error, false negative) the signal. Likewise, when a signal is absent from the environment, individuals may identify it as being absent (true negative) or mistakenly respond as if the signal was present (i.e., commission error, false positive). True positives

and true negatives represent accurate decisions. Omission errors imply the adoption of an overly conservative cut-off threshold whereas commission errors imply the adoption of overly-liberal cut-off thresholds. Finally, the ratio of correct responses to commission errors provides an index of an individual's sensitivity to signal in the environment. The most sensitive individuals are able to maximize true positives while minimizing false positives.

Two salient stresses in sport environments that may affect these indices of attentional vigilance are fatigue and dehydration (DEH). Subjective symptoms of fatigue are associated with even modest levels of DEH. For example, in a study by Cian et al. (2001), subjective ratings of fatigue indicated that subjects felt less fatigued in a control session (where subjects were allowed to drink) than under DEH conditions (induced by passive heat and exercise stress). In addition, Shirreffs et al. (2004) found that self-ratings of alertness and ability to concentrate decline and ratings of tiredness increase when fluid intake is restricted over a 37 h period to induce 1-2% DEH. Research also suggests that as little as 2-3% DEH impairs various aspects of cognitive performance, such as arithmetic ability, short-term memory, visuomotor tracking, response time, and coordination (Cian et al., 2001; Cian et al., 2000; Gopinathan et al., 1988; Sharma et al., 1986).

Moran (1996) suggests that fatigue depletes our attentional resources, which leads to a reduced capacity for controlled information processing. Moran further suggests that fatigue may impair attentional performance by enhancing distractibility. According to this theory, increases in distractibility would be expected to decrease an individual's ability to detect relevant stimuli (i.e., increase in omission errors and decrease in sensitivity). Likewise, decreases in information processing resources for signal detection would be expected to result in longer response time for correctly identifying relevant stimuli.

Several studies have evaluated the effect of DEH on perceived fatigue and/or cognitive performance (Cian et al., 2001; Cian et al., 2000; Gopinathan et al., 1988; Sharma et al., 1986; Shirreffs et al., 2004; Szinnai et al., 2005); however, no study has tested the effect of DEH on attentional vigilance in basketball players. Furthermore, it is not yet known whether fatigue associated with DEH makes basketball players more

liberal (i.e., prone to commission errors) or conservative (i.e., prone to omission errors) information processors. Therefore, the purpose of the present study was to determine the effects of DEH on attentional vigilance in 17-28 year old male basketball players. We hypothesized that basketball players would become more conservative information processors as a result of DEH. According to this hypothesis, DEH would lead to slower response time, which would in turn mitigate increases in commission errors; however, the time demands of the task would cause increased omission errors and decreased sensitivity.

Methods

Subjects. Eleven skilled male basketball players (17-28 yr) volunteered to participate in this study. Participants were informed of the experimental procedures and associated risks before providing written informed consent. This study was approved by the Institutional Review Board for the Protection of Human Subjects of The Pennsylvania State University. Preliminary screening included a resting 12-lead electrocardiogram, skinfold measurements to determine adiposity, blood analysis (CHEM-24), a graded exercise test on a treadmill (modified Balke protocol) to determine maximal oxygen uptake (VO_{2max}), and a physical exam. Criteria for exclusion included abnormal resting or exercise electrocardiogram, smoking, and taking of medications or supplements that may influence physiological or attention variables of interest. Subject characteristics are presented in Table 7.1.

TOVA Procedures. The visual form of the TOVA (Universal Attention Disorders™) was used to measure the subjects' visual information processing and attention. The TOVA program was run on a PC in MS-DOS mode. During the test the subject sat in a chair directly in front of the computer in a small quiet room. The researcher read the directions for the test as outlined in the TOVA manual (Dupuy and Cenedala, 1996) prior to the subjects' first test (i.e., baseline TOVA of 1st trial). During the test two different visual stimuli were presented (one at a time) on the screen for 100 ms at 2 s intervals in a fixed random order. The stimuli were white squares and differed only in that the target had a black hole near the top and the non-target had a black hole

near the bottom. The subject was asked to press a hand-held button when the target square appeared on the screen and to refrain from pressing the button when the non-target appeared on the screen. Both speed and accuracy of responses were emphasized in the instructions.   After the instructions were given the subject completed a 2.5-min practice test. Next, the TOVA (21.8 min in duration) was taken by the subject. The test consisted of 4 quarters (5.45 min each) or 2 halves (10.9 min each). The first half consisted of quarters 1 and 2 and the second half consisted of quarters 3 and 4. In the first half the target-to-non-target ratio was 1:3.5 (target-infrequent condition) and in the second half the ratio was 3.5:1 (target-frequent condition). The total number of stimuli presented for the test was 648 (324 of each). The TOVA program measured 4 different dependent variables of interest: errors of omission (inattention) and commission (impulsivity), mean correct response time, and sensitivity (ratio of correct responses to errors of commission). TOVA scores were presented by quarters (Q) and halves (H) for each variable.

Experimental Procedures. All subjects completed six experimental trials under the following hydration states: (1) euhydration (EUH) with a commercially available lemon-lime flavored carbohydrate-electrolyte solution (CES; 6% carbohydrate and 18.0 mmol/L NaCl), (2) EUH with a placebo (lemon-lime flavored water and 18.0 mmol/L NaCl), (3) 1% DEH, (4) 2% DEH, (5) 3% DEH, and (6) 4% DEH. Experimental trials were assigned in random order and scheduled at least one week apart. Additionally, both the subject and investigator were blinded to the fluid type during the euhydration trials.

Subjects reported to the laboratory on the morning of test days after having swallowed a disposable temperature sensor (CorTemp™) the night before and fasted overnight. Immediately upon arrival, they voided and then were weighed wearing shorts only. Next the subject ate a low-carbohydrate standardized breakfast (36% CHO, 25% fat, 39% protein) and drank 5 mL of distilled water per kg body mass. After breakfast the researcher administered the baseline TOVA (Test 1) to the subject. Next, a nurse placed an 18-gauge Teflon catheter in an antecubital vein in the subject's arm. After emptying their bladder the subject entered an environmental chamber set at 40°C and 20% relative humidity. Next, the subject (wearing shorts only) was weighed (initial body mass) and then asked to stand on a treadmill while the baseline blood sample and core temperature (T_c) were obtained. Next, the subject walked at an intensity of 50% of VO_{2max} for 15

min, followed by 5 min of rest. This interval walking protocol continued until the subject completed nine 15-min bouts of walking, separated by 5-min rest periods.

Subjects were weighed (wearing shorts only) during each rest period (i.e., after each walking bout) to determine periodic sweat loss. During the EUH trials, subjects drank enough fluid (either CES or placebo) during rest periods to fully replace sweat and urine losses and maintain their initial body mass. During the DEH trials, fluid was restricted until the subjects reached their target body mass (i.e., incurred the desired fluid deficit). Target body mass was determined by calculating the body mass that corresponds with the desired % DEH (e.g., 2% DEH body mass = initial body mass*0.98). If the subjects' body mass fell below their target body mass, they ingested distilled water to maintain the desired % DEH body mass. Ten min into each walking bout a blood sample and T_c were obtained.

At the end of the 3 h interval walking protocol the subject exited the chamber and emptied their bladder. Next, the subject completed the Fatigue Survey, a visual-analog rating scale with questions pertaining to physical well-being, and then completed the TOVA (Test 2). Following completion of Test 2, the subject had the catheter removed from his arm. Next, the subject sat in a thermoneutral room for a 50-min recovery period to rest their legs before doing the basketball drills. During this recovery period the subject was weighed at 15-min intervals and drank water, CES, or placebo as needed to maintain the desired hydration state. T_c was also recorded at 15-min intervals during the recovery period. The subject emptied his bladder at the end of recovery.

Following the 50-min recovery period, the subject was transported to a gymnasium where he completed a sequence of drills designed to simulate a fast-paced basketball game. The drill session commenced 20 min after the 50-min recovery period. Basketball drills were 80 min in duration and consisted of four 15-min quarters with 5-min breaks between quarters and a 10-min break at halftime. The desired hydration state was maintained throughout the basketball drill session by weighing the athletes at the end of each quarter and having them drink the appropriate volume of fluid during the rest periods. The Fatigue Survey was administered at halftime and at the end of the basketball drills. Lastly, the subjects were administered their final TOVA (Test 3) at the

laboratory 20 min after the end of the basketball drills. A schematic of this experimental protocol is provided in Figure 7.1.

Measurements. Body mass was measured to the nearest 0.05 kg using a Seca 770 scale. A CorTemp™ Disposable Temperature Sensor (COR-100) and CorTemp™ Recorder (CT-2000) were used to measure T_c.

Blood Analysis. Venous blood samples (10 mL each) were drawn without stasis. A 2-mL aliquot was transferred into an EDTA-treated test tube and immediately analyzed for hematocrit (microhematocrit centrifugation) and hemoglobin (Hemacue Hb 201$^+$) in triplicate. The percent change in plasma volume from baseline (ΔPV) was calculated from hematocrit and hemoglobin (Dill and Costill, 1974). The remaining aliquot was transferred into a serum separator tube, allowed 30-60 min to clot, and then centrifuged at 4°C for 15 min. Serum was analyzed for glucose concentration (S_{gluc}; hexokinase UV method, Olympus Model AU5200).

Subjective Ratings. The Fatigue Survey was completed at min 180, 310, and 350 to determine the effect of DEH vs. EUH on lightheadedness ("not lightheaded" to "very lightheaded"), hotness ("not feeling hot/overheated" to "feeling very hot/overheated"), and total body fatigue ("no total body fatigue" to "severe total body fatigue"). The subjects answered these questions by placing a mark on a 100 pt scale between the extreme answers at opposite ends of the line.

Statistical Analysis.   Significant differences between hydration states in the TOVA scores, subjective ratings, and physiological variables were determined using a two-way analysis of variance (ANOVA) (hydration state vs. time) with repeated measures. A three-way repeated measures ANOVA (hydration state vs. time vs. test) was used to compare TOVA performance among tests within hydration states. The Bonferroni *post hoc* test was used to correct for multiple comparisons in the analyses of the subjective ratings and physiological variables. PROC MIXED in SAS 9.1 was used to perform all statistical analyses. The significance level for all statistical tests was set at alpha = 0.05. All data are presented as means ± SD.

Results

No statistically significant differences in TOVA performance were observed among the 4 DEH levels or between CES and placebo EUH. To simplify presentation, every subject's ($N = 11$) score for 1, 2, 3, and 4% DEH were averaged to one score (DEH) and every subject's ($N = 11$) score for placebo EUH and CES EUH were averaged to one score (EUH) for comparison. All TOVA results are presented as change from baseline (Test 1). There were no significant differences in baseline TOVA performance between trials.

Physiological Variables. Results for T_c, S_{gluc}, and %ΔPV are presented in Table 7.2. There were no differences in T_c or S_{gluc} between trials at baseline. S_{gluc} was significantly higher in CES compared to placebo and DEH trials, after the exercise/heat exposure, i.e., at the start of Test 2. Also, S_{gluc} was significantly higher in EUH vs. DEH trials at the start of Test 2. PV was significantly lower and T_c was significantly higher during DEH compared to EUH trials at the start of Test 2. There were no significant differences between trials in T_c at the start of Test 3.

Subjective Ratings. Figure 7.2 shows the subjective ratings for lightheadedness, hotness, and total body fatigue at the end of the exercise/heat phase, halftime, and at the end of the basketball drills. The subjects felt significantly more lightheaded and hot/overheated during DEH compared to EUH at all three time points. Ratings of total body fatigue were significantly higher during DEH compared to EUH at the end of the exercise/heat phase and at halftime, but not at the end of the basketball drills.

Attentional Performance with Target-Infrequent Stimuli. The left side of Figure 7.3 presents four indicators of attentional performance relative to baseline for target-infrequent stimuli. During DEH trials, sensitivity decreased significantly relative to baseline following both the exercise/heat phase (-1.1 ± 1.3) and the drills (-0.9 ± 1.3). Neither response time, omission errors, nor commission errors differed from baseline levels after either the exercise/heat phase or the drills. Additionally, following the drills, significantly higher sensitivity (+0.4 ± 1.2 vs. -0.9 ± 1.3), faster response time (-8 ± 20 vs. +16 ± 28), and fewer omission errors (-0.4 ± 0.7 vs. +1.3 ± 2.4) characterized EUH trials compared to DEH trials. There were no differences in commission errors between hydration states.

Attentional Performance with Target-Frequent Stimuli. The right side of Figure 7.3 presents four indicators of attentional performance relative to baseline for target-frequent stimuli. Neither sensitivity, response time, nor omission errors differed from baseline following either the exercise/heat phase or the drills. During the EUH trials, commission errors decreased significantly from baseline following the exercise/heat phase (-1.9 ± 3.2) but not following the drills. Following the exercise/heat phase, EUH trials were characterized by significantly fewer omission errors (-4 ± 15 vs. +5 ± 7) and commission errors (-1.9 ± 3.2 vs. 0.6 ± 1.4) than DEH trials. Following the drills, EUH trials were characterized by significantly greater sensitivity (+0.7 ± 2.6 vs. -0.7 ± 1.1) and faster response time (-21 ± 28 vs. +5 ± 31) than DEH trials.

Discussion

The novel findings from this study were that: 1) DEH impairs vigilance-related attentional performance, 2) attentional impairment is linked to athletes becoming generally more conservative information processors, and 3) DEH-related decrements in performance are most pronounced in stimulus-frequent situations. The basketball-specific relevance of each of these conclusions is as follows: 1) slowed response time and inattention to relevant cues would likely lead to costly errors during a basketball game (e.g., turnovers, missed shot attempts, or being out of position on defense), 2) conservative decision making is the process by which DEH impairs attention in basketball players (i.e., causes slowed response time and increased number of omission errors), and 3) the game of basketball is an example of a stimulus-frequent situation (i.e., a dynamic environment), thus the results of this study are directly applicable to basketball performance.

The impaired attentional performance in the present study can be partially explained by increased feelings of fatigue associated with DEH compared to EUH. These results support previous studies characterizing the relation between DEH, fatigue, and cognitive function (Cian et al., 2001; Cian et al., 2000; Gopinathan et al., 1988; Moran, 1996; Sharma et al., 1986; Shirreffs et al., 2004). Further, the deleterious effects

of DEH on performance can now be extended to attentional aspects of cognitive function in basketball players.

The results of the current study are in agreement with Moran's theory. He suggests that fatigue may impair attention by enhancing distractibility and depleting information processing resources (Moran, 1996). Thus, we would not expect the fatigue associated with DEH to cause excessive neural activation (high arousal); instead, we would expect these two stressors to cause a decrease in neural activation (low-arousal) in response to relevant stimuli. The signal-detection model of vigilance performance (Abernethy, 1993) predicts that under low-arousal conditions, the most common type of error is failure to respond to target-stimuli (i.e., increased number of omission errors). Conversely, the model predicts that over-aroused or anxious athletes are more likely to make responses when they are not required (increased number of commission errors). In the present study, there were more significant differences between hydration states in omission errors than commission errors throughout Q1-Q4 in Tests 2 and 3. These results show that, during DEH trials, the subjects' minimized false alarms at the cost of failing to detect some target stimuli. The greater increase in omission errors compared to commission errors suggests that subjects became more conservative decision-makers during the DEH trials.

In general, attentional performance was impaired to a greater extent during the target-frequent compared to the target-infrequent presentation of the TOVA. For instance, there were more significant differences between hydration states in response time and omission errors throughout Q3-Q4 (target-frequent presentation) compared to Q1-Q2 (target-infrequent presentation). One interpretation of these results could be that DEH is more detrimental to attentional performance when tasks are performed in a highly dynamic environment. Alternatively, because the target-frequent condition was presented during the second half of the TOVA, fatigue associated with the time demand of the task may have led to the attention decrements. Due to the design of the current study (order of stimuli frequency presentations was the same for each trial) we cannot determine the relative contributions of task duration vs. stimuli frequency on the attentional-performance impairment. Nonetheless, both interpretations seem plausible

and have practical significance; that is, DEH may have more deleterious effects on performance during the latter half of complex tasks (i.e., end of a basketball game).

For each DEH and EUH trial, TOVA was completed following two different types of stress. Subjects performed Test 2 after exercise and heat stress (walking at 50% VO_{2max} in 40°C), while Test 3 was performed following exercise stress only (basketball drills were performed at room temperature). TOVA performance was significantly impaired with DEH compared to EUH during both Test 2 and Test 3. Thus, DEH impaired attention regardless of the environmental conditions in this experiment (i.e., whether the test was administered following exercise with or without environmental heat stress). Cian et al. (2000 and 2001) have determined that inducing DEH by passive heat stress or by treadmill exercise produced similar impairments in cognitive function (perceptive discrimination and short term memory) when compared to EUH. Thus, DEH *per se*, and not necessarily the heat exposure or exercise stress associated with establishing DEH, is responsible for impairing various aspects of cognitive function, including vigilance-related attention. Conversely, submaximal aerobic exercise has been shown to improve information processing above that of pre-exercise/baseline conditions provided EUH is maintained (Tomporowski, 2003). The current study shows a trend ($P < 0.1$) towards improvement in attentional performance in Q1 and Q3 of Test 2 (RT) and Q2 (sensitivity) and Q4 (RT) of Test 3 compared to baseline (Test 1) of EUH trials. In addition, commission errors decreased significantly below baseline during the second half of Test 2 in the EUH trials.

Sweating due to heat exposure and/or exercise results in fluid loss from the extracellular space (Costill, 1977). Body water distribution is a function of solute distribution, and given that sodium is the major cation in the extracellular space, sodium replacement is necessary to promote more complete rehydration (smaller % decrease in PV) compared to water alone (Costill and Sparks, 1973; Nose et al., 1988; Takamata et al., 1994). Thus, in the current study, both fluid replacement beverages (CES and placebo) used in the EUH trials included sodium (18 mmol/L NaCl) to provide an osmotic impetus to retain the ingested fluid in the vascular space. In addition, inclusion of sodium in the rehydration solution enhances palatability (Murray and Stofan, 2001; Nose et al., 1988; Takamata et al., 1994), which becomes especially important when

athletes need to drink large volumes of fluid to completely replace sweat loss (e.g., mean sweating rate during the basketball drills of EUH trials in the current study was 2.2 ± 0.7 L/h).

In the current study, there were no differences in attentional performance when EUH was maintained with CES vs. placebo (data not shown). There have been mixed results in the literature regarding the effects of glucose administration on cognitive performance. Some investigations have suggested that consumption of glucose drinks prior to testing improves memory (Sunram-Lea et al., 2001) and results in faster and more consistent information processing (Owens and Benton, 1994). In contrast, others have found no effect of glucose administration on memory and attention (Scholey and Kennedy, 2004) or performance on the Stroop Color-Word Test (Welsh et al., 2002).

Prior to Test 2 of the EUH-CES trials, the subjects' S_{gluc} was 90 mg/dL (5 mmol/L); which is lower than that of the studies showing that glucose administration improves cognitive performance (≥ 6 mmol/L). However, S_{gluc} does not explain the discrepancy between studies because Flint and Turek (2003) found no differences in most aspects of TOVA performance (including omission errors, response time, or sensitivity) between a saccharin control group and the groups that consumed a 10, 100, or 500mg/kg or 50 g dose of glucose prior to testing. In their study, S_{gluc} varied from ~80 mg/dL (saccharin) to ~115 mg/dL (50 g dose). Thus, alterations in S_{gluc} do not seem to affect most variables of attention. Again, it appears that DEH impairs attentional performance independent of factors that may be associated with the process of inducing DEH, such as alterations in S_{gluc} due to exercise or fasting.

There were statistically significant differences between EUH (CES and placebo averaged) and each level of DEH (1, 2, 3, and 4%) at various time points for the TOVA scores. However, there were no statistically significant differences in attentional performance among 1, 2, 3, and 4% DEH (data not shown). Therefore, the current data suggest that DEH at any level (up to 4%) equally impairs vigilance-related attention.

T_c was significantly higher with 3 and 4% than 1 and 2% DEH at the start of TOVA Test 2 and 3; however, a significantly higher T_c did not result in significantly poorer attentional performance in 3 and 4% vs. 1 and 2% DEH. For example, T_c at the end of the drill session (start of TOVA Test 3) for each of the trials was as follows: EUH

(CES and placebo averaged): 38.10 ± 0.70°C, 1% DEH: 38.09 ± 0.62°C, 2% DEH: 38.06 ± 0.34°C, 3% DEH: 38.52 ± 0.41°C, and 4% DEH: 38.41 ± 0.55°C. Attentional performance during TOVA Test 3 was impaired during each level of DEH compared to EUH; however, T_c was only increased above that of EUH during 3 and 4% DEH. Therefore, our current data suggest that the DEH-induced impairment of attentional performance is not related to increases in T_c.

Summary. In summary, vigilance-related attentional performance of 17-28 year old male basketball players is impaired by DEH. Specifically, DEH decreases sensitivity to relevant cues, increases the number of omission errors, and slows response time. Further, the significant increase in omission errors and minimal change in commission errors suggests that DEH makes basketball players more conservative as opposed to liberal information processors. The deleterious effects of DEH on attention are particularly evident during the target-frequent presentations (representing a highly dynamic environment, such as a basketball game). These results suggest that fluid replacement is critical in preventing the decline in attentional vigilance associated with DEH. Therefore, basketball players should be advised to maintain EUH for optimal concentration and attentional skills during competition.

Table **7.1**: Subject characteristics. Values are means ± SD. VO_{2max}, maximal oxygen consumption.

	Men ($N = 11$)
Age (yr)	21 ± 3
Height (cm)	184 ± 10
Weight (kg)	80 ± 12
Body Fat (%)	9 ± 4
Playing Experience (yr)	10 ± 2
College	$N = 6$
High School Starters	$N = 5$
VO_{2max} (ml·kg^{-1}·min^{-1})	57 ± 5

Table **7.2**: Physiological variables. Values are means ± SD. T_c, core temperature; HR, heart rate; MAP, mean arterial pressure; ΔPV, change in plasma volume; $S_{[gluc]}$, serum glucose concentration; EUH, euhydration trials; DEH, dehydration trials; CES, carbohydrate-electrolyte solution. * $P < 0.05$, vs. DEH; ** $P < 0.025$, vs. DEH; *** $P < 0.0167$, vs. DEH; † $P < 0.025$, vs. Placebo. All variables were analyzed using repeated measures two-way ANOVA. Alpha levels were adjusted by the Bonferroni technique for comparisons at three time points for T_c (i.e., *** $P < 0.05/3 = 0.0167$) and two time points for $S_{[gluc]}$ (i.e., ** $P < 0.05/2 = 0.025$).

		Baseline (Test 1)	Exercise/heat (Test 2)	Basketball drills (Test 3)
T_c (°C)	DEH	36.71 ± 0.28	37.99 ± 0.58	38.27 ± 0.52
	EUH	36.73 ± 0.25	37.42 ± 0.43***	38.10 ± 0.70
ΔPV (%)	DEH		-10.2 ± 4	
	EUH		-3.4 ± 4*	
$S_{[gluc]}$ (g/dl)	DEH	89 ± 10	77 ± 10	
	EUH	87 ± 9	82 ± 11**	
	Placebo	86 ± 7	74 ± 8	
	CES	89 ± 11	90 ± 7** †	

Figure **7.1**

Figure **7.1**: Schematic of study protocol for each trial. During the Exercise/Heat phase, subjects walked at 50% VO_{2max} at intervals of 15 min exercise/5 min rest in conditions of 40°C and 20% rh. During the rest periods, subjects were weighed and drank CES, placebo, water, or no fluid to establish the desired hydration state (EUH, 1, 2, 3, or 4% DEH). The drink treatment was continued throughout recovery and the basketball drills to maintain the desired hydration state. TOVA Tests 1, 2, and 3 (represented by gray bars) were conducted in a quiet thermoneutral room. Hatched bars represent 20-min transition periods between the laboratory and basketball court. Fatigue Surveys were administered at min 180, 310, and 350. R, recovery; BS, blood sample; BP, blood pressure; HR, heart rate; T_c, core temperature.

Figure **7.2**

Figure **7.2**: Responses to subjective questionnaire on a 100-point scale. Data presented as means ± SD. * P < 0.0167, EUH vs. DEH (repeated measures two-way ANOVA with Alpha level adjusted by the Bonferroni technique for comparisons at three time points, i.e., P < 0.05/3 = 0.0167).

Figure **7.3**

Figure **7.3**: Results for the target-infrequent and target-frequent halves of TOVA for Test 2 and Test 3 presented as change from baseline (Test 1). A. sensitivity (ratio of correct responses to commission errors); B. mean correct response time; C. omission errors (measure of inattention); D. commission errors (measure of impulsitivity). Baseline TOVA results for each half are shown at the right of their respective panels (D, dehydration; E, euhydration). There were no statistically significant differences between DEH and EUH at baseline. Data presented as means ± SD. * P < 0.05, DEH vs. EUH (repeated measures two-way ANOVA); † P < 0.05, vs. baseline (Test 1).

Chapter 8

CONCLUSIONS

The five studies comprising this dissertation were designed to 1) elucidate the role of fluid intake behavior, changes in body mass (ΔBM), beverage [Na^+], and sweat sodium loss on fluid and sodium (Na^+) balance in young adult and older men and women during prolonged exercise in the heat (Studies 1, 2, and 3) and 2) to determine the effects of 2 and 4% dehydration (DEH) on prolonged running performance in endurance-trained male and female athletes (Study 2) and progressive 1 to 4% DEH on basketball skill performance and measures of attention in male players (Studies 4 and 5). This final chapter is intended to summarize the results of each study, discuss the implications of the results in a practical context, and suggest future directions of research.

Voluntary Fluid Intake by Older Active Adults

The major findings from the first study were 1) when cool palatable fluids were readily available, active adults aged 54-70 yrs drank enough fluid to match sweating rates and maintain their body weight, 2) their fluid intake behavior was repeatable, 3) a carbohydrate-electrolyte solution (CES) promoted greater voluntary fluid intake and restored plasma volume losses faster than water, 4) there were sex differences in the fluid intake behavior of older active adults, with women drinking more water per kg BM than men, and 5) women drank more CES and water than was lost through sweating, leading to symptomatic EAH in one small woman during the water trial.

Implications

These results suggest that voluntary DEH does not occur in older adults provided that cool palatable fluid (either CES or water) is readily available between repeated bouts of moderate intensity exercise in the heat. An important distinction between this and previous studies on *ad libitum* drinking in older adults is that subjects were provided

121

ample opportunity to drink between exercise bouts, whereas previous studies induced hyperosmolality, hypovolemia, or both (by 12 to 24-h fluid restriction or hypertonic saline infusion) and then allowed *ad libitum* drinking only during recovery (Mack et al., 1994; Phillips et al., 1984; Phillips et al., 1991; Phillips et al., 1993; Rolls et al., 1990). The lack of voluntary DEH may also be explained by the subjects' low sweating rate (370 g/h over the entire protocol). In 1947, Rothstein et al. reported that when sweat losses are low (defined as < 400 g/h) young men working in the heat have little difficulty consuming sufficient fluid to replace periodic losses (Rothstein et al., 1947). A practical implication of our results is that healthy older active adults can avoid voluntary DEH provided that exercise is of low to moderate intensity and fluid is readily available during regular rest intervals. Further, given that osmotic thirst is intact in older adults (Stachenfeld et al., 1997) and that our study results confirm CES is more effective in stimulating voluntary drinking and restoring plasma volume than water, CES would be an appropriate fluid replacement beverage for this population; especially when exercise is more intense and/or prolonged, when sweating rates are greater than 400 g/h, and/or when fluid availability is more limited.

During the water trials the $S_{[Na+]}$ of the women was lower than that of the men during the final 30 min of the protocol because the women drank significantly more water relative to BM compared with the men. One woman became clinically hyponatremic ($S_{[Na+]}$ = 126 mmol/L) with symptoms by the end of the protocol during the water trial. This individual (65 yrs, 45.7 kg) drank 2.8 L of water and gained 2.4 kg during the experiment. The present study supports the notion that overconsumption of water is more common among women than men, rendering small women more susceptible to a decline in $S_{[Na+]}$. Further, the sex-related differences in voluntary fluid intake behavior and associated increased risk for EAH can be extended to older active women.

Quantitative Analysis of Serum Sodium Concentration

The main findings from the second study were 1) the Nguyen-Kurtz equation accurately predicted post-experiment $S_{[Na+]}$ when subjects drank to match sweating rate (0% ΔBM) or restrict fluid consumption and lost BM (-2% or -4%) during endurance

exercise, 2) the Nguyen-Kurtz equation did not accurately predict $S_{[Na+]}$ when athletes overdrank relative to sweat loss and increased BM (+2%), 3) consumption of beverages with Na^+ attenuated the decline in $S_{[Na+]}$ from pre-to post-exercise, and 4) prolonged running performance was impaired when subjects incurred a 2 and 4% BM deficit due to fluid restriction.

Implications

It is clear that both the volume and $[Na^+]$ of fluid consumed during exercise has implications for sodium balance. As predicted, drinking at a rate greater than sweating rate (+2% ΔBM trials) leads to dilution of $S_{[Na+]}$ and restricting fluid intake (a decrease in BM) leads to an increase in $S_{[Na+]}$. Moreover, Na^+ consumption influences the relation between ΔBM and the $\Delta S_{[Na+]}$ by attenuating the decline in $S_{[Na+]}$, and the higher the $[Na^+]$ in the beverage, the greater the attenuation. The model accurately predicts that to maintain pre-experiment $S_{[Na+]}$ while also consuming enough fluid to replace sweat losses and maintain BM, a beverage $[Na^+]$ comparable to that of the athletes' sweat is required. Additionally, a $\geq$ 2% BM deficit augments T_c, cardiovascular strain, perceived exertion, lightheadedness, windedness, and total body fatigue leading to impaired endurance performance. Altogether, these results suggest that the optimal hydration practice for endurance athletes is to consume fluids at a similar rate (to avoid $\geq$ 2% BM loss) and composition to that of their sweat losses.

Future Directions

EAH typically occurs in endurance events lasting $\geq$ 4 h (Almond et al, 2005; Casa et al., 2000; Davis et al., 2001). While our study protocol involved prolonged running, it was only 2 h in duration. Because of the number of trials (12) that comprised this study, it would have been difficult for subjects to run for $\geq$ 4 h with as little as 1 week between trials. Nonetheless, in future studies it would be relevant to track changes in $S_{[Na+]}$ for $\geq$ 4 h to investigate the impact of ΔBM, sweat Na^+ losses, and beverage $[Na^+]$ on the etiology of EAH in more prolonged events.

It is generally agreed upon that overdrinking is the primary factor involved in most cases of EAH (Noakes et al., 2005; Sawka et al., 2007). However, it may be possible that some athletes, especially women, are more susceptible to a significant decline in $S_{[Na+]}$ because of an impaired renal free-water clearance (i.e., syndrome of inappropriate antidiuretic hormone secretion, SIADH) in the face of fluid overload (Noakes et al., 2005; Stachenfeld et al., 2001). Further, it has been suggested that EAH occurs in athletes who osmotically inactivate circulating Na^+ or fail to mobilize osmotically inactive Na^+ from internal stores (Noakes et al., 2005). In our study, we did not detect an inappropriate renal response or a sex-related difference in free water clearance during the hyperhydration (+2% ΔBM) trials. Further, the change in $S_{[Na+]}$ could be predicted simply by the mass balance of water, Na^+, and K^+ (i.e., volume and Na^+/K^+ output of sweat and urine vs. volume and Na^+/K^+ beverage input using the Kurtz-Nugyen equation); precluding any role for inappropriate activation/inactivation of osmotically inactive/active Na^+ in determining post-exercise $S_{[Na+]}$. However, we tested endurance athletes with no history of EAH; thus, in future studies it would be interesting to test the accuracy of the Nguyen-Kurtz equation in those male and female athletes with a history of, and presumably a susceptibility to, EAH to determine whether the mass balance explanation still holds true.

In our study, the Kurtz-Nugyen equation predicted that a CES with a combined $[Na^+]$ and $[K^+]$ of 58 mmol/L would be required to maintain pre-experiment $S_{[Na+]}$, when consumed at a rate equal to sweating rate. That is, to maintain Na^+ balance, the CES should be similar in composition to that of the athlete's sweat (the mean sweat $[Na^+]$ and $[K^+]$ of athletes in our study was 56 and 5 mmol/L, respectively). This point was confirmed in one subject who happened to have a combined sweat $[Na^+]$ and $[K^+]$ (35 and 5 mmol/L, respectively) similar to the composition of beverage Na^+30 (30 and 11 mmol/L, respectively). When this subject consumed a volume of Na^+30 during and after exercise to maintain her pre-experiment body weight (0% ΔBM trial), she experienced minimal net $\Delta S_{[Na+]}$ (+0.1 mmol/L). Most CES contain only ~20-30 mmol/L of Na^+, but the average sweat $[Na^+]$ is ~50 mmol/L, and sweat $[Na^+]$ can be $\geq$ 80 mmol/L in some athletes (Casa et al., 2005; Costill, 1977; Maughan, 2001; Stofan et al., 2005). Thus, the direction of future studies should be to formulate a CES with a higher $[Na^+]$ to be

consumed by those athletes who have a naturally high sweat [Na^+] and who exercise strenuously for extended durations. A challenge to formulating such a beverage would be to increase [Na^+] without also increasing the beverage osmolality. Consumption of hyperosmotic fluids delays intestinal fluid absorption (Leiper, 2001; Shi et al., 1995), which would have the undesired effect of impairing the hydration process. To compensate for the increased osmotic effect of a higher beverage [Na^+], one could decrease the overall carbohydrate concentration or replace carbohydrate monomers with dimers and/or polymers (maltodextrins).

Relation between the Change in Body Mass and the Change in Body Water

The major findings from the third study were: 1) pre- to post-exercise ΔBM is an accurate and reliable method to assess the change in total body water (ΔTBW) in endurance-trained men and women after prolonged running in the heat and 2) ΔBM is a better method to predict ΔTBW than serum osmolality (S_{osm}), urine osmolality (U_{osm}), and urine specific gravity (U_{sg}).

Implications

Our study results suggest that the ΔBM is accurate to within $\pm$ 1.1 kg of the ΔTBW. It is probable that most of this variability can be attributed to the variability associated with the deuterium oxide measurements and TBW calculations. Other factors that may impact the relation between ΔBM and ΔTBW include metabolic water production and non-sweat sources of BM loss (respiratory water loss and fuel oxidation). However, these factors, which would overestimate sweat losses and the severity of DEH, are likely offset by the mass of sweat trapped in the athletes' shoes, clothing, and hair at the time of post-exercise BM measurements (Cheuvront et al., 2007; Cheuvront and Haymes, 2001); especially in the field setting where controlling for this issue may be difficult. Altogether, the available evidence suggests that pre- to post-exercise ΔBM provides a reasonable estimate of sweat losses and this practice can confidently be used to assess athletes' fluid needs during and after exercise. Thus, it is also logical to

conclude that endogenous water gain does not contribute significantly to the size of the TBW pool or to the etiology of EAH. Additionally, while measures of serum and urine concentration (e.g., S_{osm}, U_{sg}, and U_{osm}) are not as highly correlated with ΔTBW as ΔBM, they are still effective and can be used to gauge hydration status when pre- and post-exercise BM measurements are not available.

Dehydration and Basketball Skill Performance

The main findings from the fourth study were: 1) 17-28 yr old skilled male basketball players experienced a progressive deterioration in performance as DEH progressed from 1% to 4%, 2) the threshold, or % DEH at which the overall performance (i.e., combined scores for all drills during the entire 80-min simulated game) decrement reached statistical significance, was 2%, and 3) euhydration (EUH) with a CES did not enhance basketball performance over EUH with a carbohydrate-free solution.

Future Directions

Contrary to our hypothesis, maintaining EUH by consuming a CES did not lead to improved basketball skill performance over EUH with a carbohydrate-free drink (EUH control). In our study the volume of fluid intake was controlled. By contrast, during timeouts and substitutions of actual basketball games both CES and water are readily available to players for *ad libitum* consumption. Research on voluntary fluid intake behavior suggests that when both CES and water are readily available athletes, including basketball players, consume significantly more CES than water (Osterberg et al., 2007; Passe, 2001). Thus, in a setting where *ad libitum* fluid intake is allowed CES may be more effective than water in preventing the DEH-induced impairment of basketball skill performance. Another way to improve upon the practical relevance of a basketball study would be to measure skill performance during competitive drills. In our study, the players completed the session of basketball drills individually. While the players were very self-motivated to complete the drills as quickly and shoot as accurately as possible, a competitive situation would better represent how hydration status and carbohydrate

intake might affect performance during an actual game. The basketball performance studies described in this dissertation (Studies 4 and 5) have provided insight as to the impact of DEH on basketball skill and attentional performance in male players during a simulated game. Future basketball studies should employ *ad libitum* fluid consumption during breaks of competitive drills and should apply these same hydration- and carbohydrate-related research questions to performance in female basketball players.

Dehydration and Attentional Performance

The main findings from the fifth study were that: 1) DEH impairs vigilance-related attentional performance, 2) attentional impairment is linked to athletes becoming generally more conservative information processors, and 3) DEH-related decrements in performance are most pronounced in stimulus-frequent situations.

Implications

The basketball-specific implications of each of these results is as follows: 1) slowed response time and inattention to relevant cues would likely lead to costly errors during a basketball game (e.g., turnovers, missed shot attempts, or being out of position on defense), 2) conservative decision making is the process by which DEH impairs attention in basketball players (i.e., causes slowed response time and increased number of omission errors), and 3) the game of basketball is an example of a stimulus-frequent situation (i.e., a dynamic environment), thus the results of this study are directly applicable to basketball performance.

For each DEH and EUH trial, the Test of Variables of Attention (TOVA) was completed following two different types of stress: 1) after exercise and heat stress (walking at 50% VO_{2max} in 40°C) and 2) following exercise stress only (basketball drills were performed at room temperature). TOVA performance was significantly impaired with DEH compared to EUH during both conditions. Thus, DEH impaired attention regardless of the environmental conditions in this experiment (i.e., whether the test was administered following exercise with or without environmental heat stress). Thus, DEH

per se, and not necessarily the heat exposure or exercise stress associated with establishing DEH, is responsible for impairing various aspects of cognitive function, including vigilance-related attention. Additionally, although CES ingestion resulted in a higher serum glucose concentration ($S_{[gluc]}$) there were no differences in basketball skill (Study 4) or attentional (Study 5) performance when EUH was maintained with CES vs. a carbohydrate-free placebo. Thus, it appears that hydration status has a greater impact on performance than alterations in carbohydrate availability and $S_{[gluc]}$.

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Appendix

INFORMED CONSENT FORMS

Older Active Adults Study

Informed Consent Form For Clinical Research Study
The Pennsylvania State University

Title of Project: Older Subjects' Thermal Responses to Interval Cycling in the Heat
(IRB #15613)

Principle Investigator: Thayne Munce, Ph.D.
Address: 229 Noll Laboratory
Phone: (814) 863-2948

Co-Investigator: W. Larry Kenney, Ph.D.
Address: 102 Noll Laboratory
Phone: (814) 863-1672

Other Investigators:
Lindsay Baker, B.S., M.S. Candidate; Jane Pierzga, M.S., Research Assistant

This is to certify that I, _____ have been given the following
information with respect to my participation as a volunteer in a program of investigation
under the supervision of Dr. W. Larry Kenney.

1. Purpose of the study: Past research has been shown that my body's responses to heat
stress change as I age. These changes make me less able to keep a normal body
temperature during heat stress. This study further explores these changes in active older
adults. The researcher is interested in how my body responds to exercise in the heat. The
researcher also wants to know how these responses affect my feelings about the
experimental conditions and my sense of well-being. I understand that this document
explains the study as well as possible at this time. When I finish the study, the researcher
will tell me the results and the meaning of the study in more detail.

2. Procedures: *You will participate on the circled days. Please read the descriptions*
of the circled days. Then write your initials by the circled days. See section 3
for full descriptions of the measurements.

_____ initial **Day 1: Blood Draw:** I will not eat anything after midnight on the
night before I report to the General Clinical Research Center (GCRC). When I arrive, the
nurses will draw 15 mL (1 Tbsp) of blood from a vein in my arm.

_____ initial **Day 2: Screening and Bike Test:** I report to the GCRC. The medical staff gives me an exam including a medical history, check-up, blood pressure, and heart rate. They also measure my percent body fat, height, and weight. If I am a women of childbearing age, I will collect urine for a pregnancy test. The medical staff monitors my heart with an electrocardiograph (ECG) while I rest. I may request staff who are the same gender as myself to perform parts of the medical screening. Then I take an exercise test on a bike.

_____ initial **Day 3: Experiment:** I will not eat or drink anything after midnight on the night before I report to the GCRC. The medical staff records my blood pressure and heart rate when I arrive. Then I complete the first GVA survey. I provide a urine sample, and change into a bathrobe so I may be weighed. I don exercise clothing and insert the rectal probe. The researcher straps a heart rate monitor around my chest. Then I lie down so that a nurse can insert a tube into a vein in my arm and remove a 15 mL (1 Tbsp) blood sample. The tube remains in my arm until the experiment ends. Next, I enter a room in which the temperature is 30 C (86 F) and the humidity is 50%. I mount the bike, and the researcher tapes wires to my chest, arm, leg and thigh to measure skin temperature. The experiment proceeds as follows:

15-minute baseline rest period: After 10 minutes, my heart rate and blood pressure are measured, and a 15 mL (1 Tbsp) blood sample is drawn.
15-minute exercise: I pedal the bike. After 10 minutes, my heart rate and blood pressure are measured, and a 15 mL (1 Tbsp) blood sample is drawn. I report my Rate of Perceived Exertion (RPE) number.
15-minute rest period: I have the opportunity to drink. After 10 minutes, my heart rate and blood pressure are measured, and a 15 mL (1 Tbsp) blood sample is drawn.

I repeat the exercise and rest periods for a total of 4 rest and 4 exercise periods. During the 2nd exercise period, the researcher will also collect my expired air. During the 2nd rest period, I will complete the BV998 survey.

30-minute recovery period: I have the opportunity to drink. After 10 minutes, my heart rate and blood pressure are measured, and a 15 mL (1 Tbsp) blood sample is drawn. I will complete another copy of the BV998 survey. At 25 minutes, a final heart rate and blood pressure are measured, and a 15 mL (1 Tbsp) blood sample is drawn. The experiment ends.

All probes and the catheter are removed. I change into a bathrobe so I may be weighed, and I provide a urine sample. I complete another copy of the GVA survey. I may take a shower in the locker room across the hall. The researcher provides a snack. Before I leave, I have my heart rate and blood pressure measured.

_____ initial **Days 4, 5 and 6: Experiment:** I repeat the experiment with at least 1 week between repeats.

3. Discomforts and risks: *You will experience the circled procedures. Please read the*

descriptions of the circled procedures. Then write your initials by the circled procedures.

_____initial   a. <u>Blood Draw:</u> Blood draws often cause mild pain, swelling or bleeding. There is also a slight chance of infection. To keep the chance of infection minimal, the medical staff uses the same techniques used in hospitals. The total for each experiment is 165 mL (0.7 cups). The total for all 4 experiments plus the screening is 675 mL (1.4 pint). A typical blood donation to the Red Cross is 473 mL (1 pint).

_____initial   b. <u>Bike Test:</u> This test measures my fitness. I wear exercise clothes and pedal a stationary bike. During the test, I breathe into a mouthpiece, a clip holds my nose shut, and the ECG monitors my heart. Pedaling the bike gets more difficult during the test. I need to try my best to exercise as long as I can; however, I may stop at any time. Typical reactions are tiredness, sweating, breathlessness, increased heart rate, and muscle fatigue. Although unlikely, I could experience irregular heartbeats, heart attack (< 0.05%) or death (< 0.02%). The lab staff and a doctor conduct the test.

_____initial   c. <u>ECG:</u> The ECG's wires are taped to my body to measure the electrical activity of my heart. There are no risks, but the tape may irritate my skin.

_____initial   d. <u>Percent Body Fat:</u> In a private room, the medical staff gently measures the thickness of skin folds at several places on my body with a tool that looks like tongs. There are no risks to this measure.

_____initial   e. <u>Blood Pressure:</u> The researcher uses the method used in a doctor's office. During the short time the cuff is inflated, my arm may feel tingly or numb. Rarely, the cuff may cause a temporary bruise.

_____initial   f. <u>Heart Rate:</u> The monitor is in a band placed around my chest. This measure has no risk.

_____initial   g. <u>Skin Temperature:</u> The wires taped to my skin are not harmful, but the tape may irritate.

_____initial   h. <u>Rectal Temperature:</u> The probe is a soft tube with a small ball near the end that I insert into my rectum. I place a very small amount of KY jelly on the tip of the probe to make it slippery and easy to insert. The probe stays in place when the small ball is placed inside the large muscle at the entrance to the rectum. Assistance by a person of the same gender is available. The soft tube causes no harm to healthy tissue. However, the probe could irritate or harm diseased tissue or hemorrhoids. I will inform the researcher or medical staff if I have hemorrhoids or any diseases of the lower bowel. Although unlikely, a bad reaction to the jelly could cause redness, itching, rash, and/or swelling. A worse reaction could cause fever, breathing problems, changes in pulse, convulsions, and/or collapse.

_____initial   i. <u>Bike Exercise in the Heat:</u> A special bike seat reduces possible discomfort. I exercise at 65% of my greatest effort measured during the screening. Lights or sounds help me to maintain the right effort. The researcher assists me with the bike to reduce the chance of falls resulting in scrapes, sprains, bruises, or broken bones. Tiredness, sweating, breathlessness, increased heart rate, and muscle fatigue are normal. There is a small chance that the short exercise bouts may cause nausea, dizziness, or muscle cramps. The chance of irregular heartbeats, heart attack, or death is less than that for the screening bike test. I will tell the researcher about any problems and may stop at any time. Medical help is nearby.

_____initial   j. <u>Screening, RPE Scale, GVA, and BV998 Surveys:</u> I may decline to answer questions and decline to join the study. The right responses are my honest ones. The screening surveys see if I fit the requirements to be in the study. I report numbers relating to descriptions (light, hard, etc.) from the RPE chart to describe how hard I feel I am working. I describe my sense of well-being and how I feel about parts of the study by placing marks on the 1-page GVA and BV998 surveys. The researcher codes the surveys so that I cannot be identified. Only the researcher has access to the code key.

_____initial   k. <u>Urine Sample:</u> I use the restroom and void all I can into the container given to me. Privacy and the researcher's care protect my feelings. Then I return the container with the sample.

4. a. <u>Benefits to me:</u> *Please initial the item circled by the researcher*

_____initial   a. Pilot Experiment: None
_____initial   b. Experiment. I receive a medical screening and measure of my body fat that could inform me about my health. I could also gain some knowledge about how my body works during thermal stress.

   b. <u>Potential benefits to society:</u>
The pilot work helps Dr. Kenney's group to practice techniques they use in the study. This study helps us to learn more about how exercise in the heat affects older people's bodies and, in turn, how these effects impact their sense of well-being.   These results could lead to better plans for meeting the special needs of active older people. This can help and encourage people to maintain an active lifestyle. An active lifestyle can yield health and financial benefits, and enhance the quality of life.

5. <u>Alternative procedures that could be utilized:</u> The researcher could measure my temperature with a probe inserted in my throat, under my tongue or in my ear. These techniques cause greater discomfort or are less accurate than the rectal probe. The other techniques in the study are used in research worldwide. They are the best means by which to meet the goals of this study with minimal discomfort and risk to me.

6. Time duration of the procedures and study: The circled statements apply to you. Please read the circled statements. Then write your initials by the circled statements.

_____initial   Pilot work. The pilot work may last 1-4 hours.
_____initial   Experiment. I visit the Noll Lab on 6 days. The screening blood draw on Day 1 lasts no more than 20 minutes. The screening on Day 2 lasts no longer than 1.5 hours. The 4 experiment days last no longer than 4 hours each.

7. Statement of confidentiality: The data is available only to the investigators. Volunteers will be coded by an identification number for statistical analyses. All records are kept in a secure location. All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records (e.g., such as records maintained by physicians, hospitals, etc.), and in the event of any publication resulting from the research no personally identifiable information will be disclosed.

8. Right to ask questions: If I have any questions or concerns about the research or my participation in the present investigation, I may contact Lindsay at work (814-863-2948) or at home (814-862-1525). Or I may contact Jane Pierzga at work (814-865-1236) or at home (814-692-4720). If there are findings during the research that could relate to my wanting to help with the study, my will be told of the findings. I may contact the Office for Research Protections, 212 Kern Graduate Building, University Park, PA 16802, (814) 865-1775 for additional information concerning my right as a research participant.

I have been given an opportunity to ask any questions I may have, and all such questions or inquiries have been answered to my satisfaction.

9. Compensation: The circled statements apply to you. Please read the circled statements. Then write your initials by the circled statements.

_____initial   a. Pilot: I receive no compensation.
_____initial   b. Experiment: I receive $50.00 for completing each experiment for a total of $200.00. I get a T-shirt.

If I am an employee of Penn State University, the compensation I receive for participation will be treated as taxable income and therefore taxes will be taken from the total amount. If I am not employed by Penn State University, total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require me to claim the compensation that I receive for participation in this study as taxable income.

10. Injury Clause: I understand that medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. I also understand that I am not waiving any rights that I may have against

the University for injury resulting from negligence of the University or investigators.

11. <u>Voluntary participation:</u> I understand that my participation in this study is voluntary, and that I may withdraw from this study at any time by notifying the investigator. My withdrawal from this study or my refusal to participate will in no way affect my care or access to medical services. I may decline to answer specific questions. However, my acceptance into the study may be contingent upon answering these questions. My helping with the study may be ended without my consent if the researcher deems that my health or behavior adversely affects the study or increases risks to me beyond those approved by the Office for Research Protections and agreed upon by me in this document.

12. In the event that abnormal test results are obtained, I will be apprised of the results immediately and recommended to contact my private medical provider for follow-up.

This is to certify that I consent to and give permission for my participation as a volunteer in this program of investigation. I understand that I will receive a signed copy of this consent form. I have read this form, and understand the content of this consent form.

Volunteer   **Date**

I, the undersigned, have defined and explained the studies involved to the above volunteer.

Volunteer   **Date**

<center>**Endurance Running Study**</center>

<center>**Informed Consent Form For Clinical Research Study**
The Pennsylvania State University</center>

Title of Project: Sodium and Water Balance During Endurance Activity in Trained Athletes

Sponsor: Gatorade Sports Science Institute

Principal Investigator: Lindsay Baker, Ph.D. Candidate

Address: 129 Noll Laboratory	
Phone: 814-863-2948	

Faculty Advisor: W. Larry Kenney, Ph.D.
                                Address: 102 Noll Laboratory
                                Phone: 814-863-1672
Research Assistant: Jane Pierzga, M.S., Research Assistant
                                Phone: 814-865-1236

        This is to certify that I, _____ have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. W. Larry Kenney.

1. Purpose of the study: Prolonged workouts such as marathons or longer races can cause the amount of sodium and water in athlete's blood to change. Sometimes the amount of sodium in an athlete's blood falls too much. This is called exercise-associated hyponatremia (EAH). Severe cases of EAH can cause health problems and hurt performance. EAH may happen in two ways. One is by drinking dilute fluids so that the blood dilutes. A second way could be from the loss of salt from the blood through massive sweating. In this project, we will measure sodium and water balance to gain insight into what causes changes in blood sodium during long workouts in the heat. Also, some research suggests that the sodium in sports drinks is not enough to slow the fall in blood sodium. We will compare the effects of three fluids upon the blood's sodium and water balance during a long workout. The three fluids are:
        1. Flavored water (placebo, contains sugars and coloring)
        2. Gatorade (GTQ)
        3. Gatorade Endurance Formula (GEF, contains more sodium than Gatorade does)
Also, an athlete's state of hydration can impact health and performance. Therefore, we will compare the effects of four states of hydration upon some key measures of the body's well being and the athlete's ability to run. The hydration states are:
        1. Normal hydration   (no change in body water)
        2. 2% Dehydration   (mild loss of body water)
        3. 4% Dehydration   (moderate loss of body water)
        4. 2% Hyper-hydration   (moderate gain in body water)

2. <u>**Procedures:**</u> *You will participate on the circled days. Please read the descriptions of the circled days.*
Then write your initials by the circled days. You may request personnel of the same gender to perform procedures.
We will combine each fluid with each state of hydration. Therefore, you will have 12 trials plus 2 screening visits. This study involves running on a treadmill, and collecting blood and urine samples. The trials are at least 1 week (men) or 1 month apart (women). You may be asked to repeat a trial or screening at the discretion of the researcher or medical staff. You do not have to repeat a trial or screening if you do not wish to. The researcher may request a repeat if something happens that causes the trial to stop or hurts the quality of the data. The researcher could ask for a repeat because of equipment failure, for instance. You will receive payment for every trial you have. You will keep a daily training diary.

_____ **initial** <u>**Screening Day 1:**</u> You do not eat or drink after midnight during the night before your exam. You report to the General Clinical Research Center (GCRC) for your appointment. When you arrive, the staff draws 15 mL (1 Tbsp) of blood from a vein in your arm. You have an examination by the GCRC medical staff that includes blood pressure, check-up, height, weight, and 12-lead ECG**.** We send the blood sample to a lab that tests it for wellness markers. The lab destroys the sample after testing it. If you are a woman of childbearing-age, you submit a urine sample for a pregnancy test.

_____ **initial** <u>**Screening Day 2**</u>: You report to the GCRC for a medical history and graded exercise test (GXT). Bring clothes in which you can exercise. You may use clothing we provide, but we do not provide shoes. GCRC clinical staff is present or in the building. We measure your blood pressure and the electrical activity of your heart. During the test, you wear a nose clip and breathe into a tube to measure the oxygen and carbon dioxide you breathe out. The researcher adjusts the harness that holds the tube so that you are comfortable. During the test, you rate how hard you are working by using a numbered scale matched to short phrases (rating of perceived exertion or RPE scale). For the GXT, you exercise on a treadmill to measure your fitness level. The treadmill's grade increases a little every 2 minutes. The exercise becomes harder. The test is most accurate if you do your best to exercise as long as you can. However, you can stop whenever you want to stop. The test is 10-15 minutes long. The researcher measures the thickness of skin folds at various sites on your body to determine your % body fat.

_____ **initial** <u>**Trials (Days 3 to 14 or 15*):**</u> *Your trials must occur within 30 days of having your height, weight, and % body fat measured. We will re-record the measures before any trial that is not within the 30 days.*
 * A few people may be asked to repeat one trial so that we may learn if the results are the same between trials. If you are asked to repeat a trial, you do not have to repeat the trial if you do not wish to.
Preparation: About 2 days before the each trial begins, you will visit the lab to pick up supplies. If you are a women, you will submit a urine sample for a pregnancy test. We will give you a diet log, a temperature pill (T-pill), and instructions. For 24 hours before the start of the trial, you will eat and drink your normal pre-race diet and record all you

consume in the diet log we give you. However, you will not drink alcohol or caffeinated fluids, or eat caffeinated foods for the 24 hrs prior to the trial. Around 10:00 PM on the night before the trial, you will activate the T-pill and swallow it with fluid. You will not to engage in heavy exercise for 24 hrs prior to the trial. You will write a summary of your activity during the 24 hours on the activity log.

Trial: When you arrive, the medical staff will draw a 7.5 mL (0.5 tbsp) blood sample. We will check the sample to see if you have a normal amount of water and sodium in your body. If you do not the trial is cancelled, and you will be asked to come back another day. If your body's water is normal, you will drink 30 gm (1 oz) of special water that is a little heavier than normal water. Drinking the heavy water enables us to measure the total amount of water in your body. Then you will drink 100 mL (0.4 cups) of distilled water. Then you have a 3-hour wait. You will not eat or drink anything else, and you will collect any urine that you produce during the 3 hours. During this time, you may relax at the lab or you may leave the lab. After the 3 hours, you will consume a breakfast bar, and the rest of the trial begins. During the trial, men wear shorts. Women wear shorts and a sports bra. We can provide this clothing. Bring your running shoes. The medical staff will insert the tube in your arm for the blood samples. The trial proceeds as follows. The timeline is a guide that uses 8:00 AM as your arrival time at the lab after the 3 hour-wait.

Time (min)

8:00 AM   Medical staff inserts a small tube into a vein in your arm through which they will draw the blood samples during the trial. You obtain urine sample 1. We place a heart rate strap around your chest and measure your weight.

8:45 (min 0)   Exercise #1. You enter a room set at 30°C (86°F) and 40% RH. We take blood sample 1 (BS#1), and record your heart rate (HR), T-pill reading (Tc), and blood pressure (BP). Then you run on the treadmill at about 70% of your maximum effort. You will have a fan blowing air on you while you run.

8:55 (min 10)   While you run, we take BS#2, and record HR, Tc, and BP. We also record your rating of perceived exertion (RPE).

9:00 (min 15) Rest #1. You rest sitting in the same room. We measure your weight. You drink fluid if necessary.

9:02 (min 17) Exercise #2. You run on the treadmill. You drink if you did not finish the fluid you had at rest.

9:12 (min 27) We take BS#3, and record HR, Tc, BP, and RPE.

9:17 (min 32) Rest #2. We measure your weight. You drink fluid if necessary. We attach sweat patches to 5 sites (chest, back, forearm, thigh, forehead).

9:19 (min 34) Exercise #3. You run on the treadmill. You drink if you did not finish the fluid you had at rest.

9:29 (min 44) We take BS#4, and record HR, Tc, BP, and RPE.

9:34 (min 49) Rest #3. We measure your weight. You drink fluid if necessary. You complete Sensation Survey #1. We remove the sweat patches from your skin.

9:36 (min 51) Exercise #4. You run on the treadmill. You drink if you did not finish the
                fluid you had at rest.
9:46 (min 61) We take BS#5, and record HR, Tc, BP, and RPE.
9:51 (min 66) Rest #4. We measure your weight. You drink fluid if necessary. We will
                attach a sweat patch to your forehead.
9:53 (min 68) Exercise #5. You run on the treadmill. You drink if you did not finish the
                fluid you had at rest.
10:03 (min 78) We take BS#6, and record HR, Tc, BP, and RPE.
10:08 (min 83)Rest #5. We measure your weight. You drink fluid if necessary. We
                remove the sweat patch from your forehead.
10:10 (min 85)Exercise #6. You run on the treadmill. You drink if you did not finish the
                fluid you had at rest.
10:20 (min 95) We take BS#7, and record HR, Tc, BP, and RPE.
10:25 (min 100)   Rest #6. We measure your weight. You drink fluid if necessary.
10:27 (min 102)   Exercise #7. You run on the treadmill. You drink if you did not
                finish the fluid you had at rest.
10:37 (min 112) We take BS#8, and record HR, Tc, BP, and RPE.
10:42 (min 117)   Rest #7. We measure your weight. You drink fluid if necessary.
                You complete Sensation Survey #2.
10:45 (min 120)   Start the performance run. You decide upon the length of the
                performance run. You run until you cannot run any longer. Then you
                walk 10 min to cool down. We take BS#9, HR, Tc, BP, and RPE right
                after you stop running.
Recovery and   Rest sitting in the same room for 60 min. We weigh you after 30 min.
                You drink fluid if
End   necessary. You complete the Sensation Survey #3, and we take BS#10,
                HR, Tc, BP, and RPE when the 60 minutes end. We remove the tube in
                your arm. You exit the trial-room, and obtain urine sample #2. We will
                give you a snack, and the medical staff will check you out before you
                leave the lab. Showers are available on site if you wish to use them.

Measurements: *You may have a person of the same sex do the measure, if you wish.*
Skin Fold Measurements: Your percent body fat is measured using a tool that looks like
tongs. The tongs gently measure the thickness of skin folds at several places on your
body.
Blood draw: During the screening, skilled staff or nurse will take blood from your arm
using a needle. During the trials, the staff or nurse puts a small plastic tube in your arm
from which to draw blood samples. The staff or nurse uses safety measures and sterile
techniques that are used in hospitals. The staff or nurse draws blood for the screening (15
mL, 1 Tbsp). During trials, you have one 7.5 mL (0.5 Tbsp) baseline blood sample.
Then you have a blood draw every 17 minutes that are 9.5 mL (0.6 Tbsp) each. The total
for each trial is 102.5 mL (6.9 Tbsp). Only about 3% of your blood volume is removed
during each experiment. This blood is rapidly replaced before the next experiment.
Therefore, there is no cumulative deficit over the entire project. The total for the whole
study is about 1.24 L (2.6 pints). If you repeat a trial, an additional 102.5 mL (6.9 Tbsp)
of blood is drawn during that trial. For women, it takes at least year or more to draw this

total amount of blood during the whole study. Women can only be in one trial each month due to their menstrual cycles. The trial is run during the same time of the cycle each month. If a woman's schedule (work, vacation, illness, etc.) makes her skip a month's trial, it may take more than a year to complete all 12 trials. For men, it takes about 3.5 months and likely more to draw this total amount of blood. A man can be in a trial once a week. If a man's schedule (work, vacation, illness, etc.) makes him skip a week's trial, it may take more than 3.5 months to complete all 12 trials.

ECG: The staff attaches twelve ECG electrodes (sticky patches) to your chest on Days 1 and 2 to measure your heart's activity and rate.

Heart Rate (Polar Monitor): The researcher straps a Polar Monitor belt around your chest to measure heart rate.

Blood pressure: A cuff is inflated on your upper arm. The staff slowly releases the air from the cuff and listens to the area at the inside of your elbow with a stethoscope.

Graded Exercise Test (GXT): The test measures your fitness. First, you walk on a treadmill to warm up. Then you run on the treadmill. You can choose the speed at which you walk and run. The treadmill's steepness gets greater every 2 minutes. The staff measures your blood pressure and heart rate. A clip holds your nose shut. You breathe into a tube that collects the air you breathe out. You rate how hard you are working by pointing to a number on a chart (rating of perceived exertion or RPE scale). Although running becomes harder, the test's results are best if you do your best to run as long as you can. You may stop at any time. The test is 10-20 minutes long.

Metabolic Measurements: You breathe into a tube that collects the air you breathe out. The researcher also measures the volume, oxygen, and carbon dioxide you breathe out.

Ratings of Perceived Exertion (RPE) Scale: You point to a number next to the phrase that best describes how hard you are working.

Sensation Survey: The survey has sets of words that may describe how you feel. A line connects the words in each set. You make a mark on the line closer to the words in a set that best describe how you feel. The closer your slash is to the words, the better the words describe how you feel.

Core Temperature (T-pill): The T-Pill has been used for many years. Researchers have used the pill in astronauts, fire fighters, scuba divers, and people climbing mountains. The system has 2 parts:

1. T-pill – The T-pill is a small sensor that uses a radio signal to report the temperature in your body. On the night before each trial, you wake the battery in the T-pill by removing and throwing away the T-pill's wrapper. Then you swallow the T-pill with water as if it were a vitamin. The T-pill is slippery when wet so you must be careful when swallowing it. The T-pill stays inside of your body for about 2 days. After the trial, you look for the T-pill to pass from your body. We will give you an instruction sheet.

2. The recorder – The recorder reads and stores the radio signal from the T-pill. The researcher straps the recorder around your waist.

Body Weight: You dry dripping sweat from your skin and stand on a scale to be weighed.

Urine Sample: Also, you urinate into a container at certain times during the trial.

Diet and Activity Logs: You will record the type and amount of food you eat during the 24 hours before the trial on the diet log. You will also write a brief summary of your physical activity during the 24 hours on the back of the activity log. Honesty and accuracy is very important. We will give you verbal and written instructions.

Total Body Water: We measure the amount of water in your body. To do this, we use special water that is 10% heavier than normal water. A small amount of this heavy water is in your body normally. To make this measure, we take a baseline blood sample. Then you drink 30 gm (1 oz) of the heavy water. Then you drink 100 mL (0.4 cups) of distilled water. The heavy water that you drink goes throughout your body. We measure the amount of heavy water in the blood samples that we collect during the trial.

Sweat Patch: The patch is like a large band-aid that sticks to the skin for the collection of sweat. It is about 5 cm x 7.6 cm (2 in. x 3 in) in size.

3. Discomforts and risks:

Skin Fold Measurements: You may feel embarrassed having this measure.   The researcher makes this measure in a private and professional way.

Blood draw: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or a small clot. You may become lightheaded or may faint. During the trial, the plastic tube used lets blood be drawn without having to stick the vein more than once. Sometimes, the tube stops working, and the staff or nurse must put a new tube in your vein. You may request numbing cream on your skin to reduce the pain from the needle. To keep the chance of infection small, the staff or nurse uses the same methods used in hospitals. In time, your body replaces the solid parts of the blood the staff removes. The body replaces the water in the blood faster. Drinking fluids helps your body to replace the water in your blood. The staff measures elements in the blood samples. The measure can help to show that your body is not harmed by the draws. The total blood drawn for the whole program is about 1.24 L (2.6 pints). For men, the shortest possible period over which we draw this total amount of blood is 13-14 weeks. For women, it will be removed over the course of a year or more.

ECG: The staff tapes ECG wires to your body. There are no risks, but the tape may redden or irritate your skin for a while.

Heart Rate (Polar Monitor): There are no risks to this measurement.

Blood pressure: The researcher uses the method used in a doctor's office. During the short time the cuff is inflated, your arm may feel tingly or numb. Rarely, the cuff may cause a temporary bruise.

Graded Exercise Test (GXT): You will likely have tiredness, sweating, and breathlessness. You will also have increased heart rate and muscle fatigue. You may also have lightheadedness, fainting, nausea, or muscle cramp, but these occur less frequently. More severe reactions include irregular heartbeat, heart attack, and death. These severe reactions are very rare in your age group. It is possible for you to stumble or fall on the treadmill leading to cuts, scrapes, dislocations, broken bones, head injury, abnormal heart rhythms, or even death. You will be taught the safe use of the treadmill and watched closely during the test. All changes in speed will be made slowly, and you will be assisted on and off the treadmill.

Metabolic Measurements: The tube's holder can feel awkward. You may feel disturbed by the slight change in airflow due to the valves in the tube. These changes do not last long.

Ratings of Perceived Exertion (RPE) Scale and Sensation Survey: You should not worry if your answer is "right enough." The only "right" answer is one that best describes how you feel.

Sensation Survey: You may feel shy about your feelings or think that there is a right or wrong response. The only right response is one that accurately and truthfully describes how you feel. The researcher handles your data in a professional manner.

T-pill: Swallowing the T-pill presents risks like that of taking a vitamin pill such as choking or gagging on the pill or water. You know that the pill becomes slippery when wet so you must be careful. There have been no reports of abdominal problems with the T-pill. However, the pill could cause cramps, irritation, blockage, or infection. You may feel shy about watching for the T-pill to pass. However, seeing the T-Pill pass is the best way to know for sure that it has left your body. If you have not seen the T-pill pass within 4 days, you report to the lab. The researcher uses the recorder to check for the T-pill's presence. The recorder's failure to find a radio signal at this time likely shows that the T-pill passed without your seeing it. In the unlikely event of severe stomach or intestinal problems after the 4 days, an x-ray may be needed to show for sure that the pill has passed from your body. If the T-pill fails to pass from your body, you may need surgery to remove it. Only once has a T-pill been removed by surgery. This happened years ago in another lab. Before swallowing the T-pill, the person had surgery that made a pocket in his gut. The T-Pill became stuck in the pocket. You will not be in this study if you have had surgery in your abdomen. Magnetic Resonance Imaging (MRI) is a medical test that can cause the T-pill to overheat and be dangerous. You cannot have an MRI within 2 weeks after having swallowed a T-pill unless you have seen the T-Pill pass from your body. If you need an MRI and have not seen the T-Pill pass, you will tell your doctor that you have swallowed a T-Pill.

Body Weight: You may feel embarrassed having this measure. The researcher makes this measure in a private and professional way.

Urine Sample: You may feel put upon or shy about collecting the urine. However, urine collection is an important part of the study. The researcher gives you instructions and handles the samples in a professional manner.

Diet and Activity Logs: You may feel put upon or shy about recording your diet and activity. However, keeping track of your diet and activity is an important part of the study. The researcher gives you instructions and handles your data in a professional manner.

Total Body Water: The fancy name for this water is deuterium oxide. Despite its fancy name, this water is not radioactive. A small amount of this heavy water is in your body normally. Heavy water has been used in our lab and is used routinely in many labs to measure the amount of water in a person's body. The heavy water that you drink goes though out your body. You will drink 30 gm or (1 oz) for each trial and a total of 360 gram (12.7 oz) over the course of the entire study. If you weigh 70 kg (154 lb), this amount would cause 0.075% of your body's fluids to be heavy water on the day of the trial. If you weigh 54 kg (120 lb), this amount would cause 0.097% of your body's fluids to be heavy water on the day of the trial. Although heavy water eventually leaves your body like normal water does, if none of the heavy water were to leave your body over the course of all 12 trials, only 0.51% of your body's fluids would be heavy water by the end of the project if you weigh 70 kg (154 lb). If you weight 54 kg (120 lb), your total would

be 1.16%. There are no known risks from drinking this small amount of heavy water. To obtain 10% heavy water in the body, a 70 kg (154 lb) human would need to quickly drink 5 L (1.3 gallons) of pure heavy water. This is highly unlikely to occur even by accident. Heavy water levels as high as 23% in humans are known to be safe.

Latex: Some gloves and medical materials are made of latex rubber. You will inform us if you are allergic to latex and decline to participate in the study.

Sweat Patches: There are no risks, but the tape may redden or irritate your skin for a while.

4. a.   Benefits to me: You will learn about your personal health and fitness. This knowledge can help to maintain or enhance your personal training and provide feedback. You will also learn about the role of hydration in endurance running. The results of the study could provide information to you about effective ways to best maintain optimal hydration status in endurance running.

   b. Potential benefits to society: The results of this study could assist athletes and those who work with them. The study can provide data about safe and ideal levels of hydration. This research could yield ways for keeping the best level of hydration. The results could help coaches and athletes to reduce the impact of poor hydration on race results and health. Also, this study could help to define when reduced hydration has real effects.

5. Alternative procedures that could be utilized: The researcher could measure the warmth of your body in other ways besides the T-pill. Some of these techniques are not accurate enough for this project. Some of the techniques cannot be used when someone works out or drinks. Some of these techniques are like the T-pill in many ways, but are more unpleasant. The other techniques in the study are used in research worldwide. They are the best means by which to meet the goals of this study with minimal discomfort and risk to you.

Time duration of the procedures and study: You will need to visit the Noll Lab for the following:

_____   initial Day 1 is for the first part of the screening that should last no longer than 1/2 hour.

_____   initial   Day 2 is for the 2nd part of the screening that should last no more than 1 hour.

_____   initial   Days 3-14 : Trials 1-12: about 7 hours each + 0.5 hours to pick up supplies in advance.

The total number of hours for the entire study is 91.5. For women, it takes at least a year to complete the whole study. For men, it takes at about 3.5 months. The study could take longer depending upon your schedule.

_____   initial Day 15 Repeatability Trial. You may be asked to repeat one trial so that we may learn if the results are the same between trials. If you are asked to repeat a trial, you do not have to repeat the trial if you do not wish to.

7. Statement of confidentiality: The data is available only to the investigators. Volunteers are coded by an identification number for statistical analyses. All records are kept in a secure location. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records (e.g., such as records maintained by physicians, hospitals, etc.), and in the event of any publication resulting from the research no personally identifiable information will be disclosed. The Office of Human Research Protections in the U.S. Department of Health and Human Services, the U.S. Food and Drug Administration (FDA), the Office for Research Protections at Penn State and the Biomedical Institutional Review Board may review records related to this project.

8. Right to ask questions: If you have any questions or concerns about the research or your participation in the present investigation, or feel that you have been harmed by the research, you may contact Lindsay Baker (W: 814-8839454, H: 814-238-4349) or Jane Pierzga (W: 814-865-1236, H: 814-692-4720). If there are findings during the research that could relate to your wanting to help with the study, you will be told of the findings. You can also call this number if you have complaints or concerns about the research. If you have questions about your rights as a research participant, or you have concerns or general questions about the research, contact The Pennsylvania State University's Office for Research Protections at (814) 865-1775. You may also call this number if you cannot reach the research team or wish to talk to someone else.
You have been given an opportunity to ask any questions you may have, and all such questions or inquiries have been answered to your satisfaction.

9. Compensation:
You will receive $50.00 for each trial. Also, you will receive an additional amount for the performance run at the end of each trial at the rate of $1.00/minute. You will receive a lab T-shirt after completing 4 trials and a cinch-bag after completing 8 trials. You will receive an extra $50.00 after completing all 12 trials. For each trial, you are paid an amount of money equal to the part of the trial that you complete. For instance, if you complete only half of a trial you will be paid $25.00 for that trial. This is because $25.00 is one half of $50.00. The total amount that you can be paid for all 12 trials is $650.00 (at least) to $900.00 or more depending upon your performance runs. You may be asked to repeat a trial. If you agree to repeat a trial, you will be paid for the repeated trial as stated above.
Total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. Voluntary participation: Your being in this study is voluntary. You may withdraw from this study at any time by telling the researcher. If you decide to withdraw or refuse to participate, you will not have a penalty or loss of benefits you would receive otherwise. You may decline to answer certain questions. You may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures. The researcher may end

your role in the study without your consent if the researcher deems that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document.

11. <u>**Injury Clause:**</u> In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

12. <u>**Abnormal Test Results:**</u> In the event that abnormal test results are obtained, you will be apprised of the results immediately and recommended to contact your private medical provider for follow-up.

You must be 18 years of age or older to take part in this research study. If you agree to take part in this research study and have read the information outlined above, please sign your name and indicate the date below.
You will be given a copy of this signed and dated consent for your records.

_____

Volunteer   **Date**

I, the undersigned, have defined and explained the studies involved to the above volunteer.

_____

INVESTIGATOR   **DATE**

Dr. Kenney is the Chair of the Scientific Advisory Board for Gatorade Sports Science Institute.

Basketball Performance Study

Informed Consent Form for Clinical Research Study
The Pennsylvania State University

Title of Project: Effect of Hydration Status on Basketball
　　　　　　 Performance: 16-30 Year-Old Men

Sponsor: National Basketball Association

Principal Investigator:　　 W. Larry Kenney, Ph.D.　　 W: 814-863-1672
Other Investigator:　　　 David Conroy, Ph.D.　　　 W: 814-863-3451
Graduate Students:　　　 Lindsay Baker　　 W: 814-863-2948, H: 814-238-4349
　　　　　　　　　　　　 Kelly Dougherty　 W: 814-863-2948, H: 814-238-8626
Research Assistant:　　　 Jane Pierzga　　 W: 814-865-1236, H: 814-692-4720

This is to certify that I, _____, have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. W. Larry Kenney.

1. Purpose of the study: Playing sports places demands upon the bodies and minds of athletes. Athletes' abilities to meet these demands affect how well they perform. Food, rest, drink, and other factors can affect an athletes' success. This study explores the effect of dehydration on how well a basketball player's body and mind performs. Dehydration is when the amount of water in the body is less than normal. This study has 6 trials. Four trials look at the affect of 4 states of dehydration on playing basketball. These trials cause dehydration by having the player workout in a hot room until the desired amount of his body's water is lost through sweating. The amount of water-loss is tracked by weighing the body before and during the workout. Two more trials look at the effect of 2 drinks (sports drink and flavored water+sodium) on how well players perform. The amount of water in the body stays normal for these 2 trials. All trials use basketball drills to create a mock basketball game to test how well the player's body performs. Also, this study uses a computer program to test the player's alertness and thinking. A blood sample is taken to make sure that the player is able to be in the study. Other blood samples track changes caused by dehydration and drinking. For each trial, the player swallows a special pill that measures the warmth inside the body.

2. Procedures to be followed: *You will participate on the circled days. Please read the descriptions of the circled days and procedures. Then write your initials by the circled days and procedures.*
You will have 2 days of screenings and 6 days of trials. The trials are at least about 1 week apart. You may be asked to repeat a trial or screening at the discretion of the researcher or medical staff. You do not have to repeat a trial or screening if you do not wish to. The researcher may request a repeat if something happens that causes the trial to

stop or hurts the quality of the data. The researcher could ask for a repeat because of computer failure, for instance.

If you are not allergic to lidocaine, you may request that the researcher use numbing cream for the insertion of any needles into your skin.

_____initial **Day 1 (Screening 1):** On the evening before your first visit to the Noll Lab, you do not eat or drink anything after 9 PM. During your first visit, report to the General Clinical Research Center (GCRC). The staff or nurse draws 15 mL (1 Tbsp) of blood from your arm to check your state of wellness. After the blood draw, you receive a breakfast bar and juice, if you wish. You have your blood pressure, height, weight, and resting ECG measured. The researcher measures the thickness of skin folds at various sites on your body to determine your % body fat.

_____initial **Day 2 (Screening 2):** You will eat a light breakfast before coming to the lab. You will bring shorts, t-shirt, and shoes in which you can run. You will have a check-up that includes your health history by the GCRC medical staff. The researcher measures your maximum vertical leap. A graded exercise test (GXT) measures your fitness. You run on a treadmill during the test. The treadmill's steepness gets greater every 2 minutes. The researcher straps a heart rate monitor around your chest. The staff measures your blood pressure and heart rate. You wear a nose clip and breathe into a tube so the researcher can collect the air you breathe out. You rate how hard you are working by using a numbered scale (rating of perceived exertion or RPE scale). Although, running becomes harder, the test's results are best if you do your best to run as long as you can. However, you may stop at any time. The test is about 12 minutes long.

_____initial **Days 3 – 8 (Trials):**

Trials:

Dehydration Trials: On 4 days, you will workout in a warm, dry room (40 C or 104 F; 20% relative humidity) to decrease your body's water by 1%, 2%, 3%, or 4%. The researcher checks how much water you lose by weighing you during the workout. When you have lost enough water, you drink water during the rest of the workout to prevent more weight loss.

Normal Hydration Trials: On 2 days, you will workout in a warm, dry room (40 C or 104 F; 20% relative humidity). You will drink to keep your body weight the same. The researcher checks how much water you lose by weighing you during the workout and gives you the right amount of drink.

Trial Procedure: You activate and swallow the temperature pill during the evening before the study. Then you do not eat or drink for the rest of the evening before the trial. When you arrive at the lab, the staff measures your heart rate and blood pressure. The researcher straps a heart rate monitor around your chest. The trial proceeds as follows. The timeline is a guide.

7:30 – 8:00 AM	You eat a standard breakfast supplied by the lab.
	Measurements: weight, urine sample.
8:00-8:20 AM	Computer test (CPT test).
8:30-11:30 AM	You workout in a warm, dry room (40 C or 104 F; 20% relative humidity).
	Workout: Bike, treadmill.

Measurements: weight, heart rate, blood pressure, body temperature, rating of perceived exertion (RPE), blood samples, urine sample, fatigue survey.

Depending upon the trial, you may or may not drink during the workout.

11:30 AM -12:30 PM You rest at normal room temperature. The researcher gives you

the right amount of drink to keep the right amount of water in your body.

Measurement: body temperature, heart rate, Computer test (10:30-10:50 AM).

12:30-1:30 PM The researcher takes you to a basketball court. You perform basketball drills.

Measurements: Drill performance rating, RPE, heart rate, body temperature, urine sample, weight, fatigue surveys at halftime and after drill session.

1:40-2:00 PM You rest at normal room temperature

Measurement: Computer test

After the trial, you will be fed a standard lunch. Your heart rate and blood pressure are measured before you go home.

Measurements: *You may have a person of the same sex do the measure, if you wish.*

Skin Fold Measurements: Your percent body fat is measured using a tool that looks like tongs. The tongs gently measure the thickness of skin folds at several places on your body.

Blood draw: During the screening, skilled staff or nurse will take blood from your arm using a needle. During the trials, the staff or nurse puts a small plastic tube in your arm from which to draw blood samples. The staff or nurse uses safety measures and sterile techniques that are used in hospitals. The staff or nurse draws blood for the screening (15-mL, 1 Tbsp). During trials, you have a blood draw every 20 minutes (15-mL, 1 Tbsp each) while you workout in the warm room. There are 10 blood draws in each trial (150 mL, 10 Tbsp). The total amount of blood drawn for the entire program is: 915ml, (3.87 cups or 1.9 pints).

ECG: The staff attaches twelve ECG electrodes (sticky patches) to your chest on Day 1 to measure your heart's activity and rate.

Heart Rate (Polar Monitor): The researcher straps a Polar Monitor belt around your chest to measure heart rate.

Blood pressure: A cuff is inflated on your upper arm. The staff slowly releases the air from the cuff and listens to the area at the inside of your elbow with a stethoscope.

Graded Exercise Test (GXT): The test measures your fitness. First, you walk on a treadmill to warm up. Then you run on the treadmill. You can choose the speed at which you walk and run. The treadmill's steepness gets greater every 2 minutes. The staff measures your blood pressure and heart rate. A clip holds your nose shut. You breathe into a tube that collects the air you breathe out. You rate how hard you are working by pointing to a number on a chart (rating of perceived exertion or RPE scale). Although

running becomes harder, the test's results are best if you do your best to run as long as you can. You may stop at any time. The test is 10-20 minutes long.

Metabolic Measurements: You breathe into a tube that collects the air you breathe out. The researcher also measures the volume, oxygen, and carbon dioxide you breathe out.

Ratings of Perceived Exertion (RPE) Scale: You point to a number next to the phrase that best describes how hard you are working.

Fatigue Survey: The survey has sets of words that may describe how you feel. A line connects the words in each set. You make a mark on the line closer to the words in a set that best describe how you feel. The closer your slash is to the words, the better the words describe how you feel.

Core Temperature (T-pill): The T-Pill has been used for many years. Researchers have used the pill in astronauts, fire fighters, scuba divers, and people climbing mountains. The system has 2 parts:

1. T-pill – The T-pill is a small sensor that uses a radio signal to report the temperature in your body. On the night before each trial, you wake the battery in the T-pill by removing and throwing away the T-pill's wrapper. Then you swallow the T-pill with water as if it were a vitamin. The T-pill is slippery when wet so you must be careful when swallowing it. The T-pill stays inside of your body for about 2 days. After the trial, you look for the T-pill to pass from your body.

2. The recorder – The recorder reads and stores the radio signal from the T-pill. The researcher straps the recorder around your waist.

Continuous Performance Test (CPT): This tests your alertness and thinking. During the test, you will react to target on a computer by pushing a button.

Body Weight: You dry dripping sweat from your skin and stand on a scale to be weighed.

Urine Sample: You urinate into a container at certain times during the trial.

Basketball Drills: Most basketball players know these drills. The researcher makes sure that you know how to do the drills. You need to use your best effort. The drills mock a basketball game and are described below.

1 1/3 hours total: Four 12 min quarters, 10-min break between quarters, 15-min break between halves

1st Quarter
• *shooting* – X-out lay-ups (start at elbow, dribble in for lay-up, dribble to opposite elbow, dribble in for lay-up, repeat) – record # makes in one minute
• 1.5 min rest
• *speed* - one "suicide" – measure time to completion (suicide = a series of runs: baseline to foul line and back; to mid court and back; to opposite foul line and back; full court and back)
• 1.5 min rest
• *explosiveness* - vertical jumps – hit target on wall (80%max) 10 times – measure time to completion
• 1.5 min rest
• *agility* - defensive slides (zigzags) two lengths of court – measure time to completion
• 1.5 min rest
• *shooting* – around the world – record # makes in one minute

• 1.5 min rest
• *combo* – around perimeter and on midline of court: sprint forward, defensive slides across midline, sprint forward, defensive slides across baseline, backpedal to midline, defensive slides across, backpedal to baseline, defensive slides across
● 10 minutes (rest/drink)

2nd Quarter
• *shooting* – 3 pointers (from 7 spots) – record # makes in one minute
• 1.5 min rest
• *speed* – run width of court 10 times – measure time to completion
• 1.5 min rest
• *explosiveness* - vertical jump – record best of 3 (express as %max)
• 1.5 min rest
• *agility* – 20 lane slides (defensive slides across width of key) – measure time to completion
• 1.5 min rest
• *shooting* – free throws - record # makes out of 10 attempts
• 1.5 min rest
• *combo* – around the key: sprint forward, diagonal defensive slides to baseline, sprint forward, diagonal defensive slides to baseline, repeat – measure time to complete 5
● 15 minute halftime (rest, drink)

3rd Quarter – Repeat 1st Quarter drills
● 10 minutes (rest,drink)

4th Quarter – Repeat 2nd Quarter drills

3. Discomforts and risks:
Skin Fold Measurements: You may feel embarrassed having this measure. The researcher makes this measure in a private and professional way.
Blood draw: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or a small clot. You may become lightheaded or may faint. During the trial, the plastic tube used lets blood be drawn without having to stick the vein more than once. Sometimes, the tube stops working, and the staff or nurse must put a new tube in your vein. You may request numbing cream on your skin to reduce the pain from the needle. To keep the chance of infection small, the staff or nurse uses the same methods used in hospitals. In time, your body replaces the solid parts of the blood the staff removes. The body replaces the water in the blood faster. Drinking fluids helps your body to replace the water in your blood. The staff measures elements in the blood samples. The measure can help to show that your body is not harmed by the draws. The total blood drawn for the whole program is: 915ml, (3.87 cups or 1.9 pints).
ECG: The staff tapes ECG wires to your body. There are no risks, but the tape may redden or irritate your skin for a while.
Heart Rate (Polar Monitor): There are no risks to this measurement.

Blood pressure: The researcher uses the method used in a doctor's office. During the short time the cuff is inflated, your arm may feel tingly or numb. Rarely, the cuff may cause a temporary bruise.

Graded Exercise Test (GXT): You will likely have tiredness, sweating, and breathlessness. You will also have increased heart rate and muscle fatigue. You may also have lightheadedness, fainting, nausea, or muscle cramp, but these occur less frequently. More severe reactions include irregular heartbeat, heart attack, and death. Severe reactions are very rare in your age group. It is possible for you to stumble or fall on the treadmill leading to cuts, scrapes, dislocations, broken bones, head injury, abnormal heart rhythms, or even death. You will be taught the safe use of the treadmill and watched closely during the test. All changes in speed will be made slowly, and you will be assisted on and off the treadmill.

Metabolic Measurements: The tube's holder can feel awkward. You may feel disturbed by the slight change in airflow due to the valves in the tube. These changes do not last long.

Ratings of Perceived Exertion (RPE) Scale and Fatigue Survey: You should not worry if your answer is "right enough." The only "right" answer is one that best describes how you feel.

Core Temperature (T-pill): Swallowing the T-pill presents risks like that of taking a vitamin pill such as choking or gagging on the pill or water. You know that the pill becomes slippery when wet so you must be careful. There have been no reports of abdominal problems with the T-pill. However, the pill could cause cramps, irritation, blockage, or infection. You may feel shy about watching for the T-pill to pass. However, seeing the T-Pill pass is the best way to know for sure that it has left your body. If you have not seen the T-pill pass within 4 days, you report to the lab. The researcher uses the recorder to check for the T-pill's presence. The recorder's failure to find a radio signal at this time likely shows that the T-pill passed without your seeing it. In the unlikely event of severe stomach or intestinal problems after the 4 days, an x-ray may be needed to show for sure that the pill has passed from your body. If the T-pill fails to pass from your body, you may need surgery to remove it. Only once has a T-pill been removed by surgery. This happened years ago in another lab. Before swallowing the T-pill, the person had surgery that made a pocket in his gut. The T-Pill became stuck in the pocket. You will not be in this study if you have had surgery in your abdomen.

Magnetic Resonance Imaging (MRI) is a medical test that can cause the T-pill to overheat and be dangerous. You cannot have an MRI within 2 weeks after having swallowed a T-pill unless you have seen the T-Pill pass from your body. If you need an MRI and have not seen the T-Pill pass, you will tell your doctor that you have swallowed a T-Pill.

Continuous Performance Test (CPT): The test may be boring. The test only measures your current alertness and thinking. These can be changed by many factors. The test does not measure how smart you are.

Body Weight, Urine Sample: You may feel embarrassed having this measure. The researcher makes this measure in a private and professional way.

Basketball Drills: The drills make you sweat and increase your heart rate and blood pressure. The drills make you feel fatigued and breathless. This is part of playing basketball and running drills. You may stop at any time. Working your body can produce strains, sprains, and soft tissue tears. Head/neck injury and broken bones are

possible. Since the drills mock a basketball game, you are not exposed to many of the parts of the game that can result in such injuries. Therefore, the risk of these injuries is less than that from a real game. The screening will further reduce the unlikely risk of serious heart or breathing problems.

Dehydration: Dehydration means having less water than normal in the body. Researchers track the body's water-loss by measuring the change in body-weight. Children and adults lose water from their bodies when they workout in the heat. People have studied water-loss in children. In one a study, children lost 1-2% of their body weight when they worked in the heat. In another study, researchers observed children 8 to 17 years of age in a sporting event. Over 1/3 of the children lost over 1.5% of their body-weight. Some lost more than 3% of their body-weight. The water-loss in this study can make you feel thirsty. You may have a headache or a mild stomach cramp.

Exercise in the heat: You will feel hot and may get thirsty. Tiredness, sweating, breathlessness, increased heart rate, and muscle fatigue are normal. There is a small chance that the workouts may cause nausea, dizziness, or muscle cramps. The remote chance of irregular heartbeats, heart attack, or death is less than that for the GXT. You will tell the researcher about any problems and may stop at any time.

Numbing Cream: You will not use the numbing cream if you are sensitive to lidocaine. Anbesol and Orajel are common drugs similar to the numbing cream. When you use the cream, all feelings within the treated area are blocked. Therefore, you must avoid scratching, rubbing, or exposing the treated sites to very hot or cold temperatures at until complete sensation has returned. During or right after the cream is applied, mild swelling, skin redness or strange feelings may develop at the site of treatment. In studies on intact skin of cream-treated subjects, one or more such local reactions were noted in 56% of patients. The reactions were mild and short-lived. The reactions stopped without help in 1 or 2 hours. No serious reactions resulted from the use of the cream. Allergic reactions, although rare, can occur. Whole body adverse reactions from correct use of the cream are unlikely due to the small dose absorbed. If effects do occur, they are like those seen with other local numbing agents. These may include feeling faint, nervous, dizzy, or sleepy. You could also have twitching, and vomiting. Reactions may be brief or not all.

4. a. Benefits to you: **You will receive information about your health and fitness. You will be given an assessment of your basketball skills. You also learn about the importance of hydration in the performance of basketball.**

   b. Potential benefits to society: **The results of this study can benefit coaches, players, and others who care about the basketball athlete. We will learn how reduced water in the body affects the athlete. Also, this study will suggest the better of 2 drinks to fight these affects. This study could cause people to think about the impact of decreased water in the body on athletes in other sports.**

5. **Alternative procedures which could be utilized:**
The researcher could measure the warmth of your body in other ways besides the T-pill. Some of these techniques are not accurate enough for this project. Some of the techniques cannot be used when someone works out or drinks. Some of these techniques are like the T-pill in many ways, but are more unpleasant. The other techniques in the

study are used in research worldwide. They are the best means by which to meet the goals of this study with minimal discomfort and risk to you.

6. Time duration of the procedures and study:
This study requires 8 visits. Day 1 (screening 1) will take about 4 hours. Day 2 (screening 2) will take about 4 hours. Sessions 3-8 (trials) will last about 8 hours each. The trials are at least about 1 week apart.

7. Statement of confidentiality: The data is available only to the investigators and authorized personnel of Penn State University's Office of Research Protections and Institutional Review Board. Volunteers are coded by an identification number for statistical analyses. All records are kept in a secure place. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records (e.g., such as records maintained by physicians, hospitals, etc.), and in the event of any publication resulting from the research no personally identifiable information will be disclosed.

8. Right to ask questions: If you have any questions or concerns about the research or your participation in the present investigation, please contact Lindsay Baker (W: 814-863-2948, H: 814-238-4349), Kelly Dougherty (W: 814-863-2948, H: 814-238-8626), or Jane Pierzga (W: 814-865-1236, H: 814-692-4720). If there are findings during the research that could relate to your wanting to help with the study, you will be told of the findings. You may contact the Office for Research Protections, 212 Kern Graduate Building, University Park, PA 16802, (814) 865-1775 for additional information concerning your right as a research participant.

 I have been given an opportunity to ask any questions I may have, and all such questions or inquiries have been answered to my satisfaction.

9. Compensation: You are paid $150.00 for each of the first 4 trials. Then you are paid $200.00 each of the last 2 trials. For each trial, you are paid an amount of money equal to the part of the trial that you complete. For instance, if you complete only half of the 3rd trial, you are paid $75.00 for that trial. This is because $75.00 is one half of $150.00. The total amount that you can be paid is $1,000.00. You get a T-shirt. You may be asked to repeat a trial. If you agree to repeat a trial, you will be paid an additional $200.00 for each trial that you repeat.

If you are an employee of Penn State University, the compensation you receive for participation will be treated as taxable income and therefore taxes will be taken from the total amount. If you are not employed by Penn State University, total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. Injury Clause: Medical care is available in the event of injury resulting from research but neither financial compensation nor free medical treatment is provided. You

are not waiving any rights that you may have against the University for injury resulting from negligence of the University or the investigators. Questions regarding this statement or your rights as a subject of this research should be directed to the Office for Research Protections in 212 Kern Building, University Park, PA (814-865-1775).

11. Voluntary participation: Your participation in this study is voluntary, and you may withdraw from this study at any time by telling the investigator. Your withdrawal from this study or your refusal to participate will in no way affect your care or access to medical services. You may decline to answer specific questions. However, your acceptance into the study may be contingent upon answering these questions. Your helping with the study may be ended without your consent if the researcher deems that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Office for Research Protections and agreed upon by you in this document

12. In the event that abnormal test results are obtained, you will be told of the results immediately and told to contact your private medical provider for follow-up.

This is to certify that I am 18 years of age or older, and I consent to and give permission for my participation as a volunteer in this program of investigation. I understand that I will receive a signed copy of this consent form. I have read this form, and understand the content of this consent form.

Participant Signature/Legal Guardian   Date

I, the undersigned, have defined and explained the studies involved to the above volunteer.

Person obtaining consent   Date

Dr. Kenney is a member of Gatorade's Sports Medicine Review Board.

VITA

Lindsay Brooke Baker

Education:

The Pennsylvania State University   May 2008
        Ph.D., Kinesiology

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        B.S., Biology

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Publications:

Baker, L.B., T.A. Munce, and W.L. Kenney. Sex differences in voluntary fluid intake by older adults during exercise. *Med. Sci. Sports Exerc.* 37(5):789-796, 2005.

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