Sun Radiation in Moderate Environmental Conditions Does Not Affect Fluid Balance

in Female Collegiate Soccer Players

by

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ABSTRACT

Exposure to sun radiation (SUR) with ambient temperature may be an influencer on athletes' sweat loss in different environments, but the results are not currently known. The purpose of this study was to determine the effects of SUR on fluid balance (FB) and hydration status (HS) in athletes exercising indoors and outdoors.

Initial FB and HS were assessed in NCAA-DI female soccer athletes (n=10) of a single team in temperate, dry conditions (55-68°F, 18-48% humidity) who were monitored during 3 practices of equal estimated energy expenditure (EE): two outdoors in direct SUR (cold/moderate temperatures) and one indoors without SUR (moderate temperatures). Humidity, temperature, and wet bulb globe temperature (WBGT - ameasurement partly based on SUR, including ambient temperature/relative humidity) were recorded using Heat Stress Meters placed in the direct sun or in the shade. Each athlete's semi-nude dry body weight was recorded before and after exercise. Urine samples were taken before, after, and the morning after. Urine specific gravity (USG) was tested to assess HS. Athletes wore combined heart rate and activity monitors to estimate EE and were provided *ad libitum* water and/or a zero-calorie sports drink. Their total intake included weights of consumed food and drink. Sweat rate was calculated using body weight change and intakes of liquids minus urine losses/hour. Two-way repeated measures ANOVA analyzed group-level differences. No significance was found in total FB (1.01±0.32 L/hr) or EE/hr (444±97.1 kcal/hr) across all days (p>0.05). In analyzing individual athlete results, 40% had consistent USG >1.025 (p=0.001) suggesting potential dehydration. These 4 athletes selected water as their beverage, of which is known that consuming only water does not stimulate drinking

behavior as does electrolyte drinks. The remaining 60% were overall not dehydrated (USG <1.025) but must be aware of incidental dehydration in hotter temperatures. The conclusion is that in low-moderate temperatures, athletes self-regulate drinking habits and achieve fluid balance during exercise with or without sun radiation. However, athletes with average USG >1.025 are likely to remain dehydrated in moderate temperatures. The findings suggest that more education would benefit these athletes by ensuring hydration in any environment.

DEDICATION

This work is dedicated to my family, specifically my parents, without whose support and encouragement throughout my entire life I would not have been able to accomplish all that I have today. My interest in nutrition and exercise has grown and flourished through my years in school and in the opportunities I have had to share my knowledge and skills with those around me. Moving across the country to complete my schooling and internship was an impossible dream, but now is my rewarding reality. My family's continued love and encouragement will carry me to realize all of my potential, and for that I am forever grateful.

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CHAPTER 1

INTRODUCTION

Dehydration, also known as hypohydration, is an overlooked incident that poses many unfavorable risks for the performance and cognition of competing athletes. Even with a water loss of 1% of total body weight, athletes can begin to experience negative effects on their performance in practices and games, both physically and mentally (Bardis, Kavouras, Arnaoutis, Panagiotakos, & Sidossis, 2013; Logan-Sprenger, Heigenhauser, Killian, & Spriet, 2012). Strategies to ensure proper hydration are urgently needed to promote and protect the health of all athletes.

The problem found across a variety sport types is that while dehydration – a deficiency of fluids in the body – is well known, still too many athletes are dehydrated before, during, and after activities (Casa et al., 2000). When transitioning quickly from practices to games in a player's on-season, when they are training for and competing in games, many athletes never rehydrate completely and thus may remain in a cycle of light, moderate, or even extreme dehydration for consecutive days (Rosinger, 2015).

Recent research has determined that an extraordinary number of athletes perform at levels under euhydration (proper hydration) (Bardis, Kavouras, Arnaoutis, et al., 2013; Bardis, Kavouras, Kosti, Markousi, & Sidossis, 2013; Davis et al., 2015; Goulet, 2013), where the athlete is in a state of fluid balance (fluid intake aligns with fluid output). Both food and fluid are calculated as the input substances while outputs can occur as insensible losses from the lungs and skin, or obligatory losses from normal kidney functions (Cheuvront, Kenefick, Charkoudian, & Sawka, 2013).

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As water balance decreases, the state of hypohydration may cause negative side effects such as fatigue, cramps (Utter, McAnulty, Riha, Pratt, & Grose, 2012), heat exhaustion and heat stroke (Bardis et al., 2013; Fernandez-Elias et al., 2014; Thomas, Erdman, & Burke, 2016), and decreased cognitive function (Thigpen, Green, & O'Neal, 2014). In extreme cases loss of consciousness and death can occur if an athlete does not safely and properly rehydrate (Thomas et al., 2016).

An athlete's hydration status can be measured in many ways, using both laboratory-based methods and field-based methods. Field measurements are essential to researchers because of their practicality and relevance to a training or competing athlete; they can be performed on-site, produce instantaneous results, and prevent an athlete from having to enter a laboratory setting to produce samples. Field measurements are also comparably less expensive than transporting samples to be analyzed in a lab.

One manner of measuring hydration status that can be performed as a field measurement is using urine specific gravity (USG) (Arnaoutis et al., 2015), a measurement of salt and protein in the urine. USG values indicate hydration anywhere from overly hydrated to overly dehydrated. Along with the color of urine, which can be compared to a standardized color chart, an athlete's hydration status is easily determined during practices or games. A study which intentionally caused dehydration in athletes found that using USG values accurately depicted their hydration status (Hamouti, Del Coso, & Mora-Rodriguez, 2013). Since even slight levels of dehydration cause a myriad of problems in athletes across all different sport types, understanding and using accurate hydration tests are essential to potentially prevent these problems from ever occurring. A publication of Armstrong classified the USG values to represent different states of hydration. A USG value between 1.013-1.029 indicated normal hydration, and hypohydration was indicated >1.029 (Armstrong et al., 1994). A more widely accepted range was proposed in 2001 which defined euhydration between 1.006 and 1.020, with USG values above 1.020 signifying the start of hypohydration (Casa et al., 2000; Popowski et al., 2001; Zubac, Antelj, Olujic, Ivancev, & Morrison, 2017). USG reference ranges may be set specifically for gender, age, and activity level. For example, male wrestlers are required by the NCAA to be euhydrated (\leq 1.020) in order to be eligible for competition in their weight class (Armstrong et al., 2010).

Color is an additional measurement that can easily be performed in field settings. Observing and recording the color of an athlete's urine sample is a non-invasive, instantaneous method for determining hydration status. Researchers can use validated color charts to compare against the color of a urine sample and conclude if it equates to proper euhydration, hypohydration, or even hyperhydration. Athletes are also able to monitor their own urine with this method to quickly determine their hydration status and make adjustments to drinking behaviors if needed surrounding times of exercise.

For all athletes, whether they are aware of their hydration status or not, there are standard recommendations for fluid intake during exercise based upon length of time and intensity. These recommendations are generally broad, such as those set by the National Athletic Trainer's Association (NATA): consuming approximately 200-300 mL of fluid every 20-30 minutes; completing rehydration within 2 hours of exercise; including carbohydrates and sodium with exercise exceeding 2 and 4 hours, respectively; and with

any athlete, educating on the effects that dehydration has on sports performance (Casa et al., 2000). However, NATA also suggests considering additional factors – individual anthropometrics, exercise duration and intensity, environment, breaks and access to fluids, and fluid preferences – to establish accurate and personalized protocols.

Despite the past research that has revealed much about the effects of hypohydration on athletes under different environmental circumstances, no research has focused on athletes who practice or play in both indoor and outdoor conditions and the differences in hydration status between these two environments. As sunlight appears to be an important factor for fluid loss, a difference should be found in athletes training inside and outside under the same environmental temperature.

The lack of research warrants a further look into the correlation between athletes who train and compete both indoors and outdoors and how well hydrated they are during exercise. Many athletes are not counseled on rehydration methods; many more are only given a general regimen to adhere to following any type of exercise, regardless of the environment or any personal factors. Finding a difference between the hydration status or fluid balance of these athletes would justify a change in the recommendations for rehydration practices as athletes may need to hydrate more after exercising with exposure to the sunlight than they would without it.

Study Purpose

The objective of this study was to examine environments and their relationship to athletic performance, specifically measuring hydration status and tracking fluid balance in student athletes. The research design was a quasi-experimental research study and the subjects were healthy, uninjured, collegiate student athletes of a single soccer team in Arizona. As sunlight exposure was measured to determine hydration statuses in these athletes who exercise both inside and outside, the following variables were measured: exposure (in the form of ambient temperature and wet bulb globe temperature) with heat stress meters; hydration status, based upon USG markers pre- and post-exertion as well as the morning after exercise; total fluid intake; sweat rate; and energy expenditure.

Research Aims and Hypothesis

Expected outcomes were as follows:

- Sunlight and heat exposure will cause athletes to lose a greater percentage of total body weight via losses in water.
- Athletes will have increased needs to replace fluid losses to restore fluid balance after exercise and before any subsequent exercise.

The hypothesis of this study was that athletes will have increased levels of hypohydration after exercising outdoors compared to exercising indoors due to sun radiation exposure.

Definition of Terms

Fluid Balance: balance of fluids in the body, accounting for fluids inside and outside of cells; ingested fluids; electrolytes and movement of solutes; and total body water volumes (Armstrong, 2007; Cheuvront et al., 2013; Sawka et al., 2000; Tyrwhitt-Drake et al., 2015).

- Sweat Rate: the amount of fluid lost per hour (L/hr) in the form of sweat.
 - Calculation: (*Pre-exercise BM* (kg) post-exercise BM (kg)) + (fluid ingested (L) urine output (L))/protocol duration (min) x 60 (Edwards et al., 2007).
- Urine Loss: the amount of fluid lost in the form of urine; not accounting for losses from sweat, respiration, or excretion of fecal matter (Casa et al., 2000; Heavens, Charkoudian, O'Brien, Kenefick, & Cheuvront, 2016).
- USG: urine specific gravity.
 - A comparison of the densities of water and urine.
 - A urinary marker that delineates levels of general hydration status.
 - Measured in mg/dL (Rosinger, 2015).
- Dehydration: decreased body mass from loss of water.
 - Can be experienced after a single bout of exercise (Carlton & Orr, 2015).
- Hypohydration: USG \geq 1.020 mg/dL (Arnaoutis et al., 2015; Sawka et al., 2007).
 - In this study, hypohydration is primarily defined using USG values.
- Ambient Temperature: room temperature, or temperature surrounding a person/object.
 - Measured with thermometers, heat stress meters (Baker et al., 2016; Nuccio, Barnes, Carter, & Baker, 2017).
- Wet Bulb Globe Temperature (WBGT): measurement of the amount of sun radiation experienced on Earth (Broad, Burke, Cox, Heeley, & Riley, 1996).

• In this study, WBGT was measured using a Heat Stress Meter which contains a black bulb to measure the exposure to the sun.

Delimitations and Limitations

Delimitations:

- Healthy, uninjured adult student athletes
- College campus
- No known health issues were reported
- Eligible participants were screened for supplement use
- Written informed consent was received from all participants before commencing the study

Limitations:

- No randomization
- Estimating for insensible water losses
- Small sample size

CHAPTER 2

REVIEW OF LITERATURE

Introduction

The following includes a literature review on the findings of research that pertain to the topics of hydration status and fluid balance of athletes. This review contains information about and the latest research for hydration status and fluid balance of a variety of competitive and non-competitive athletes and study volunteers over a wide range of ages, types of sport, and levels of performance. A review of both lab-based and field-based testing methods and their practicality in different research and every-day settings are analyzed and discussed. The databases used for this study were Arizona State University's Library Online Database, CINAHL Plus, EBSCOhost, Google Scholar, MEDLINE ProQuest, Pubmed, and SPORTDiscus.

Key words searched in various combination were, *athletes, adult athlete, athlete dehydration, color chart, dehydration, electrolytes, euhydration, fluid balance, humidity, hydration, hydration status, hypohydration, intentional dehydration, knowledge, osmolality, sodium, sun exposure, sweat, radiation, temperature, thermoregulation, thirst, training, urine* and *USG.* From the literature review the following themes emerged: fluid balance, fluid and electrolyte movement in the body, assessment methods, hydration, sweat, thirst, athletes and nutrition/fluid needs, sport types (specifically soccer), urine analysis, and environmental conditions.

The goal of the literature review was to identify the current findings of athlete hydration and fluid balance studies and determine the holes in past research. This

extensive overview of a large scope of topics demonstrates the need for research in the areas of environmental factors affecting hydration, and thus leads to the methods that were developed to perform a hydration study for elite female soccer players in different environmental conditions, which will add to the current research in sports nutrition.

<u>Fluids</u>

Dehydration cannot be as easily defined as simply fluid loss from the body. There are many complexities that surround shifts of fluids, electrolytes, and total volumes throughout the body, known as the body's fluid balance. There are a myriad of fluid balance factors that cause different types and different levels of dehydration, and which consequently may cause increased heart rate, decreased blood pressure, central nervous system problems, and decreased thermoregulation (Tyrwhitt-Drake et al., 2015); as well as shifts in osmolality, thirst responses, and diuretic responses (Cheuvront et al., 2013).

Fluid Balance

Fluid balance is studied in many elite athletes who are younger than collegiateaged athletes (Cleary, Hetzler, Wasson, Wages, & Stickley, 2014; Da Silva et al., 2011, 2012; Zubac et al., 2017), but the data is still pertinent to studies with adult athletes. For different age groups there are different levels of activity, types of exercise, and amounts of fluid that need to be ingested to counteract fluid losses, which can vary greatly from person to person (Phillips, Sykes, & Gibson, 2014).

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Phillips and colleagues explained that the intense level of play for many team sports, specifically soccer, requires athletes to replace their body water losses by means of oral fluid intake to avoid extreme rises in core body temperature. How much fluid an athlete needs to replenish can be determined in various ways. One measurement, urine osmolality (U_{osm}), can determine a person's fluid balance and hydration status. U_{osm} is a measurement of urine solute concentration and has a normal level \leq 700 mOsmol/kg; hypohydration begins if a urine sample reads >900 mOsmol/kg (Thomas et al., 2016).

A simpler and more common method of monitoring fluid balance is weighing an athlete's dry body weight with minimal clothing before and after exercise to determine how much weight they lose, which corresponds to losses in total body water. For example the NCAA recommends that one pint of fluid should be ingested for every pound lost during exercise (NCAA, 2016). It is essential for every athlete to understand his or her fluid needs because of the various shifts of fluid that occur constantly within the body.

Total Body Water

The main component of fluid balance is total body water (TBW), which describes all of the fluids in the body that are found both intra and extracellularly, making up approximately 60% of the body's total weight (Armstrong, 2007; O'Toole, 2003). A person's TBW weight can vary, typically anywhere from 45-70% of their complete body mass. The variance depends on a person's composition of muscle or adipose tissue as these portions of the body are composed of approximately 75% water for muscle tissue and 10% water for adipose tissue. Thus, most athletes will have a higher TBW percentage as they have more lean muscle and a smaller body fat percentage that most non-athletes (Sawka, 1988; Sawka et al., 2000).

Hypohydration is revealed through decreased TBW while hyperhydration would be represented through increased TBW (Sawka et al., 2000). Hypohydration can occur through total extracellular fluid volume depletion, shifts in extracellular concentrations, or both (Heavens et al., 2016). The testing method of bioelectrical impedance (BIA) measures TBW by sending electrical currents through the body's fluids, measuring resistance (R), which can provide a more accurate reading, although it is found to be costly and difficult to use in field testing (Heavens et al., 2016; Reljic et al., 2013; Utter et al., 2012).

Heavens' research group analyzed studies that had participants perform exercise trials either euhydrated, dehydrated extracellularly (through the use of diuretics), or dehydrated intracellularly (through exercising in the heat). The researchers used BIA to measure the R in the athletes. The results showed that, while the R values were not significantly different between the athletes of different trials, BIA was still able to detect changes in hydration status. The detection of changes permits health care professionals to learn how to treat dehydration cases more appropriately (Heavens et al., 2016).

Zubac and colleagues performed a study to identify the effectiveness of determining hydration status through various tests. An interesting result they found was that U_{osm} can increase even if body weight does not change and even if total body weight is stable with consistent days of exercise-induced hypohydration. Additionally, fluid shifts intracellularly and/or extracellularly can still be occurring even if a person is noted

as euhydrated (with no changes in body mass or TBW shifts), thus the reason multiple hydration markers should be used in conjunction with each other for the most accurate representation of hydration status (Zubac et al., 2017).

Intra and Extracellular Movement of Fluids

Besides the movement of fluids from physical intake to physical output, which are viewed on the 'macro' level of fluid balance, fluids move within and around the cells of the body. Solids compose about 40% of the body's total weight; inside of the cell, intracellular fluid makes up another 40% of the body's total weight; and outside of the cell, extracellular fluid composes the remaining 20%. Along with the different percentages of fluid, there are also variations in fluid type. One type is interstitial fluid, which is the low-protein fluid that surrounds cells and creates their external environment for carrying substances that cells need to function. The lesser portion of extracellular fluid is composed of intravascular fluid, or plasma, the fluid that flows through blood vessels (O'Toole, 2003).

Water moves more freely than solutes across cell membranes, and it moves into the areas of higher concentration of solutes to equilibrize both intra- and extracellular spaces. When water shifts into the cells to compensate for higher solute concentrations, extracellular dehydration occurs. Conversely, when water moves out of cells to compensate for lower solute concentrations in the extracellular spaces, intracellular dehydration occurs (Hooper et al., 2016). Insufficient fluid intake or excess fluid losses cause the latter to ensue. For athletes, these occurrences can happen and go undetected throughout exercise, so it is important to monitor athletes for shifts in fluid and their overall hydration throughout all types of practice, training, and competition game play.

Electrolytes

An electrolyte is a chemical substance that creates electrical currents when separated into ions. The main anions are bicarbonate, chloride, and phosphate; the main cations are calcium, magnesium, potassium, and sodium. Two positive electrolytes, potassium (K⁺) and sodium (Na⁺), have concentration gradients which produce cell membrane potentials. These potentials of electrical currents are transmitted to nerve and muscle fibers which are essential to the working athlete (O'Toole, 2003).

Intra and Extracellular Movement of Electrolytes

The intracellular fluids (ICF) and extracellular fluids (ECF) in the body are composed of a varied concentration of all electrolytes. For example, ICF contains more K^+ and phosphate (PO₄³⁻) while ECF contains more sodium (Na⁺) and chloride (Cl⁻). K⁺ is approximately 30 times more concentrated intracellularly than extracellularly; conversely Na⁺ is 15 times more concentrated extracellularly than intracellularly (O'Toole, 2003).

Hypernatremia and Hyponatremia

Na⁺ is one electrolyte that is particularly essential to the working athlete. There are two states of Na⁺ balance in the body that can arise from imbalances of fluids within both intracellular and extracellular spaces – hypernatremia, and hyponatremia. The

normal range of sodium in the blood is between 135-145 mEq/L, and any higher or lower value could cause problems if not correctly promptly.

<u>Hypernatremia</u>

While it is the lesser frequent of the two Na⁺ balance states in athletes, hypernatremia is a state of increased blood Na⁺ that can occur in various forms. It occurs at levels \geq 145 mEq/L, can show symptoms at >147 mEq/L, and can be caused by insufficient fluid intake, excessive sweating, excessive losses through fecal matter (diarrhea), or improper kidney function and resorption (O'Toole, 2003). Examining the extracellular fluid volume levels associated with hypernatremia reveals three additional components of hypovolemic, hypervolemic, and euvolemic hypernatremia.

Hypovolemic hypernatremia, also called volume depletion, salt loss, or dehydration of the extracellular spaces (Hooper et al., 2016), involves decreased total body Na⁺ with an even greater decrease in TBW when in conjunction with hypernatremia; hypervolemic hypernatremia includes an increase in total body Na⁺ and usually no change in TBW; and euvolemic hypernatremia is the opposite with no change in total body Na⁺ but a decrease in TBW (O'Toole, 2003).

Each of these conditions involve a different treatment method, as replenishing fluids will not always treat high blood Na⁺. Hypovolemic hypernatremia is best treated with an increased volume of hypotonic saline to replace both losses in Na⁺ and water which may occur through the kidneys, or any extrarenal losses (respiratory, sweating, or feces). Water replacement is sufficient to treat euvolemic hypernatremia. Hypervolemic hypernatremia can be treated in multiple ways, including the use of water replacement, dialysis, or diuretics (O'Toole, 2003).

<u>Hyponatremia</u>

Hyponatremia is a state of decreased blood Na⁺, and it is more prevalent in competing athletes. Symptoms of hyponatremia can begin to arise in athletes under 130-135 mEq/L, including nausea and vomiting, headaches, and seizures (Thomas et al., 2016), and can be caused by inadequate Na⁺ intake, excessive fluid intake, or fluid retention (O'Toole, 2003).

The treatment of hyponatremia necessitates the consideration of plasma osmolality, or the concentration of solutes in the blood, specifically the water-electrolyte balance. The normal range for plasma osmolality is 280-292 mOsmol/kg H₂O. (Plasma osmolality values have been touted as the 'gold standard' for measuring hydration status in the laboratory setting; however, a discussion of practical tools and measurements of hydration will be performed in the Urine Analysis section.) Normal plasma osmolality can falsely diagnose hyponatremia while in fact an athlete could be suffering from hyperlipidemia or hyperproteinemia instead (Weisberg, 1989).

When plasma osmolality is increased, it may indicate hyperglycemia. When plasma osmolality is decreased, ECF volume may be interpreted the same as with hypernatremia, but with the advantage of physical signs and symptoms to direct toward the correct diagnosis. Hypovolemic hyponatremia can be indicated by tachycardia, hypotension, and decreased skin turgor (Schrier & Bansal, 2008). The TBW is decreased with an even greater decrease in total body Na⁺. Volume expansion, the same treatment method for hypovolemic hypernatremia, is also indicated here.

Euvolemic hyponatremia involves no change in total body Na⁺ but an increase in TBW. Athletes will have a normal pulse, blood pressure, skin turgor, and no signs of edema. However, since the athlete is still deficient in Na⁺, they will typically be placed on a water restriction to equalize the water-electrolyte balance in the body. Finally, hypervolemic hypernatremia involves an increase in total body Na⁺ with an even greater increase in TBW. With signs of edema and even acute or chronic renal failure, Na⁺ and water restrictions should be instated (Canada et al, 2012).

Factors Affecting Hydration

A key difference between the severities of dehydration has been noted with an athlete's performance regarding the factor of heat. Exercise alone causes an increase in fluid loss and eventual dehydration, but in a recent study exercise along with heat stress caused an even greater increase in instances of hypohydration in athletes (Nuccio et al., 2017). Additionally, these and other researchers noted that athletes who train in the heat also experience cognitive impairments and greater perceptions of exhaustion, exertion, and thirst (Bardis, Kavouras, Arnaoutis, et al., 2013; Benton, Jenkins, Watkins, & Young, 2016; B. A. Davis et al., 2014; Nuccio et al., 2017; Vandermark, 2016).

Typically, there is at least some level of hypohydration an athlete reaches before he or she notices or perceives thirst. Depending on the individual, the reaction to hydrate by drinking fluids can vary greatly – being influenced by the type of fluid, the fluid's temperature, and its taste (Apostu, 2014). A great number of athletes experience skewed perceptions of thirst in this manner.

The type of sport also affects levels of fluid balance in athletes. Those athletes whose sports involve intentional weight cutting through dehydration, for example the weight-class sports of boxers, mixed martial artists, judo athletes, and wrestlers, can experience greater chronic levels of hypohydration even in their seasons of maintenance where body mass reduction methods are not employed (Jetton et al., 2013; Pettersson, Ekström, & Berg, 2013; Zubac et al., 2017).

Sweat Rate

During exercise, sweat is a response to increases in metabolism, heat production, and core body temperature. The act of sweating decreases these processes and cools the body as sweat evaporates and releases heat (James, Moss, Henry, Papadopoulou, & Mears, 2017) in the form of fluids and electrolytes (Greenleaf, 1992). It is important to understand sweat losses to accurately determine fluid replacement requirements for athletes. Higher losses in sweat indicate that the athlete must consume more fluids to replace those losses and avoid dropping below the 2% body weight loss cutoff and prevent their performance from being negatively affected (Bardis, Kavouras, Arnaoutis, et al., 2013; Cleary, Hetzler, Wasson, Wages, & Stickley, 2014; Goulet, 2012; 2013).

Sweat rate can be calculated from variables measured during exercise to determine how much an athlete sweats over a specific period of time, typically presented as liters per hour (L/hr). An equation from a study performed by Edwards et al. (2007) is

the following: (*Pre-exercise BM* (kg) - *post-exercise BM* (kg)) + (*fluid ingested* (L) – *urine output* (L))/*protocol duration* (*min*) x 60. This equation was used in the current study to calculate the sweat rates of the female soccer athletes.

Insensible Losses

Insensible fluid loss describes pure water losses that do not occur from the major organs (sweat glands, kidney processes) but from smaller sources of water loss. These include losses from the respiratory tract, lungs, and losses from fecal matter (Greenleaf, 1992). When interpreting body mass changes before and after exercise, sweat loss and insensible losses are both included; this is a valuable tool more so for laboratory than field testing methods (Vandermark, 2016). No insensible losses (fecal loss, loss of water through respiration, or production of water during energy production) are considered in field studies as they can compensate for one other and create minimal net losses compared to losses from urine excretion and sweat production.

Thirst

Thirst can be defined as a desire to drink due to a loss or shortage of water (Greenleaf, 1992). For a non-athlete, thirst perceptions typically prompt a person to drink enough to avoid dehydration or extreme fluid losses. However, for athletes who exercise intensely under stressed conditions, their thirst perceptions may not be enough to meet their fluid needs.

Within sports teams where hydration is encouraged before training and games and rehydration is encouraged after, athletes can still experience extensive levels of fluid imbalances due to distorted perceptions of thirst and dehydration (Burchfield et al., 2014; Davis et al., 2014; Fernandez-Elias et al., 2014; Thigpen et al., 2014; Zubac et al., 2017). Participating in endurance sports, especially those which have stark fluctuations in energy expenditure, can lead to continued sweating even after the exercise is completed.

Athletes may rehydrate only to the extent of their thirst perception which may cause them to stay below a state of euhydration. Various factors include: an athlete's involvement or position in the sport (Phillips et al., 2014); wearing protective equipment and gear (Nuccio et al., 2017); being a single athlete or participating in a team sport (Da Silva et al., 2012; Thigpen et al., 2014); and personal preference, such as intentional dehydration to avoid disruptions during games to urinate (Utter et al., 2012). If another training session or game is scheduled shortly after this, an athlete can subsequently start again in a state of hypohydration and possibly continue in this path for hours or days to follow. Additionally, if the losses are not replaced a water deficit will arise and lead to increased levels of hypohydration (Da Silva et al., 2011).

Apostu (2014) conducted a study which verified hydration status using heart rate. In the study two groups of athletes who were either hydrated or dehydrated performed anaerobic exercise while their heart rates were recorded. Those athletes who began dehydrated had higher exercising heart rates (156.5 beats/min) compared to the athletes who were properly hydrated (151.6 beats/min) which is an indicator of decreased health and athletic performance and ability. Similar to athletes who are unable to properly estimate their fluid losses, a study found that distorted thirst perceptions can inhibit athletes from properly rehydrating. Athletes in the study participated in uphill cycling tests and were either given fluids to replace their sweat losses or were not provided fluids at all. Those athletes who were not provided fluids during exercise but then rehydrated with fluid boluses experienced a relief of thirst faster; however since the athletes had their thirst quenched more quickly, they were not hydrated completely to achieve euhydration (to replenish body mass lost during exercise) (Vandermark, 2016).

James et al (2017) found that during states of hypohydration, athletes rated their thirst higher than when they were properly hydrated. Additionally, oral fluid consumption was an important contributor to thirst perception. Some studies performed rehydration thorough infusions of isotonic saline, so thirst was never stimulated in these athletes (Cheung et al., 2015; Wall et al., 2015). However in James' study, they provided hydration through an orogastric feeding tube (or removed fluids from this tube to simulate dehydration) as well as through oral ingestion which blinded the participants to the intervention and further validated the importance of proper hydration methods for fluid balance and thirst perception during times of exercise (James et al., 2017).

Drinking to thirst is appropriate in some situations without concern for hypohydration. Prescribed drinking protocols are used frequently in studies to regulate an athlete's hydration status and determine associated changes that occur during dehydration. Kenefick verified that athletes can rely on their thirst perception to guide how much they should consume after exercise when: the exercise time is less than 60-90 minutes; exercise is completed in cooler temperatures (~24°C); and the intensity of exercise is low. However it is still not recommended to drink so much so that the athlete gains any additional weight as this can lead to hyperhydration and distorted electrolyte balance (Kenefick, 2018).

Athletes may experience increased levels of sodium in the blood following activity that models 'high intensity intermittent exercise' as this form of intense workout with pauses in between movement can cause buildups of both Na⁺ and lactate. Mears and his fellow researchers sought to determine if thirst sensations would adequately meet their needs through *ad libitum* fluid intake; however, groups of participants were either given water freely after exercise, made to wait 30 minutes after exercise, or were not given water at all during the 60 minutes after exercise. The study concluded that while serum lactate and Na⁺ concentrations did not differ significantly after this type of exercise, total water intake was the greatest in the groups who were allowed to drink *ad libitum*, indicating that thirst sensations were accurate and that the athlete was not satiated until allowed to drink (Mears, Watson, & Shirreffs, 2016).

Antidiuretic Hormone, Aldosterone, and Diuretics

Antidiuretics play a large role in not only thirst response, but also fluid shifts in the body. Antidiuretic hormone (ADH), also known as vasopressin or arginine vasopressin (AVP), is produced naturally by the hypothalamus and is stored in the pituitary gland. Its main function is to balance water in the body. Receptors in the hypothalamus can sense changes in ECF solute concentrations and can adequately release or inhibit the release of ADH to compensate for these changes and return the environment of the ECF to normalcy (Segar & Moore, 1968). Alongside ECF changes, the variances in the ICF are equally controlled by ADH (Cheuvront et al., 2013).

ADH is also regulated by renal functions especially during fluid shifts and changes in hydration status (Utter et al., 2012). In the ADH cycle, dehydration causes an increase in plasma osmolality which increases the release of ADH, subsequently reducing water losses through the kidneys and normalizing plasma osmolality. Conversely, rehydration does not necessitate the release of ADH; thus, the decrease in plasma osmolality inhibits ADH and will increase water losses through urination.

Other stressors that can cause an antidiuretic effect on the kidneys are different environmental variations (altitude changes; shifts from extremely hot to cold) which could occur even if an athlete is not dehydrated (Cheuvront, Kenefick, & Zambraski, 2015). ADH levels have also been found to fluctuate based upon these environmental temperatures as well as core temperatures. Segar & Moore (1968) determined that when participants were subjected to high and low temperatures, their ADH levels rose and fell, respectively (from 1.6 ± 0.4 to 5.2 ± 0.8 µU/mL at 50°C, and to 1.0 ± 0.26 µU/mL at 26°C). In the hotter temperatures, water and Na⁺ losses were minimized whereas they were increased in the colder temperatures.

Aldosterone

While ADH is needed for water balance in the body, another hormone called aldosterone is essential for the regulation of tonicity, or pressure gradients, in the body (Armstrong 2007; Shore et al., 1988). Aldosterone, produced in the adrenal cortex, is specifically geared towards regulating the absorption of Na^+ in the kidneys while K^+ is excreted through the sodium-potassium channels.

Aldosterone is majorly a part of the renin-angiotensin aldosterone system (RAAS). Any small changes below or above the normal plasma osmolality range of 280-292 mOsm/kg will initiate the RAAS to address solute deficiencies (Cheuvront et al., 2015) and restore normal plasma osmolality values through fluid and electrolyte retention or excretion (Vandermark, 2016). Mears et al. (2016) also found a connection between increased aldosterone and increased stimulation of thirst.

Diuretics

Opposite of the antidiuretic responses in the body are diuretic responses, a topic that has received much attention for competing athletes. In the field of exercise and sports, diuretics are typically supplements that athletes take to increase or support water losses. While these drugs can be taken for medical purposes (to treat high blood pressure, edema) (Sawka et al., 2000), or have been used in research to purposefully induce hypohydration (Armstrong et al., 2012; Davis et al., 2014; Godek et al., 2010), diuretics can be overused or abused by athletes. One example of common pills used by athletes are water pills which cause the kidneys to excrete an excess of Na⁺ and water through the urine. These tactics are employed to achieve more rapid or more extreme weight loss and are seen mostly in weight-class athletes such as wrestlers. The NCAA has banned the use of diuretics for any athlete in any type of sport (NCAA, 2016). The reasoning for restrictions on diuretics is the likelihood of the supplements causing overt hypohydration. When athletes cut weight with diuretics through the excretion of water and solutes, the body lacks euhydrated balance (Reljic et al., 2013), especially when the weight loss is rapid. However even when athletes are using diuretics without the intent of weight loss, the use of these drugs can cause athletes to begin exercise in a state of hypohydration (Da Silva et al., 2012; Sawka et al., 2007) which research has proven to cause many negative side effects for the athlete's health and performance (Cheuvront et al., 2010; Hamouti et al., 2010).

Thermoregulation

With all of the side effects associated with dehydration, including impairing an athlete's metabolism (Pettersson et al., 2013), and mental, muscular, and subsequently performance abilities (Broad et al., 1996; Sobana & Many, 2014), one mechanism that occurs automatically in the body is the ability to internally compensate for changes in environmental temperature; this is known as thermoregulation. Thermoregulation, though efficient in a non-exercising person, can be drastically impaired when an athlete is exercising and loses excess amounts of electrolytes or water, especially when exercising in the heat (Sobana & Many, 2014).

Even slight levels of dehydration cause stress on the working body especially for cardiac functions and heat dissipation (Arnaoutis et al., 2015). Additionally, a conditioned athlete can suffer from the effects of improper thermoregulation in certain situations. Being in a state of hypohydration negates any advantages an athlete might have gained in terms of becoming acclimated to exercising in the heat or increasing their aerobic exercise abilities. Sawka and colleagues discussed that when these conditioned athletes begin and continue exercising in states of hypohydration, their bodies' abilities to thermoregulate are decreased just as their performance declines, increasing their risk for developing additional and more serious side effects of severe dehydration (Bardis, Kavouras, Arnaoutis, et al., 2013; Fernandez-Elias et al., 2014; Sawka et al., 2000; Thigpen et al., 2014; Thomas et al., 2016; Utter et al., 2012) if they do not return to states of proper hydration.

Sweating is a mechanism that aids in thermoregulation by expelling heat in the form of sweat from the body. Dehydration caused by excess sweat rate puts a strain on cardiovascular and thermoregulatory functions, which in turn can affect athletes' performance during practices and games. Furthermore the greater an athlete is dehydrated, as identified by percentages of body weight lost, the greater the strain on these cardiovascular and thermoregulatory functions (Adams & Casa, 2003; Bardis, Kavouras, Arnaoutis, et al., 2013).

When athletes are dehydrated and exercising in moderate-cool temperatures, the risk of inappropriate thermoregulation is not increased as the athletes' bodies are better able to maintain proper core temperature without much strain. However for those dehydrated athletes exercising in hotter temperatures, the body can reach a state of "thermic shock" in where thermoregulation insufficiently maintains or improperly returns core temperatures back to normal (Apostu, 2014). Other research has also verified the increased strain on thermoregulation when athletes exercise in the heat as compared to
more temperate environmental conditions, revealing how dehydration directly influences thermoregulatory responses (Arnaoutis et al., 2015; Bardis, Kavouras, Kosti, et al., 2013; James, Mears, & Shirreffs, 2015; Sawka et al., 2000).

Laboratory and Field Hydration Assessment Methods

In a laboratory setting, one common method of measuring intra and extracellular movement of fluids is bioelectrical impedance analysis (BIA) (Reljic et al., 2013). This testing method, also called multifrequency bioelectrical impedance analysis (MFBIA), is used for weight-driven athletes and tests TBW as well as total body fat. This system offers a noninvasive technique that does not require the use of biological samples (Utter et al., 2012). However, for field testing of fluid balance and fluid shifts, these tools are not practical because of their cost, burden on the athlete, and time restraint.

Urine Specific Gravy (USG) and Urine Color

The fluid balance of adult athletes in both training and games can begin in a hypohydrated state, which is characterized by a urine specific gravity (USG) value of $\geq 1.020 \text{ mg/dL}$ (Casa et al., 2000; Cheuvront et al., 2010; Hamouti et al., 2010; Sawka et al., 2007). Multiple studies have concluded that a balance of fluid intake and fluid output, whether it be from sweat losses or urine excretion, has a direct correlation to hydration status of active athletes (Cheuvront et al., 2010; Da Silva et al., 2011). Arnaoutis et al. (2013) and Fernandez-Elias et al. (2014) were also able to detect dehydration in elite

athletes based on urine color values using a validated color scale which rates urine as light in color (well hydrated) to darker in color (poorly hydrated).

Fluid Intake

In research, typical methods of assessing intake of water during training and exercises include free access to personalized fluid bottles (Arnaoutis et al., 2013); *ad libitum* intake from individualized fluid bottles provided by the study (Da Silva et al., 2011, 2012); or prescribed amounts of fluid to replace losses or resume dehydration (Nuccio et al., 2017), which is a steady state of hydration without fluid gains or losses (Goulet, 2013). While a valid method, solely using fluid balance as a measure of hydration status in athletes does not provide a complete account of their physical and mental states. Recording fluid intake must be used in accordance with other measurements, including USG results taken at different points of exercise or assessing body weight changes before and after exercise to provide a full account of hydration.

Athletes

Athletes have a comparatively higher requirement for fluid and nutrition than most other populations because of their increased levels of intense exercise (Carlton & Orr, 2015). Additionally, requirements vary between athletes even throughout a single sports team. Some of the factors that differ between athletes are the micronutrient and macronutrient needs, the timing of food and fluid intake, the amount of fluid taken in, the amount that is lost through sweat and urine losses, and the movement of intra and extracellular fluids in the body (Heavens et al., 2016; Hooper et al., 2016; Judge et al., 2016).

Nutrition Requirements

Maintenance of proper nutrition is essential in an athlete's daily activities. However, an identical plan for any two people does not exist, let alone athletes within a single sport. Athletes have higher energy requirements to accomplish not only their daily activities but perform well through all of the added exertion that is needed during practices and games (Supriya, 2013). There are methods for estimating energy expenditure (EE) which is essential in determining athlete needs to replace what they lose during exercise.

One manner of determining EE is to equip athletes with tracking devices which monitor and record various parameters that can be input into estimating equations. A Zephyr BioHarness is one such tracker which can be worn by an athlete during exercise and measures heart rate, breathing rate, skin temperature, and activity level second-bysecond (Zephyr Technology, 2012). These measurements can be used in an equation to determine metabolic equivalent (METs) which relates the amount of activity a person performs compared to their metabolic rate. The equation is METs = -1.1644 + 7*HR +5.8985*ActivityScore + 3*BR (Rosenberger Hale et al., 2013). METs were then calculated towards energy expenditure (kcal) using the following formula: METs*body weight (kg)*hours of exercise (Jette, Sidney, & Blumchent, 1990).

The common standard in research is often to determine EE using indirect calorimetry (IC). IC monitors gas exchange with either oxygen consumption or production of nitrogen waste or carbon dioxide through an air-tight chamber. These readings are also input into equations, similar to variables from a tracker device, to estimate EE. This method has been considered most accurate as it measures gas exchange alongside basal metabolic rate, resting EE, and total daily EE (Cunningham, 1990; Levine, 2015). However, estimating with variables measured using tracking devices worn by athletes are more practical methods that allow them to quickly determine on average how many calories are burned during exercise so that they can more accurate determine their daily caloric needs.

Loucks noted in her review that many athletes, especially competitive females, are energy deficient, meaning that their energy expenditure outweighs their energy intake. Through the combination of inadequate nutrition and the stress of exercise, athletes can experience decreased performance and health issues (Loucks, 2004). To compensate, athletes can manipulate nutrition protocols to enhance their performance. Many soccer players desire a gain in lean muscle mass with a loss in body fat to optimize their strength and game play during the on-season. Out of an average of 309 athletes from 16 different studies, data points revealed that the average energy intake was 188 kJ/kg (126-282 kJ/kg); and the average percentages of carbohydrate, protein, and fat intake were 51%, 16%, and 33%, respectively (Burke, Loucks, & Broad, 2006). Another study found averages to be 50%, 15%, and 35% for females and 49%, 17%, and 34% for males (Holway & Spriet, 2011).

Specific carbohydrate requirements have been made for both genders, and ranges can be followed depending on sport type and intensity level. The 2006 FIFA/FMARC recommendations are 5-7 g/kg/day of carbohydrates for soccer players. The consensus statement from the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine recommends 6-10 g/kg/day. However reports of actual intakes by both female and male soccer players are only 4.0 g/kg/day and 5.6 g/kg/day, respectively (Holway & Spriet, 2011).

Athletes can prepare for intense practices or competitions by fueling up in advance, typically 24-48 hours before exercise begins. When comparing highcarbohydrate to low-carbohydrate protocols, athlete study participants who were provided higher carbohydrate intakes had improved glycogen stores, improved work performance, increased time to fatigue, and the ability to complete more high-intensity movement than those who were permitted lower carbohydrate intakes (Burke et al., 2006).

Fluid Requirements

No one recommendation of fluid demands can be made for all athletes either, although one can determine some ranges that will fulfill fluid needs. Thomas et al. (2016) outlined methods for determining fluid needs for athletes and modifying plans based on the individual. Fluids that count towards intake in the fluid balance come from beverages such as water, milk, coffee or tea, juices, sports drinks, and energy drinks. However, fluids are also found in many food sources which must be taken into account in the fluid balance as well (Pettersson & Berg, 2014).

Casa and colleagues established extensive guidelines for initiating hydration protocols for athletes. Considering the athlete's age, anthropometrics, type of exercise performed, duration and intensity of exercise, total body weight change (including sweat rate and urine losses), environmental factors and heat acclimation status, rehydration opportunities, type of fluid consumed, and pre-exercise hyperhydration or euhydration are all essential if an athlete desires to reach optimal performance (Casa et al., 2000).

There are stages in which an athlete should particularly focus on ingesting fluids, and they typically surround practices or game play time. Before exercise, fluid deficits can be normal occurrences that cause an athlete to begin exercising in a state of hypohydration. An athlete should attempt to consume enough fluids to begin exercising euhydrated (Magal, Cain, Long, & Thomas, 2015), or even marginally hyperhydrated in some cases (Apostu, 2014) to prepare for the impending exercise and sweating.

While some research has shown that requiring athletes to only drink when they sense thirst can properly hydrate them (Dion, Savoie, Asselin, Gariepy, & Goulet, 2013), it is not a technique that can be employed by all active athletes. Normally individuals at rest or performing minimal activities can maintain fluid balance and homeostasis of electrolytes in the body, but with increase energy expenditure – especially in hotter temperatures and environments – athletes' bodies have a more difficult time maintaining proper fluid balances and thus their performance in practices and games can be negatively affected (Cheuvront et al., 2010).

Males versus Females

It is understood that males and females are different in their body composition and thus their needs in terms of both nutrition and fluid. Specifically, for fluids during exercise, fluid needs are higher for males who have larger body masses and faster metabolisms than females. Moreover, male sweat rates and electrolyte losses are more significant. It has been found that women suffer more side effects from dehydration than males, especially when exercising in endurance events (Sawka et al., 2007). Although these variances are not always significant, they are still important to consider when making fluid protocol recommendations for athletes of different genders.

Fluid Type

Apostu (2014) suggests that different types of fluids be consumed for different lengths of time of exercise. In exercises that are completed under an hour, water alone is an acceptable fluid to rehydrate an athlete and restore fluid balance. Exercise exceeding an hour, especially past two or more hours, requires more than only rehydrating with water. The concentration of solutes in and around body cells can decrease with the sole ingestion of water (or fluids without added electrolytes) and decreased blood Na⁺ levels can arise (Apostu, 2014; O'Toole, 2003). (Refer to the Fluid Balance section).

Some of the more seriously competitive athletes also consider carbohydrate beverages as research has shown that different types or combinations of carbohydrates can produce different levels of oxidation during exercise. Burke and colleagues explained that athletes should consume the correct amount and type of carbohydrate beverage (glucose, fructose, etc.) based on time and intensity of exercise so that both the central nervous system and the muscles have optimal availability of carbohydrates for energy. The researchers determined that rinsing the mouth or taking small sips of carbohydrate beverages is appropriate for ~1 hour of higher intensity exercise; any increasing time would require anywhere from 30-90 g/hr of a carbohydrate beverage for most athletes (Burke, Hawley, Wong, & Jeukendrup, 2011).

Fluid choices during and surrounding exercise have also been studied to determine the effects on performance. In a study comparing cyclists' leg force after 120 minutes of training and consumption of different beverages (water; sports drinks with different concentrations of carbohydrates and Na⁺), they found that the sports drinks preserved their performance over the entirety of the investigation (Coso, Estevez, Baquero, & Mora-Rodriguez, 2008). In a study that looked at post-exercise effects of sports drinks with added K⁺, these drinks did not assist in maintaining euhydration any more than normal sports drinks (Pérez-Idárraga & Aragón-Vargas, 2014).

An earlier investigation compared a carbohydrate drink, a combined carbohydrate and protein drink, and a placebo drink on post-exercise recovery of females after running for 30 minutes on a treadmill (Green, Corona, Doyle, & Ingalls, 2008). Their observations revealed that there were no differences between the three drink types, and that the beverages align more with athlete drink preferences. This is similar for athletes who choose between regular and zero calorie sports drinks. For those who are concerned about ingesting extra calories, but prefer to have a beverage with taste while exercising, a type of artificially sweetened beverage would be appropriate (such as the product *Powerade Zero* which contains sucralose) (Spencer et al., 2016).

Fluid Recommendations

The amount of fluids that athletes need during exercise varies substantially based upon many variables, including but not limited to: gender, age, height and weight, exercise type, length of exercise, amount and duration of breaks, environmental conditions, pre-exercise hydration status, type of fluids to be consumed, and many other factors. Recommendations have been made from previous research that analyzed fluid needs of training and competing athletes and how their needs fluctuate depending on what stage of exercise they are in, which will be discussed below.

Pre-Exercise Hydration

A review provided by Sawka et al. (2007) made recommendations for fluid intake before exercise is initiated. Their suggestion advised drinking 5-7 mL/kg of body weight of fluid, slowly, and approximately 4 hours before a practice or game. If an athlete is struggling with an underproduction of urine, drinking an additional 3-5 mL/kg of body weight of fluid 2 hours before exercise will assist with meeting their fluid needs. If an athlete is struggling with an under-stimulation of thirst, adding a beverage or snack that contains Na⁺ will assist with stimulation to meet electrolyte needs (Sawka et al., 2007).

Goulet (2012) recommended for endurance athletes specifically that 5-10 mL/kg of fluids 2 hours before exercise is a realistic and adequate goal for beginning exercise in a state of hydration, maintaining peak levels of performance, and avoiding a drop below the 2% body weight loss cutoff. Cleary et al. (2014) found that when athletes drank at least 2 bottles of fluid (591 mL each), regardless of the fluid type (a sports drink or plain

water), the athletes were better hydrated before practices. Pre-hydrating in this manner allows the athlete to begin exercise in a state of euhydration instead of going into a practice or game hypohydrated and possibly continuing this pattern throughout exercise.

During Exercise Hydration

The goal of hydration during exercise is to maintain the athlete's body weight and avoid reaching extreme levels of hypo- or hyperhydration with inadequate or excessive fluid intake. Thomas et al. (2016) suggested that an athlete ingests at least 0.4-0.8 L of fluid per hour during exercise depending on sweat rate (most athletes lose anywhere from 0.3 to 2.4 L of fluid per hour from sweat). When monitoring changes in dry body weight, an athlete can also determine how much fluid needs to be consumed based upon the rule of 1 kg of body weight lost equaling 1 L of sweat lost.

Cleary et al.'s study identified athletes' specific needs based on their sweat rate and prescribed specific amounts of fluid every 20 minutes for the 2 hour practice in which they participated, as well as ingesting fluid *ad libitum* at other time points (Cleary et al., 2014). The result was an improvement in hydration with an overall decreased loss of body weight before and after exercise when they were provided with a personalized hydration plan. They learned how to rehydrate at the appropriate times and how much fluid would suffice to replace their needs.

Post-Exercise Hydration

While pre-exercise and during exercise hydration are essential for attaining and maintain euhydration, rehydrating after exercise is just as important. Many athletes are found to underestimate their fluid losses during exercise (O'Neal et al., 2012), which

makes it even more essential for post-exercise hydration protocols to be established which will accurately replace losses.

O'Neal recommended that athletes consume 125-150% of the estimated fluids lost from sweat after exercise is performed, especially if an athlete is to exercise again shortly thereafter. Thomas et al. (2016) also made suggestions for post-exercise fluid needs in addition to the fluid needs during exercise, recommending that athletes should consume up to 1.25-1.5 L for every 1 kg of body weight lost after exercise.

Sport Types

The type of sport in which an athlete participates is impactful on that individual's ability to maintain fluid balance. Some major categories of sports include individual, team, and endurance, along with many other subsections that can be defined between and around these groupings. Individual sports where dehydration is typically an issue include tennis (O'Neal et al., 2012), soccer (Da Silva et al., 2012), and bi- or triathlons (Carlton & Orr, 2015) because of the athletes' time spent either practicing or competing outdoors. The main focus of the following section of this literature review will be on team sports and the dynamic between players, coaches, and other members of the team which affect individual players in a myriad of circumstances.

Team Sports

Focusing on team sports, some of the teams that can experience major differences in fluid balance are those who practice or compete both indoors and outdoors. The change in environment, temperature, and exposure to sun radiation all affect fluid balance and hydration status (Sawka et al., 2007). The occurrence of dehydration in team sports is seen to be higher because of the sheer number of players and the ratio to coaches, dietitians, or other trainers who assist the athletes in determining nutrition and fluid needs. If a team does not have a coach or trainer who is properly qualified, the players have an even greater risk of developing dehydration from improper advice or ineffective plans for maintaining fluid balances (Nuccio et al., 2017; Sobana & Nirmala, 2014).

In two studies performed by Sobana, they noted that athletes on a team sport usually switch between high- and low-intensity bouts of exercise as they shift positions, are stopped during a game, or are taken in and out of plays – for example soccer or football. These changes throughout exercise can cause greater amounts of water losses through sweat (Sobana & Many, 2014; Sobana & Nirmala, 2014). Proper education on rehydration protocols, though, is not always provided to all coaches and trainers, and thus is never passed on to the athletes (Cleary et al, 2014; McDermott et al., 2009).

Soccer Players

Now the focus shifts to the team style that was researched during this study – of soccer players who are a unique type of athlete. Soccer has great variability in terms of exercise type and energy expenditure, depending on the athletes' individual positions and the distances that the athletes are moving around the field during any given practice or game. There are many frequent bouts of sprints performed across the field multiple times during a game, which is self-paced by each athlete (Da Silva et al., 2011). Athletes may

be provided very little time to hydrate during exercise with the setup of soccer games; as athletes have long play times (two 45-minute periods), minimal breaks, and no permitted access to fluids on the field (Broad et al., 1996), their risks for dehydration are one of the highest of any team sport (Da Silva et al., 2012).

Soccer players have been recorded to have maximal oxygen consumption, or VO₂max, levels of 70-80% during typical sessions, depending on the player and their position on the team. The subsequent increase in body temperature and decrease in TBW from sweating and other losses causes more concern for athletes to rehydrate throughout all times surrounding exercise, not just during practices or games (Phillips et al., 2014).

The effects of dehydration on soccer athletes have not gone unresearched over the past few decades. Predictively, soccer athletes have been found to perform more poorly when dehydrated, even mildly (Da Silva et al., 2012), affecting both their overall performance and their specific skills related to ball handling (Da Silva et al., 2011). Ultimately the goal for preventing dehydration is to ensure adequate fluid intake before, during, and after soccer practices and games.

One of the most quoted concerns is avoiding dehydration when athletes exercise outdoors where they are exposed to environmental conditions such as sun radiation, wind, and thermal stress. While this data is helpful in understanding soccer players' needs, most research studies have been performed only on single days and typically use adult athletes as research participants which excludes pertinent information that can be taken from a younger population of athletes and from a comparison across multiple study days (Da Silva et al., 2011).

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In 2013, Arnaoutis and colleagues were able to study a younger population of over 100 male soccer players (ages 11-16) to determine if drinking *ad libitum* during soccer camp was enough to prevent dehydration. The conditions during the study were an average temperature of 27.2±2°C (~81°F) and 57%±9% relative humidity. Their findings were that for almost all athletes, no player was able to drink enough to stay hydrated or return to proper hydration, especially when they began exercising already hypohydrated. It is difficult, they found, to reach euhydration when starting hypohydrated and exercising; even when drinking fluids and in moderate temperatures, athletes can remain hypohydrated or reach greater levels of hypohydration (Arnaoutis et al., 2013).

In a 2017 literature review, multiple articles examined soccer specifically – the protocols employed, hydration levels, and other factors that provide a great insight into the hydration status of these athletes. Out of 75 studies identified, soccer was listed as one of two sports (followed by rugby) with a high risk for athletes becoming dehydrated. In analyzing both male and female soccer athletes, decreased body mass during exercise with increased instances of hypohydration were common with an average of 0.7–2.5% body weight lost. Many of the listed effects that hypohydration had on the athletes were decreased physical performance and decreased mental performance in terms of cognition, fine motor skills, speed, and memory (Nuccio et al., 2017).

Soccer is considered one the most popular sports in the world, but especially in Latin American countries. Chilean soccer players are a group that have diverse geographies and climates, from deserts to glaciers, implicating that some teams face a myriad of conditions. In a 2015 study, 156 soccer players from 6 professional Chilean teams were evaluated for weight, height, and USG before exercising. The study took place in October and November with conditions of 17-23°C (62-73°F) in north-central Chile and 10-15°C (50-59°F) in south-central Chile. The subjects were not informed of any fluid or food protocols in order to determine the actual habits of the players.

Urine samples were taken, and USG was measured immediately. Out of the 156 players, 0.6% were euhydrated; 9% were minimally dehydrated; 77% were significantly dehydrated, and almost 14% were severely dehydrated; with a mean USG value of 1.026±0.005 from all teams. The main results of this study indicate that practically all professional soccer players were arriving to practices already dehydrated; and of these, 90% are significantly or severely dehydrated. These athletes are at high risks for continuing exercise in severe states of dehydration which affects their performance as well as health (Castro-Sepúlveda et al., 2015).

Perceived Hydration and Exertion

Athletes can have a skewed perception on the importance of hydration which was a trait found in elite female soccer players at different levels of competition – friendly matches, training sessions, and official matches (Castro-Sepulveda et al., 2015). While the athletes did not rate themselves differently in terms of their hydration and how it would affect their performance, the mean USG value across all activity types was 1.027 and 47% of the 18 studied athletes were found to be severely dehydrated with a USG >1.030. Table 1 includes a summary of important themes found throughout research that

represent a majority of common findings that have been used to create today's

recommendations for athletes (Burke, 2007).

Торіс	Themes and Findings	Sources
Sweat Loss & Fluid Intake	With <i>ad libitum</i> fluid intakes, players do not consume adequate fluids to keep up with sweat losses.	Broad et al., 1996; Maughan et al., 2014; Shirreffs et al., 2005; Stofan et al., 2003, 2005.
	Less fluids are consumed when warmer beverages were offered.	Godek et al., 2005.
Environment & Fluid Intake	Colder temperatures, decreased sweat rate and decreased fluid intake (comparing winter/summer).	Broad et al., 1996.
	Winter, lower fluid consumption, hypohydration.	Maughan et al., 2005.
Pre-exercise Hyperhydration	Over hydrate to compensate for previous exercise's sweat losses.	Godek et al., 2005; Maughan et al., 2005.
	Monitor weight change/urine "characteristics" to determine fluid needs under heat stress conditions.	Dabinett, 2001.
	Voluntary and hyperhydration (~2.7/4.6 L/day) in a week before competition in the heat; increased temperature regulation, but no significance on performance.	Rico-Sanz et al., 1996.
Carbohydrate Beverages	Carbohydrate beverages/food during some exercises enhance performance; sports drinks for fluid and fuel needs during times where recovery periods are shorter.	Kirkendall et al., 1988; Muckle, 1973; Ostojic & Mazic, 2002; Welsh et al., 2002.
	Research that did not have the above findings.	Criswell et al., 1991; Nassis et al., 1998; Zeederberg et al., 1996.

Table 1. Categorized Research Studies. Pertinent findings from studies cited in Burke, 2007.

Urine Analysis

Along with the complexities of types of dehydration are the dilemmas in assessing dehydration through a person's hydration status and fluid balance. A perfect universal 'gold standard' of measurement has yet to be discovered; thus some studies may culminate in improper assessment and therefore treatment of dehydration (Armstrong, 2007; Cheuvront et al., 2013). Various researchers have found that plasma osmolality is the most accurate indicator when compared to urinary markers of USG and U_{osm}, which may be associated with the renal response to ADH (as discussed in the Diuretics and Antidiuretic Hormones section) (Popowski et al., 2001). This, however, may be impractical and can lead to resistance from athletes in participating in research if they are asked to have blood drawn to obtain results.

As previously mentioned, there are many ways to determine an athlete's hydration status, including: recording dry body weight before and after exercise; monitoring fluid intake and fluid output; accounting for insensible losses; and using a color chart to determine urine color and thus hydration status. This section will continue the discussion on ways in which urine can be analyzed in terms of hydration, how to analyze different aspects of urine, how to take, record, and measure samples, and factors that affect the measurement of samples.

Urine and Hydration

Urine samples have been used frequently in research studies to identify levels of hydration in both exercising and non-exercising populations. The most useful studies in

terms of determining extreme levels of dehydration are with those participants who are active athletes training or competing; since their levels of exercise are higher, their energy expenditure is greater, and additionally their opportunities for proper rehydration between training or competitions may be limited. While these urine samples can assist in showing specific time points of hydration status, a single urine sample alone is not accurate in showing an athlete's overall hydration status or representing the complexities of fluid movements intra and extracellularly. Combining at least two of these field-based measurements can more accurately assess hydration (Armstrong, 2007).

Analysis with Urine Osmolality

The urinary measurement of urine osmolality (U_{osm}) is one laboratory-based method of determine hydration status. U_{osm} represents the number of solutes that are dissolved in urine. In a recent study, an inverse association was found between urine osmolality and drinking behaviors – as elite boxers neglected to drink sufficient water to avoid dehydration, their U_{osm} values increased (Zubac et al., 2017).

There were similar findings in this inverse association with non-elite or noncompeting athletes. A study monitored over 100 undergraduate college students who were either permitted or not permitted to drink water. They were taken into closed rooms to perform cognition tests, without performing any physical exercise during these trials. Even without the aspect of physical exercise, the participants who did not drink water had high U_{osm} values (Benton et al., 2016) which shows that urine osmolality can be a sensitive indicator of hydration even in people not exerting large amounts of energy.

Analysis with USG

Urine Specific Gravity (USG) is a more commonly used urine analysis tests as it is easy to perform at low costs (U_{osm} measurements require laboratory equipment). An increased USG value represents an increase in urine concentration in response to increases in dehydration, with or without significant changes in TBW (Cheuvront et al., 2015). USG is especially used in field testing settings, as it is a non-invasive method, highly practical, and provides instant results (Armstrong, 2007; Arnaoutis et al., 2015; Rosinger, 2015).

Countless studies have validated its use in assessing hydration status (Armstrong et al., 2010; Hamouti et al., 2013; Minton et al., 2015; Sommerfield et al., 2015). Tools that are used to measure USG may not always accurately represent hydration status, which will be discussed in a section further on about the factors that affect urine measurements. Urine samples can easily be taken in duplicate and averaged to test USG (Hamouti et al., 2013; O'Neal et al., 2012) which assists in validating the USG values of urine samples at different time points surrounding exercise.

A 2006 study sought to determine the hydration status of football players participating in consecutive days of two-a-day practices, monitoring their pre-exercise USG values, changes in body weight, and to determine if their current fluid protocol was sufficient to prevent dehydration. The athletes' USG values were >1.020 on most of the days prior to beginning exercise without implementing the drinking protocol. Additionally, the athletes were losing significant body weight throughout the practices. The second part of the study which included implementing a standard drinking protocol showed improvement in USG values (decrease from 1.021-1.016 on average) and the athletes were gaining weight instead of losing weight. Using not only body weight change data but also USG values, it is much easier for researchers, coaches, and trainers to determine the needs of athletes and assist in improving their hydration and overall performance in practices and games (Stover, Stofan, Murray, & Horswill, 2006).

USG and Urine Osmolality Correlation

Multiple researchers have noted in past publications that when assessing hydration status, USG can be used interchangeably with urine osmolality as they present with a very high correlation between measurements ($r^2=0.81-0.91$) (Armstrong et al., 2010); ($r^2=0.995$) (Logan-Sprenger et al., 2012). With a USG cutoff value of >1.020 denoting the beginning of dehydration, the comparable value of >701 mOsm/kg denotes the cutoff value for urine osmolality (Zubac et al., 2017).

Analysis with Urine Color

The use of color for determining hydration status is one of the simplest techniques that can be used in field or laboratory settings. Urochrome is the aspect of urine that is being measured when classifying urine color. When urine is excreted in larger volumes with low concentrations of solutes, the color will be pale; however densely concentrated solutes in urine will cause the color to be dark yellow, or even brown (Fernandez-Elias et al., 2014). An 8-point color chart has been used for countless studies and validated for many adult urine sample testing (Armstrong, 2007), when a color indicated by 1 will be pale and by 8 will be dark brown (see Appendix D).

Prior to 2015, using a color scale for testing the hydration status in children and adolescents had never been performed. Kavouras and colleagues therefore completed a study to determine urine color in over 200 children ages 8-14 and verified that their urine osmolality values matched the previously validated 8-point color chart and thus was an accurate tool for assessing children's hydration status (Kavouras et al., 2015).

Proper methods for classifying urine color are crucial as the determination of color can be affected by many factors. One method employed in Arnaoutis' study was placing the urine samples in clear glass vials; using proper lighting; holding the vials against a plain white background; and comparing the samples to the validated 8-point color chart (Arnaoutis et al., 2015). Along with having either the same person performing this series for each sample, or having two people to validate the samples' colors, these methods ensure the most accurate determination of urine color and how closely it matches to the colors on the validated scale.

Analysis at Different Time Periods

Along with the variety of the above stated methods, there are options for when to test urine samples and their accuracy in measuring hydration status at different time points. A spot measurement, which can be defined as taking urine samples at one specific time point, is usually recorded either before exercise, during exercise, directly after exercise, or the morning after exercise (typically a first morning void) (Arnaoutis et al., 2015). These types of measurements represent only a small part of a person's total hydration status; as the ICF/ECF movements in the body change so rapidly surrounding times of exercise, a single-point urine sample will not accurately represent an athlete's overall hydration (Cheuvront et al., 2015).

Cheuvront and colleagues went on to discuss what samples taken at different time points represent. A "first morning" urine sample has been agreed upon as the best marker of hydration as a person's body fluids equilibrate overnight and the follow morning's urine concentrations will be accurate of their actual hydration status (Cheuvront et al., 2010; Hamouti et al., 2010). Measurements taken directly before exercise or directly after exercise, while having the potential to be compared against first morning, are not accurate representative measurements of hydration status. Fluid ingestion surrounding these times can actually undermine a person's possible dehydration (Phillips et al., 2014). These single-point measurements taken directly before or after exercise do not pick up on nuances of TBW movements (Cheuvront and Kenefick, 2014).

A 24-hour collection of urine has the potential to represent the complete hydration status of athletes surrounding times of exercise. Collecting a full 24-hour sample would represent the average level of hydration or dehydration compared to single-point spot sample measurement. Additionally, some studies have found a correlation between number of voids and hydration status. One study was designed with non-athletes partaking in the collection a 24-hour void where half of the participants were a control and the other half were restricted to 500 mL of water. The study participants who were classified as more euhydrated produced a greater number of urine voids over 24 hours. Additionally, this group also had higher overall urine outputs, lower USG and U_{osm}

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values, and lower classification on the color scale, all correlating to proper hydration statuses when not restricted in fluid intake (Burchfield et al., 2014).

Analysis with Urine Metabolites

Urine can also be analyzed for the composition of chemical compounds with dipsticks, which can produce point-of-care results on the contents of urine samples, specifically: glucose, USG, bilirubin, ketones, blood, pH, protein, nitrites, and leukocytes. The results can verify any color or USG measurements performed manually. In addition to identifying these components, the results can assist healthcare professionals in identifying any underlying issues, such as a person's liver or kidney functions, acidbase balance, or even urinary tract infections (National Committee, 1996).

Urine Analysis Tools

Multiple tools have been created to measure these urinary markers of hydration, specifically to measure USG as it is one of the most widely accepted measurements. One tool used is a refractometer which calculates light velocity ratios in urine (Pettersson & Berg, 2014). Manual-style refractometers are typically used with placing drops of urine onto a glass plate in the tool, holding it up to the light, and manually reading where the line touches the USG value scale. A digital-style refractometer is a device than can be inserted into a urine sample and it will display a USG value on the screen of the tool (Cleary et al., 2014; Rosinger, 2015). While easier to use, digital refractometers may underestimate USG and thus a person's actual level of dehydration (Minton et al., 2015).

A hydrometer, which is similar to a thermometer in that it is weighted with mercury to float in the substances in which it is placed, also measures USG. Urine can be placed in a cylinder and the hydrometer is insterted into the urine. It is read by the line that touches the meniscus of the urine which will represent its USG value. Refractometers are generally considered more accurate than hydrometers, but the accuracy of the digital over the manual or vice versa has not been clearly identified (Carlton & Orr, 2015).

Factors Affecting Urine Measurements

In order to ensure that the best and most accurate measurements are being produced by these tools, proper methods must be followed closely. Refractometers must be properly calibrated before measuring urine samples – most can be calibrated to read zero with distilled water and should be cleaned between reading urine samples with distilled water as well (Brandenburg & Gaetz, 2012; Chen et al., 2016; Da Silva et al., 2011; Zubac et al., 2017).

Temperature is another factor that must be monitored when testing urine samples. Chen performed his analyses at room temperature which is around 20°C (Chen et al., 2016). In terms of storing urine samples for later analysis, Adams and colleagues concluded that refrigeration of samples prolonged the urine's stability longer than if the samples were kept at room temperature (7 versus 2 days, respectively) and that the readings of both U_{osm} and USG were similar to samples tested immediately after collection. They found, however, that freezing the samples altered the validity of the U_{osm} and USG measurements, specifically decreasing the accuracy of identifying true hydration compared to the immediately tested urine samples (Adams et al, 2017).

With the combination of tools, testing measurements, and proper methods, there is potential room for error from either the manual or the technological side. All procedures must be followed closely to ensure that the urine composition and concentration measurements are read accurately. Any tools used should be calibrated and tested frequently for accuracy and validity. Some testing methods are not entirely accurate solely because of peoples' frequent fluctuations in TBW and fluid balance, so sometimes measurements such as USG can produce errors of greater assumptions of dehydration than are actually present (Arnaoutis et al., 2015). Using USG, U_{osm}, urine color, urine composition, or any other method, multiple samples at multiple time points should be collected and measured surrounding exercise for the most accurate representation of a person's hydration status. It must be performed in a standardized way to meet the optimal requirements of measurements.

Training Environments

The environment in which an athlete is training or competing may be the most important determinant for their hydration status and fluid balance surrounding exercise over an extended period of time. Studies have been performed with athletes who exercise indoors and who typically do not experience any effects from the sun, and studies with athletes who exercise outdoors and will have exposure to many environmental conditions (temperature, humidity, sun radiation, wind speed, precipitation). The following studies give insight into the difficulties that athletes can face in both environments, with a focus on sunlight radiation, and different behaviors these athletes may employ to ensure proper hydration while maintaining optimal performance.

Indoors

Indoor environments for training and competition have more stability and predictability than do any outdoor environments. When indoors, the afore-mentioned conditions have no effect on the athletes and the game play. In some instances, indoor areas can be climate controlled which creates ideal environments for whatever type of exercise that will be done. Basketball players train and compete almost exclusively indoors, and their courts can be adjusted to provide moderate-temperature conditions; this is unlike sports such as soccer who typically practice and compete outdoors and have no control over environmental factors (Brandenburg & Gaetz, 2012).

Studies can be created to establish climate-controlled environments. If temperature, humidity, or any other environmental factor is not a variable for which the researchers want to analyze, they can control for these factors by creating rooms or chambers with conditions that meet their exact requirements. For example, in Capitán and Aragón-Varags' study, they wanted to test thirst response with dehydration through heat exposure. To ensure consistent conditions for each participant every trial, they created climate-controlled chambers in which the participants cycled and were able to produce either heat with humidity or dry heat (Capitan & Aragon-Vargas, 2014). Unexpected changes can still occur indoors, however. Wrestling rooms or any smaller room without proper ventilation can accumulate heat and humidity, changing the environment during exercise and potentially affecting the athletes and their fluid requirements to compensate for increased sweating during exercise (NCAA, 2016).

Outdoors

Outdoor conditions, while predictable to some degree, can present a myriad of volatile circumstances for athletes during training or competition. Training sessions that take place without shade or cover from the sun or precipitation mean that athletes may potentially need to come prepared with different gear or clothing. Practices or games may be cancelled or moved to a different location if conditions are very extreme. Athletes are affected in different ways depending on the environmental conditions, and just as indoors, they will have to adjust their behaviors accordingly.

Sunlight Radiation

The greatest significance between indoor and outdoor environments is the presence or lack of sunlight radiation. The exposure of ultra-violet (UV) light from the sun in part of sunlight radiation involves the transfer of heat between the sun and the earth. Sunlight radiation can cause varying degrees of stress on the human body, including but not limited to heat stress or illness, skin burns, poor core body temperature regulation, or decreased exercise duration or intensity. Because the body needs to stay within a certain range for internal temperature (35-39°C) the duration and amount of

sunlight that an athlete is exposed to can greatly affect their health and performance (Baker & Kenney, 2016).

In a study by Otani and colleagues, cyclists exercised with differing levels of sunlight exposure, measured as sun irradiance in watts per meter squared (W/m^2). The highest exposure was 800 W/m^2 , decreasing to 500, 250, and 0 W/m^2 for the remaining trials. No differences were found in sweat rate or core body temperature, but the cyclists had the fastest time to exhaustion compared to all lower levels of sunlight exposure (Otani, Kaya, Tamaki, Watson, & Maughan, 2016). This finding is important in understanding the significance of exercising outdoors with exposure to sunlight radiation as compared to indoors where no radiation is experienced as athletes may have significant increased time to fatigue and even longer times for recovery.

The amount of sunlight radiation from the sun that is experienced on earth can be directly measured with a black light from a heat stress tracker that provides the measurement of wet bulb globe temperature (WBGT). Using this value alongside ambient temperature and other environmental measurements provides a description of the climate and the amount of heat stress that athletes may experience when outside in conditions with exposure to the sun (Otani et al., 2016). If a team is unable to measure WBGT with a heat stress tracker, it can be estimated with a chart comparing ambient temperature to percent relative humidity (Binkley et al., 2002).

There are protocols that have been established to safeguard athletes from exercising too long in conditions that are too hot or too high in exposure to sunlight radiation. Organizations such as NATA, U.S. Military, and the Georgia High School Athletics Association have created guidelines that are similar in their recommendations for ambient temperature and WBGT ranges and the type/intensity of exercise that should be performed or avoided. For example, the U.S. Army Center for Health Promotion created a table that depicts Heat Categories as colors ranging from white, green, yellow, red, and black that recommend as the WBGT index increases, the amount of moderate and hard work should be decreased as rest is increased, alongside an increase in water intake (University of Connecticut, n.d.).

Temperature

The range of temperature that can be reached in an outdoor environment is so extreme that it can be difficult to make comparisons between different locations and different types of exercise or sports. There are studies on climate and the effects on body composition, with one of them identifying that those with higher muscle composition (compared to higher fat composition) are able to adapt better to varying hot and cold conditions (Wells, 2012). Colder temperatures present dilemmas such as hypothermia, which is an uncommon occurrence in athletes since they should be properly clothed for those environments and additionally, are actively expelling energy. More research is needed in the area of athletes training and competing outdoors in colder temperatures to determine any negative effects on hydration and performance (O'Neal et al., 2012).

In hotter temperatures, hypohydration has been shown to occur more frequently than in colder temperatures as the factor of increased sweating and possibly insufficient fluid intake increases an athlete's dehydration throughout exercise. James and colleagues performed a study which monitored the outcome of performance in athletes who became hypohydrated with losing greater than 2% of their body mass. With trials at temperatures of 34°C, athletes performed exercises as either euhydrated or hypohydrated. Those athletes who exercised hypohydrated in hot temperatures had decreased performance; and increased thirst, heart rate, and rate of perceived exertion (RPE) (James et al., 2017).

In research studying the effects of heat stress on athletes, Wingo and colleagues summarized the average ambient temperatures between 22-35°C (~71-95°F). 22°C was considered a 'cool' ambient temperature between these studies although in most cases, this is still a moderate-hot environmental condition (Wingo, Ganio, & Cureton, 2012). With hotter temperatures, athletes have greater risks of hypohydration from increased sweating, but also decreased fluid intake – not just during exercise but times outside of practices or games when athletes need to rehydrate. One mechanism to ensure proper hydration and rehydration is to supplement with an electrolyte beverage to increase thermoregulation and improve performance measures as well (Sawka et al., 2000). Humidity

Humidity can be present both indoors and outdoors and can occur with either hot or cold temperatures. Heat alongside humidity is a harmful combination to athletes as they have greater dangers of excessive sweating, increased fluid needs, yet longer recovery and rehydration periods (Rosinger, 2015). Sweat rate is a more important factor than total sweat loss in these environments (Thigpen et al., 2014). When exercising indoors and outdoors with exposure to humidity, which can range from 0-100%, other factors must still be considered. If indoors, ambient temperature is also an important

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factor to monitor. If outdoors, ambient temperature, WBGT, and wind speed are important as well (Broad et al., 1996).

Another consideration in humid environments is the risk for higher levels of pollutants in the air. After the 2008 Olympics, an article was published regarding the athletes who trained for this event and how they prepared for the highly polluted region of Beijing, China. High humidity with high pollution can cause respiratory distress in any person, but especially for an exercising athlete they are at risk for asthma or exerciseinduced bronchospasms (Braniš & Větvička, 2010). Monitoring the air quality and relative humidity is thus essential to protect athletes from any unnecessary distress during exercise ranging from small practices all the way to Olympic games.

Acclimation

Becoming acclimated to certain temperatures and environmental conditions is an advantage that some athletes can have over others. This may take place naturally for athletes who are training during the warmest months of the year consecutively, for example June-September for most of the areas throughout the United States (Baker et al., 2016).

Some advantages of heat acclimation reported by Sawka and colleagues are improved thermoregulation, enhanced Na⁺ resorption, improved ECF, and solute redistribution; but negatively, these athletes have a worse core temperature response, increased sweat rate, and more potential instances of hypohydration (Sawka et al., 2000). Athletes can train to avoid uneven progression towards acclimation to their environment which also capitalizes on their adaption to thermoregulation (Coso et al., 2008).

Conclusion

How an athlete responds to his or her environment is influenced by countless factors, some that can be controlled, yet many others that cannot. For athletes who transition quickly between multiple bouts of exercise with minimal recovery time, the unknown of how their bodies will react is a topic that must be researched heavily. In addition, it is very important to determine athletes' needs not just in one environment, but in all possible exercise situations. For soccer players, the variability of exercise types, energy expenditure, and most importantly environmental conditions yields even more importance on the understanding on their needs.

The lack of research involving athletes who participate in activities both indoors without sun exposure and outdoors with sun exposure warrants a deeper look into this special population of athletes. Especially for those athletes who are living or competing in hotter climates, with or without humidity, the stark transitions from indoors to outdoors can cause even more problems with their abilities to maintain proper fluid balance and sustain euhydration. This current study sought to determine the effect of sunlight radiation on fluid balance and hydration status by studying indoor and outdoor exercise. The results of this study will be novel in the sense of: the direct comparison of athletes training indoors and outdoors; similar estimated energy expenditure and performance of similar exercises; not informing the athletes of the exact mechanism of the study design; and not restricting or dictating their fluid intake.

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CHAPTER 3

METHODS

Design

The research design was a quasi-experimental research study as the variable of outdoor or indoor location (exposure or no exposure to sunlight radiation) was manipulated by the investigators. The study sought to determine a causal effect or impact of sun exposure on the population of female soccer players as they exercised in both conditions with varying temperatures and levels of humidity. The study was performed at the athletic training center at Arizona State University (ASU) in Tempe, Arizona, both on the soccer team's outdoor practice field and inside of the climate-controlled practice field. The study was performed in March and April of 2018. The research protocols were reviewed and approved by the ASU IRB.

Study Population

The subjects were healthy, uninjured, adult female athletes of a singular soccer team who trained and competed at the NCAA DI Pac12 level for ASU. Thirteen female soccer athletes on the active roster consented to participating in the study. The athletes were ages 18-21 (19.8 ± 1.23).

Procedures

The athletes were followed initially during three typical days of practice. As sunlight exposure was the factor being tested, the measurements and data collection were

performed both outdoors with direct sunlight exposure and indoors without direct sunlight exposure. The first and third days were the team's outdoor practices in direct sunlight, and the second day was an indoor practice without sunlight exposure but with controlled climate. Besides the location of practice, which was an independent variable, all other environmental variables were accounted for or controlled. The investigators requested that all monitored training sessions were to be of equal duration and estimated energy expenditure without statistical significance.

The athletes were randomly assigned a study ID number (1-13) by a research assistant who made a private document associating the athlete's name and information with their ID number. The number identified the athlete, their drink bottles, their urine sample cups, and their performance scores during exercise. The primary investigator (PI) and the Co-PI were blinded to the assignment of study ID numbers to athlete names. These investigators identified athletes by their ID numbers to avoid bias in associating results with names.

Each athlete was sent text message reminders from the Co-PI about the study days. The phone numbers were assigned by study ID number, and the texts were sent without an association between athlete numbers and names. The messages contained information about refraining from taking any multivitamin or Vitamin B complex vitamins, what time to arrive before practice, and to use the urine cups provided to collect samples at home. Athletes were told that they could email the PI or Co-PI with any questions regarding the information provided or any additional questions or concerns they had about participating in the study. Once at practice, each athlete was asked to use a numbered facility bathroom stall that matched their ID number and provide a urine sample. They voided their bladders completely into a urine specimen hat. Athletes were provided with a large container to void their bladders at home if they could not wait until practice, and this urine was brought to the facility and included in the weighing and testing of the athlete's preexercise urine sample. A research assistant transferred the urine into previously weighed large containers, measured the weight of the urine voided, and transferred it into 30 mL test tubes to measure USG. After exercising, another urine sample was collected and analyzed in a similar manner.

Each athlete was given a small urine sample cup (labelled with their ID number) to take home. These were used to collect the first morning urine samples the morning after each study day. The athletes were instructed to fill the cup as much as possible with a mid-stream sample the first time they used the bathroom in the morning. If an athlete had to use the bathroom during the middle of the night, they were asked to report this when they handed in their sample. This urine was not weighed, but all other protocols were followed to transfer the urine, determine the USG, the urine content, and color.

Measurements

At practice all data collection was completed within thirty minutes before and after training. The following is a list of measurements taken during the study: urine samples, body weight, fluid intake, and food intake; total distance covered, total minutes for tests, and heart rate; temperature, WBGT, and humidity; total practice time, exercises performed, and total break time. An explanation of how each measurement was performed can be found in the following sections.

Screening Questionnaire

Previous to beginning data collection during practices, a verbal recruitment script was provided to each athlete (see Appendix A), and all athletes volunteered to participate. At an information session held for the athletes, coaches, and staff, the athletes received and completed forms of consent before testing began (see Appendix B). At this session, the heights of the athletes were taken twice, recorded, and averaged. All data was recorded in an online document which could be seen and edited by the investigators and research assistants. There was no specific relationship or dependence between athletes and members of the research team.

The athletes were also asked to participate in an online screener questionnaire which asked questions regarding anthropometrics; activity level; medication, supplement, over-the-counter, and prescription drug use; and what type of fluid they prefer to drink during exercise: either water, sports drinks, or both (see Appendix B and C). This information was important to ensure the athletes were safe to participate in the study. Additionally, it was used to determine the representative habits of the athletes throughout their season of practice and competition, to add to the background information for the characteristics of the team as a whole, and to determine what fluids would be provided for them during the study.

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Fluid Balance

<u>Sweat Rate</u>: Sweat rate was calculated as the amount of fluid lost per hour (L/hr) in the form of sweat. The equation is: (Pre-exercise BM (kg) - post-exercise BM (kg)) + (fluid ingested (L) – urine output (L))/protocol duration (min) x 60. (Edwards et al., 2007).

Eluid and Food Intake: The athletes were all provided drink bottles with their study ID numbers on them. Based on their responses in the questionnaire, they were provided either two water bottles (if their preference for drinks during exercise was only water) or one bottle with water and one with a zero-calorie sports drink (if their preference for drinks during exercise was water and a sports drink). The bottles were weighed (PT 1400, Sartorius AG) twice before filling with liquid and were weighed twice again after to determine the weight of fluid in the bottles pre-exercise. They were encouraged to drink *ad libitum* throughout practices (Da Silva et al., 2011, 2012). They were asked to not spit out the drink or to use it to rinse any part of their bodies. The bottles were kept in a cooler with ice; they were replaced as needed when emptied by the athlete and a coding system allowed for deidentified distribution of bottles. The weights of all bottles, whether empty or fluid-containing, were recorded again twice after practices to determine total fluid intake of each player.

Any snacks that the athletes ate within 15 minutes before and after practice were also weighed in duplicate (PT 1400, Sartorius AG) and recorded in grams to be included in the final weight calculation (weight change was calculated using pre- and post-weight measurements minus the weight from food intake). <u>Urine Collection</u>: Athletes were provided with the opportunity to use the bathroom during practice as there was a mobile bathroom set up on the side of the practice fields. Their urine was collected in the same manner as before, transferred from the specimen hats into previously weighed and labeled collection containers to determine weight loss that was not due to sweat loss. The weights of any during-exercise urine samples were added to the value of the post-exercise urine samples when determining fluid balance (fluid balance was calculated using fluid intake, body weight change, and fluid output). No fecal matter was collected at any time point.

<u>Bodyweight Difference</u>: Directly after the athletes delivered their pre-exercise urine sample, the research assistant who had access to the document of the assigned numbers wrote the ID number on the hand of each athlete to allow the investigators and other research assistants to quickly identify all players while on the practice field. The athletes' weights were taken in duplicate by stepping on a scale (896 Flat Scale, Seca) without shoes, wearing only the team's designated sports bra and spandex shorts and a pair of socks. After exercise, the athletes dried off and changed into their pre-training dry clothes and were weighed again in duplicate to determine their post-exercise weight.

Urine as Predictor of Hydration Status

<u>USG</u>: For each urine sample, taken before, during/after, or the first morning after exercise, 20 mL of urine was transferred into the labeled 30 mL test tubes for immediate analysis of USG.

<u>Urine Color</u>: For each urine sample, taken before, during/after, or the first morning after exercise, urine color of the samples in the 30 mL test tubes was identified against a standardized color chart. See Appendix D.

After each study day was completed, the urine samples were transported to the nearby study laboratory. The USG was determined with the digital refractometer (Pen - Urine S.G., Atago). Based on earlier pilot measurements it reported more precise and accurate measurements than the other two tested options: an optical USG meter and a hydrometer. The samples were measured twice and recorded, and an average was taken to determine the sample's USG value.

Urine color was tested on a separate day. Any urine samples which had been frozen in the 30 mL test tubes were brought back to 20°C in a warm laboratory water bath. All samples were inverted to redistribute particles that might have precipitated. The test tubes were held against a plain white background using the same back-lighting and were compared against the verified color chart with a 1-8 scale (1 is extremely hyperhydrated and 8 is extremely hypohydrated) (Fernandez-Elias et al., 2014). Both the PI and the Co-PI agreed upon which color on the chart the urine sample most closely matched.

It is important to note that previous research has determined an influence of freezing on urine color., Samples frozen at -20°C or colder produced underreported numbers; meaning samples that are frozen produce urine that is lighter in color and lower on the urine color scale making it read up to one shade lighter (one number lower) (Adams et al., 2017).

Exercise Performance

Athletes of the participating roster completed all designated exercises within their practices. The performance portion of the study was conducted during the normal training regime of the athletes. The investigators and research assistants communicated the expectations of testing to the athletic trainer and the coaching staff previous to the study commencing. All parameters of the performance of the study days were recorded in detail. The overall time of each practice day was recorded from the first warm-up to the last minute of scrimmage or running.

The following entails the training sessions discussed with coaching staff* before the study commenced, approximating 160 minutes of total workload per study day:

- 20-30 minutes for delivering on-site urine samples and measuring pre-training weight;
- 60 minutes for warm up, high quality ball training (short drinking opportunities in the middle);
- 5 minutes for a recovery pause;
- 25 minutes to complete the Yo-Yo Intermittent Test. Athletes who finished the test jogged the remaining time which allowed the others that dropped out earlier to finish the test on a modified pace;
- 5-10 minutes for a recovery pause;
- 20-30 minutes for delivering on-site urine samples and measuring post-training weight.

*Actual practice times and activities performed were modified on the study days based on decisions by coaching staff. The total time was not equal across all study days and the activities performed varied as well (see the Discussion section for changes). The individual times of completing the tests were recorded, as well as the type and duration of warm-up exercises, drills, and scrimmages. The number and duration of breaks were recorded to determine how much time the athletes were provided for drinking opportunities.

<u>Yo-Yo Test</u>: Yo-Yo Intermittent Recovery Test Level 1 was completed on Day 1 outdoors and Day 2 indoors. This test is a frequently used respiratory fitness test. The test is used to gain insight in performance levels and risk of injury in athletes.

<u>Running Box Test</u>: A Running Box Test was completed on Day 3 outdoors. This test consists of timed sprints and core-strengthening exercises.

<u>Total Meters Covered</u>: The athletes were provided with a tracking device (BioModule Catapult) by the athletic trainer, which they wore on their backs during all regular team practices. The Catapult trackers recorded the distance (in meters) that the players covered, and this information was provided to the investigators by the athletic trainer.

<u>Heart Rate</u>: The athletes were provided with an additional tracker (Zephyr Performance Systems) by the research team, which they wore around their ribs right below the breast bone. These trackers recorded multiple data points, one of which included heart rate that was used for the statistical analysis. The PI or research assistants checked to ensure proper fit of the Zephyr and that they were all turned on and connected for recording data before each practice began. <u>Estimated Energy Expenditure</u>: EE was calculated using heart rate (from the Zephyr tracker), METs (from the Catapult tracker), and total time for each athlete on each practice day. EE was analyzed as a variable both as total EE and EE/hr.

Environmental Variables

To measure the environmental variables, two environmental trackers (Kestrel 5400 Heat Stress Tracker) were utilized to obtain accurate average readings. For outdoor practices, one tracker was placed in the shade and one in the sunlight. For the indoor practice, each tracker was placed on opposite corners of the practice field. The second of the outdoor practice days was a day with higher sun radiation and was also compared to both the first day of outdoor practice as well as the indoor practice day. The trackers recorded ambient temperature, wind speed, relative humidity, and WBGT which was the variable that revealed the amount of sunlight radiation.

Statistical Analysis

Data was reported as mean \pm standard deviation (SD) or as median and interquartile range (IQR). Data was examined for normality. Two-way repeated measures ANOVA were used to analyze if a difference in performance outcomes, fluid balance, and hydration status existed between the athletes practicing in different environments. A non-parametric repeated measures ANOVA (Friedman's) test was run for this data set to detect differences across all three days (p<0.05). To further determine significant differences between specific days, a Wilcoxon signed rank test was ran on all variables (p<0.0167). Correlations were run to determine any further similarities between all variables. Significance was set at p \leq 0.05 and the Statistical Package for Social Sciences (SPSS 21 for Windows, 2010, Chicago IL) was used for analysis.

The data was organized within Excel and SPSS by study ID number, separating each athlete and showing their individual values for each of the three study days. The environmental values were the same for each athlete, so they were listed first before the other variables. Some variables were also split into groups (for example, water drinkers or water and sports drink drinkers) to test for differences in this population.

CHAPTER 4

RESULTS

Ten of the thirteen athletes participated in all three study days. Data was not normally distributed, and results are presented as medians and IQR. The population were elite female collegiate soccer players ages 19.8±1.23, with an average height of 171.6±6.4 cm and weight of 63.7±7.4 kg. Athlete characteristics were reviewed among those who participated on all three days, acquired from the pre-screening questionnaire. All athletes were healthy and uninjured and able to partake in normal training activities. No athletes reported taking a vitamin B supplement or using tobacco products, however 20% took a daily multivitamin and 33% of the athletes were on birth control. Out of the 10 athletes, 60% preferred drinking only water during practices while the other 40% preferred drinking both water and a sports drink.

Performance Outcomes

The research outcomes defining the study across the three study days are presented in **Table 2**. Total energy expenditure (EE) was significantly different between the three days (717 kilocalories [kcal], 627 kcal, and 693 kcal respectively; p=0.001), and statistical significance was found for total EE between days 1 and 2, and days 2 and 3 (p=0.007, 0.005). These differences were only observed when the absolute EE was calculated; when EE/hr was calculated no difference was found (p=0.078). Average heart rate was significantly different between all days (163 BPM, 156 BPM, and 153 BPM

respectively; p=0.041), but not when each individual day was compared against the

others (p>0.05). No differences were observed for mean heart rate.

	Day 1	Day 2	Day 3	
Exercise				
Energy expenditure (kcal) ◊□	718 (671-810)	627 (562-678)	693 (632-775)	
Energy expenditure/hour (kcal/hr)	454 (425-513)	448 (402-484)	425 (389-475)	
Mean HR (beats/min)	163 (156-170)	156 (140-165)	153 (143-159)	
Peak HRpint (beats/min)	200 (191-203)	205 (192-211)	204 (196-220)	
Distance covered (km) ●□	6.57 (5.29-7.39)	7.13 (6.16-8.28)	5.67 (4.67-5.91)	
Environment				
Month	March	March	April	
Type of training	Cold-outside	Moderate-inside	Moderate-outside	
Temperature (°C)	14.2 (9-19.4)	19.25 (19-19.5)	21.1 (16.8-25.3)	
Wet Bulb Globe Temperature (°C)	10.9 (6.7-15)	13.1 (12.5-13.6)	14.9 (13.6-16.2)	
Humidity (%)	46.5 (36.9-56.1)	27.8 (27.2-28.4)	18.1 (13.6-22.6)	

Table 2. Exercise and environmental characteristics per study day, Median (IQR).

A non-parametric ANOVA test was run for this data set (Friedman's ANOVA, p<0.05) as well as a Wilcoxon signed rank test (p<0.0167).

◊ statistical significance between Day 1 and Day 2

• statistical significance between Day 1 and Day 3

□ statistical significance between Day 2 and Day 3

The distance covered in meters by the athletes was significantly different between

the three days (6,571 meters (m), 7,127 m, and 5,667 m, respectively; p=0.005), and

statistical significance was found for distance covered between days 1 and 3, and days 2

and 3 (p=0.012, 0.011).

Slight differences were observed for the environmental factors of humidity and

temperature. As humidity decreased over the three days (48%, 28%, and 18%,

respectively), ambient temperature increased (13°C, 19°C, and 20°C, respectively). Day 1

was colder than 2 and 3; however, none of these differences were significant (p>0.05).

Fluid Intake, Sweat Rate, and Bodyweight Changes

Variables pertaining to the athletes' hydration status and fluid balance of the three

days compared against one another are described in **Table 3**. Six athletes chose to drink

water only, and four athletes chose both water and a zero-calorie sports drink. For the athletes who chose both, each athlete drank more from their bottles of water than they did their bottles of sports drink (an average of 0.538 L of water compared to 0.349 L of sports drink; exclusive water drinkers drank an average 0.693 L).

The athletes consumed the most amount of fluid on Day 3 (1.09 L [0.91-1.29 L]) but drank at the highest rate on Day 2 (0.76 L/hr [0.53-0.94 L/hr]). To compare to their sweat rate, the highest average rate occurred on Day 2 as well (0.55 L/hr [0.23-0.69 L/hr]). No athletes lost amounts of weight over the 2% body weight loss cutoff or even over a 1% mark; and some gained weight on study days, producing a total average range for absolute percent weight loss of -0.55-0.58%.

No significant differences were found in these categories, but the results may still represent practical relevant differences between some variables. Fluid intake rate was marginally significant across the three study days (0.49 L/hr, 0.76 L/hr, and 0.67 L/hr, respectively; p=0.07). Post weight values were also marginally but not as discernably significant across three study days (65.53 kg, 65.45 kg, and 65.30 kg, respectively; p=0.066). The medians of these values were both the highest on Day 2 (see **Table 3**). Other variables with marginal differences over the three study days were sweat rate and total fluid intake. No other perceived significances were found in any of the other variables. Splitting the data set based on drinking preference, performance outcome, fluid intake, sweat rate, or food intake did not result in significant differences (p>0.05).

	Pre weight	Post weight	Absolute	Absolute	Level of	Fluid intake	Fluid intake/	Food intake	Sweat
	(kg)	(kg)	weight loss	percent	Dehydration	(1)	Hour (l/hr)	(kg)	rate/hour
			(kg)	weight loss	(%)				(l/hr)
Day 1	65.7	65.4	-0.2	-0.29	0.29	0.77	0.49		0.48
	(56.6-68.6)	(56.5-68.4)	(-0.35:-0.10)	(-0.55:-0.17)	(0.17-0.54)	(0.66-1.06)	(0.42-0.67)		(0.24-0.64)
Day 2	65.3	65.5	0.1	0.18	-0.17	1.06	0.76	0.0	0.55
	(57.0-70.0)	(57.1-69.5)	(-0.15-0.20)	(-0.21-0.31)	(-0.27-0.29)	(0.74-1.31)	(0.53-0.94)	(0-0.03)	(0.23-0.69)
Day 3	65.3	65.3	0.0	0.02	0.04	1.09	0.67	0.04	0.45
	(56.3-68.6)	(56.6-68.2)	(-0.35-0.40)	(-0.52-0.58)	(-0.55-0.63)	(0.91-1.29)	(0.56-0.79)	(0-0.07)	(0.36-0.74)

Table 3. Hydration status and fluid balance per study day, Median (IQR), n=10.

Absolute percent weight loss only depicts the percent differences between pre-exercise weight and post-exercise weight. Level of dehydration depicts the percent differences as well as accounting for food intake.

Hydration Status Using USG

Urine Specific Gravity (USG) values were measured pre-exercise, post-exercise, and the morning after exercise resulting in USG values listed below in **Table 4**. The values ranged from 1.003 (relative hydration) to 1.032 (severe dehydration), which were both recorded from the morning after exercise USG values. No significance was found within data for each category of before, after, or the morning after exercise (p>0.05), and no significance was found between the three values compared against the three study days (p>0.05). In total 68% of the samples exceeded the cut-off value of 1.020, and 37% of the samples exceeded the cut-off value of 1.025, indicating that a large portion of the players were dehydrated before and after practice.

	USG Pre			USG Post			USG 1 st Morning		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Mean	1.021	1.024	1.022	1.021	1.024	1.024	1.022	1.021	1.019
Min	1.014	1.013	1.014	1.008	1.009	1.009	1.014	1.013	1.003
Max	1.030	1.031	1.030	1.030	1.029	1.030	1.032	1.032	1.030
% >1.020	60%	90%	70%	70%	80%	80%	60%	50%	50%

Table 4. USG Values per Study Day; for pre-exercise, post-exercise, and the morning after exercise; averages of each day, including the minimum and maximum recorded USG value.

When the data was observed graphically looking at First Morning USG Values for separate athletes on each study day, it was determined that four out of the ten total athletes consistently presented with high USG values (>1025). See **Figure 1**. The athletes

were separated into groups – dehydrated or hydrated – which delineated their average USG values over all study days as either >1.025 (dehydrated) or <1.025 (hydrated). The data is also represented as grouped results and individual data in **Figures 2 and 3** below:



Figure 1. First Morning USG Values the day after exercise, organized by the dehydrated athletes (n=4) and hydrated athletes (n=6).



Figure 2. Dehydrated and hydrated USG values. Median (IQR) and range for athletes in the dehydrated group (average USG values >1.025) and hydrated group (average USG values <1.025).



Figure 3. Individual USG values from all days and time points combined separated by dehydrated group (average USG values >1.025) and hydrated group (average USG values <1.025).

Urine Color

The color of all urine samples collected pre-exercise, post-exercise, and the morning after exercise resulted in the values listed in **Table 5**. The values ranged from 1 (extreme hyperhydration) to 7 (heavy-extreme hypohydration, being dehydrated). No significance was found within data for each category (p>0.05), and no significance was found between the three values compared against the three study days (p>0.05). The highest average color values were recorded from after practice samples, indicating that a large portion of the players were dehydrated during and post-exercise.

Table 5. Average (min-max) urine color using a validated color scale (Appendix D) from the three study days and separate time points.

	Before Practice			After Practice			Morning after Practice		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Mean	5	6	5	6	6	6	5	5	4
Min	3	3	3	1	4	3	3	2	1
Max	7	7	7	7	7	7	7	7	6

CHAPTER 5

DISCUSSION

Although differences were predicted to occur between the environmental factors and their relationship to athletic performance, hydration status, or fluid balance across the three study days, none were observed. Exercise performed outdoors with exposure to sunlight radiation did not cause increased levels of hypohydration compared to exercise performed indoors without sunlight exposure. The ambient temperature, WBGT, and humidity were similar between both of the outdoor and the indoor days. With these parameters, athletes had comparable changes in body weight, intakes of fluid, and sweat rates. While environment can play a role in dehydration instances, personal behaviors and protocols can have an impact on athlete hydration status and fluid balance, especially for athletes training in moderate environments but preparing for hotter conditions.

Fluid Intake and Bodyweight Changes

No athlete lost more than 2% of their body weight from water losses during practice on any day. Arizona characteristically has very hot, dry conditions throughout a majority of the year; however, the temperatures, humidity levels, and WBGT were not representative of typical Arizona weather. The study conditions were temperate, with moderate temperatures in the early morning practices, and without substantial sun exposure or heat. From desired conditions of very low humidity on all days, and hotter temperatures and greater sunlight radiation on outdoor days, the actual conditions presented the following: the highest ambient temperature was recorded on the final outdoor day at 25.3°C (77.5°F) with a maximum WBGT of 16.2°C (61.2°F), and a high of 56.1% relative humidity on the first outdoor day. The larger effect of sun radiation that was desired did not occur.

It may be hypothesized that when athletes were to complete the same study in hotter, dryer conditions on the outdoor practice days, higher levels of dehydration would have been seen because of an inability to drink sufficient fluids. To speak to Arnaoutis' 2015 study, he found a paradox in fluid balance with athletes practicing or competing indoors and outdoors. While athletes exercising indoors are typically given more drinking opportunities or are situated closer to areas that contain fluids for consumption, notably ice hockey or basketball, their levels of dehydration were actually found to run higher than outdoor-focused athletes (Arnaoutis et al., 2015), which could be explained by their high intensity of sport.

The previous hydration studies that have been performed in hot weather reveal higher instances of performance hindrances and negative effects on health. James and colleagues found that between euhydrated and hypohydrated athletes exercising in a hot environment of 34°C, the hypohydrated group had greater levels of weight loss and greater thirst. Sawka and Montain discussed that higher fluid needs that athletes have when exercising in the heat because of increased sweat rates. They, as well as Wingo et al., discussed that in terms of exercise performance, increasing hypohydration subsequently decreases an athlete's work capacity as temperatures rise (James et al., 2017; M N Sawka et al., 2000; Wingo et al., 2012).

Other athletes may have experienced involuntary dehydration. Thirst perceptions play a role in this type of dehydration where athletes are simply not consuming sufficient fluids to replace their losses. If athletes sense that their thirst is quenched faster than their fluids are replaced, they may inadvertently fall into hypohydration (Greenleaf, 1992).

The athletes who selected to receive both water and a zero-calorie sports drink all drank more from their water bottle than they did the sports drink bottle during the practice days. Fluid choices can affect thirst response in athletes when exercising, and fluids also hold effects depending on their content of electrolytes and/or carbohydrates. Athletes' bodies may have retained more water when drinking the sports drink compared to only water (Adams & Casa, 2003) which could have helped their hydration status by producing less concentrated urine post-exercise.

Sweat rate calculations revealed no statistical significance either indoors or outdoors. As sweat rate is determined from changes in body weight, fluid intake, and urine output, none of these variables were statistically significant which produced no significance in the calculated sweat rate. As the athletes drank sufficiently during practices, they did not lose significant amounts of body weight. Conversely, some athletes gained weight between pre- and post-exercise measurements. These gains in body weight point towards both a surplus in drinking fluids alongside a minimal amount of fluids lost in the form of sweating. The range of sweat rate seen in this study (0.23-0.74 L/hr from all study days) is substantially lower than other studies that monitor athletes' fluid intake, bodyweight changes, and sweat loss (0.6-1.3 L/hr (Cleary et al., 2014); 0.71-1.77 L/hr and 0.99-1.93 L/hr, in winter and summer training conditions, respectively (Sawka et al., 2007)).

General Hydration Status

Although the players consumed enough fluid during practices, based on urinalysis using USG measurements their general hydration status may need to be improved. Performing USG measurements with a refractometer is an accepted and widely used method for determine instances of dehydration in athletes and the tool used in this study presented timely results. USG values recorded directly after exercise are not indicative of an athlete's real-time hydration status, but this reading can be used in conjunction with measurements taken at other points surrounding exercise for an average value. USG values recorded the morning after exercise delineate an athlete's equilibrated fluid balance, which research has shown to be the best marker of hydration (Cheuvront et al., 2015; Hamouti et al., 2010). Again, USG measurements taken at multiple times will provide the most accurate depiction of an athlete's general hydration status.

Some athletes, according to their USG values, were dehydrated during some time points throughout the study, either before exercise, after exercise, or 24 hours after exercise. All mean USG values per day exceeded the cut-off value of 1.020 except for 1st morning on Day 3; overall as a team, though, the athletes averaged to be well hydrated.

Urine color analysis can confirm the results based on both USG and U_{oam} . As researched by Kavouras and Perrier, their methods reported the reliability of using urine color when monitoring hydration status. These researchers found that color can

accurately predict elevated U_{osm} values which signify dehydration. These findings have been confirmed for a variety of study populations, including children, pregnant mothers, and adults (Kavouras et al., 2015; Perrier et al., 2017).

Performing color analysis on the samples from this study did not reveal as accurate of results. The determination of color ratings from the verified color scale were performed in a well-lit laboratory with the same background of a white piece of paper behind the urine samples. Two investigators were confirming the color of each sample. The results revealed that color was rated higher for 36% of all of the urine samples taken; for this percentage, the color value delineated a higher level of dehydration than the accompanying USG value. While using the color scale can be a simple and instantaneous method for determining an athlete's hydration status, neither athletes not researchers should not rely solely on these results.

The addition of the 1.025 USG cutoff value was produced after the analysis of athletes' grouped results of pre-exercise, post-exercise, and first-morning urine samples from all study days (9 measurements per athlete in total). While 1.020 is the value used in research, as stated in previous sections, the investigators sought to determine the results of when the typically dehydrated athletes with average USG values >1.025 were compared against the typically hydrated athletes with average USG values <1.020. There were marked differences as represented in the figures from the Results section. Grouping the athletes in this manner assisted with determining characteristics of the team and allowed for better recommendations to be made for each athlete based upon their average USG values.

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Performance Outcomes

After analysis of the EE variables between all study days, the average EE was not significantly different, suggesting that the tests had no actual differences in overall intensity. The significance found for total EE arose from the differences in times between the three study days (95 minutes, 84 minutes, and 98 minutes, respectively). When EE was compared against time, the significance was no longer present; the variables for total EE were divided to produce kcal/hr which allowed a comparison between all study days to be made properly. The total energy that the athletes expended across all study days was comparable, with an overall range of 389 kcal/hr on Day 3 to 513 kcal/hr on Day 1, which was not significant.

In terms of athletes expending energy and remaining consistently euhydrated when they exercise frequently, studies have examined the impact of hydration status during multiple training sessions. Adams and Casa found that for football players who trained in back-to-back sessions, they were unable to sufficiently replace fluid losses during exercise and during the recovery period between sessions (Adams & Casa, 2003). This research adds to the knowledge of the importance of hydration protocols before, during, and after exercise as an athlete needs to properly hydrate before exercise, continue hydration practices afterwards, and avoid dipping into severe dehydration.

Another difference that was found for EE was that some athletes participated in modified exercises on some study days. While all 10 athletes completed exercise on all three study days, some athletes had modifications as requested by the coach (e.g. goalkeepers practiced drills for catching balls while forwards practiced passing balls); hence the differences between EE a possible explanation for differences between fluid intake and urine output. While performance was not specifically measured as an outcome during this study, there is ample research that has monitored the effects of hypohydration on performance. The majority of studies determined that athletes' performance is compromised and worsened when they begin endurance exercises already in a state of hypohydration (Bardis et al., 2013; Davis et al., 2014; James et al., 2017).

Limitations

Fluid intake behavior may have been influenced by having measurements taken, as athletes who have not been exposed to research may adjust their behaviors when participating in a research study. The athletes were all provided an information session which explained how the study days would operate, but they were not informed of the direct outcomes or hypotheses. Although players were instructed to drink freely during practices, some athletes may have drank differently than normal if their drinking practices were not optimal, as has been noted in previous research (Brandenburg & Gaetz, 2012).

Freezing urine samples can alter the validity of urine color, U_{osm} , and USG measurements. When frozen and subsequently thawed, there is decreased accuracy of identifying true hydration/dehydration compared to the immediately tested urine samples (Adams, Kavouras, Johnson, Jansen, Capitan, & Robillard, 2017). While all USG measurements were taken before freezing the samples, urine color was determined after which can explain the variances between color and USG values as described above.

While the PI and Co-PI presented an outline of the desired total time and exercises for the study, the days differed in total minutes of practice and play time (95, 84, and 98 minutes) based upon slight variances in activities performed each day. Variables were all adjusted for time to make results comparable, but the variances created different lengths of time for breaks and drinking opportunities.

The athletes completed the Yo-Yo Intermittent Test on the first and second study days as outlined by the investigators, but the coaches chose the Running Box Text for Day 3 which they decided would be lower in intensity and cause less of a strain on the athletes who were preparing to compete shortly after the study concluded. Because of the different style of exercise, where the first test involves more running while the second involves more stationary movement, distance was significantly different between Day 3 and the previous two study days.

This study did not calculate specific insensible losses as no fecal samples were produced during the study days. Insensible losses like water lost through fecal matter, respiration, or during energy production normally are not considered in field studies. These processes can compensate for one other and thus do not greatly impact the final calculation of sweat losses during exercise (Vandermark, 2016).

The study participants were females and some of these females were menstruating during the study days. In terms of urine color, some athletes' samples were notably more red or brown in color, and athletes did confirm that they had their menstrual cycle when they produced urine samples. Both the color and USG measurements can be affected by producing false negatives of dehydration or color/USG values that are higher than they would be without the presence on blood in the urine samples.

Other possible misinterpretations of data could include: misreported food consumption if the athletes did not bring their food items/packaging to the field; urine excreted in the home that was not collected or brought to the field; athletes not discontinuing their supplements within the required time frame (two days before each study day); or sweat being trapped within clothing during weigh-ins.

CHAPTER 6

CONCLUSION AND APPLICATIONS

In general, the hydration status of the female soccer athletes in this study was not indicative of severe dehydration on any of the study days, whether with or without sunlight exposure. While a significant difference was not found between training sessions of moderate temperatures, the potential for extreme dehydration in hotter Arizona temperatures justifies a change in the recommendations for rehydration practices. Athletes must be aware of their hydration status and fluid balance in cold-moderate temperatures by monitoring their USG values, bodyweight changes, and sweat rates; if they are beginning exercise in states of hypohydration without the factor of sun radiation, their instances for dehydration with intense sunlight radiation will cause extreme dehydration which will only further hinder their performance and negatively affect their health.

Since performing this study, it is imperative that additional research be performed to continue testing with this type of elite athlete in all environments. Performing this research study design with both male and female soccer players can produce a comparison especially between USG values and urine color of the two genders. Performing this research study with different sports also, like lacrosse, basketball, sprinters, or triathletes, will produce additional information for the comparison between types of athletes.

In the present study's conditions, the female soccer players were able to selfregulate their drinking behaviors and achieve adequate fluid balance whether exercising with exposure to the sun or not. However, some athletes were edging on dehydration during these more moderate days and have the potential to stay dehydrated when transitioning into moderate-hot temperatures. The overall findings of this study necessitate proactive education for both athletes and coaching staff alike. The application of personalized hydration protocols would benefit the athletes in determining their state of hydration in any type of environment – whether cold, moderate, hot, humid, or dry – and allow them to capitalize on their behaviors to achieve optimal performance, hydration status, and fluid balance.

REFERENCES

- Adams, J. D., Kavouras, S. A., Johnson, E. C., Jansen, L. T., Capitan, C., & Robillard, J.I. (2017). The Effect of Storing Temperature and Duration on Urinary Hydration Markers J.D., 27(1). https://doi.org/10.1123/ijsnem.2016-0098
- Adams, W., & Casa, D. (2003). Hydration for Athletes. *Coaches Quarterly*, 28(Winter 2006), 1–3.
- Apostu, M. (2014). A Strategy for Maintaining Fluid and Electrolyte Balance in Aerobic Effort. *Procedia - Social and Behavioral Sciences*, 117, 323–328. https://doi.org/10.1016/j.sbspro.2014.02.221
- Armstrong, L. E. (2007). Assessing Hydration Status: The Elusive Gold Standard. Journal of the American College of Nutrition, 26, 575S–584S. https://doi.org/10.1080/07315724.2007.10719661
- Armstrong, L. E., Ganio, M. S., Casa, D. J., Lee, E. C., McDermott, B. P., Klau, J. F., ... Lieberman, H. R. (2012). Mild Dehydration Affects Mood in Healthy Young Women. *Journal of Nutrition*, 142(2), 382–388. https://doi.org/10.3945/jn.111.142000
- Armstrong, L. E., Pumerantz, A. C., Fiala, K. A., Roti, M. W., Kavouras, S. A., Casa, D. J., & Maresh, C. M. (2010). Human hydration indices: Acute and longitudinal reference values. *International Journal of Sport Nutrition and Exercise Metabolism*, 20(2), 145–153. https://doi.org/10.1123/ijsnem.20.2.145
- Arnaoutis, G., Kavouras, S. A., Angelopoulou, A., Skoulariki, C., Bismpikou, S., Mourtakos, S., & Sidossis, L. S. (2015). Fluid Balance During Training in Elite Young Athltes of Different Sports HHS Public Access. *J Strength Cond Res*, 29(12), 3447–3452. https://doi.org/10.1519/JSC.00000000000000400
- Arnaoutis, G., Kavouras, S. A., & Kotsis, Y. P. (2013). Ad Libitum Fluid Intake Does Not Prevent Dehydration in Suboptimally Hydrated Young Soccer Players During a Training Session of a Ad Libitum Fluid Intake Does Not Prevent Dehydration in Suboptimally Hydrated Young Soccer Players During a Training Session. *International Journal of Sports Nutrition and Exercise Metabolism*, (February), 245–251.
- Baker, L. B., Barnes, K. A., Anderson, M. L., Passe, D. H., Stofan, J. R., Baker, L. B., ... Stofan, J. R. (2016). Normative data for regional sweat sodium concentration and whole-body sweating rate in athletes. *Journal of Sports Sciences*, 34(4), 358–368. https://doi.org/10.1080/02640414.2015.1055291

- Baker, L. B., & Kenney, W. L. (2016). Exercising in the Heat and Sun. *Journal of Physical Activity and Health*, 4(3), 358–358. https://doi.org/10.1123/jpah.4.3.358
- Bardis, C. N., Kavouras, S. A., Arnaoutis, G., Panagiotakos, D. B., & Sidossis, L. S. (2013). Mild dehydration and cycling performance during 5-kilometer hill climbing. *Journal of Athletic Training*, 48(6), 741–747. https://doi.org/10.4085/1062-6050-48.5.01
- Bardis, C. N., Kavouras, S. A., Kosti, L., Markousi, M., & Sidossis, L. S. (2013). Mild hypohydration decreases cycling performance in the heat. *Medicine and Science in Sports and Exercise*, 45(9), 1782–1789. https://doi.org/10.1249/MSS.0b013e31828e1e77
- Benton, D., Jenkins, K. T., Watkins, H. T., & Young, H. A. (2016). Minor degree of hypohydration adversely influences cognition: A mediator analysis. *American Journal of Clinical Nutrition*, 104(3), 603–612. https://doi.org/10.3945/ajcn.116.132605
- Binkley, H. M., Beckett, J., Douglas, ;, Casa, J., Kleiner, M., Paul, ;, & Plummer, E. (2002). National athletic trainers' association position statement: Exertional heat illnesses. *Journal of Athletic Training 329 Journal of Athletic Training*, 37(3), 329– 343.
- Brandenburg, J. P., & Gaetz, M. (2012). Fluid balance of elite female basketball players before and during game play.
- Braniš, M., & Větvička, J. (2010). PM10, Ambient temperature and relative humidity during the XXIX summer olympic games in Beijing: Were the athletes at risk? *Aerosol and Air Quality Research*, 10(2), 102–110. https://doi.org/10.4209/aaqr.2009.09.0055
- Broad, E. M., Burke, L. M., Cox, G. R., Heeley, P., & Riley, M. (1996). Body weight changes and voluntary fluid intakes during training and competition sessions in team sports. *International Journal of Sport Nutrition*, 6(3), 307–320. https://doi.org/10.1123/ijsn.6.3.307
- Burchfield, J. M., Ganio, M. S., Adams, J. D., Kavouras, S. A., Gonzalez, M. A., & Ridings, C. B. (2014). 24-hr Void Number As A Marker Of Hydration Status. *Medicine And Science In Sports And Exercise*, 46(5), 485.
- Burke, L. M., Hawley, J. A., Wong, S. H. S., & Jeukendrup, A. E. (2011). Carbohydrates for training and competition. *Journal of Sports Sciences*, 29(SUPPL. 1). https://doi.org/10.1080/02640414.2011.585473

- Burke, L. M., Loucks, A. B., & Broad, N. (2006). Energy and carbohydrate for training and recovery. *Nutrition and Football: The FIFA/FMARC Consensus on Sports Nutrition*, 0414, 19–37. https://doi.org/10.4324/9780203967430
- Carlton, A., & Orr, R. M. (2015). The effects of fluid loss on physical performance: A critical review. *Journal of Sport and Health Science*, *4*(4), 357–363. https://doi.org/10.1016/j.jshs.2014.09.004
- Casa, D. J., Armstrong, L. E., Hillman, S. K., Montain, S. J., Reiff, R. V., Rich, B. S. E., ... Stone, J. A. (2000). National Athletic Trainers' Association Position Statement: Fluid Replacement for Athletes. *Journal of Athletic Training*, 35(2), 212–224. https://doi.org/10.4085/1062-6050-48.2.25
- Castro-Sepúlveda, M., Astudillo, S., Álvarez, C., Zapata-Lamana, R., Zbinden-Foncea, H., Ramírez-Campillo, R., & Jorquera, C. (2015). Prevalencia de deshidratación en futbolistas profesionales Chilenos antes del entrenamiento. *Nutricion Hospitalaria*, 32(1), 308–311. https://doi.org/10.3305/nh.2015.32.1.8881
- Chen, Y., Yu, Y., Li, X., Zhou, H., Hong, X., & Geng, Y. (2016). Fiber-optic urine specific gravity sensor based on surface plasmon resonance. *Sensors and Actuators*, *B: Chemical*, 226, 412–418. https://doi.org/10.1016/j.snb.2015.09.056
- Cheuvront, S. N., Ely, B. R., Kenefick, R. W., & Sawka, M. N. (2010). Biological variation and diagnostic accuracy of dehydration assessment markers. *American Journal of Clinical Nutrition*, 92(3), 565–573. https://doi.org/10.3945/ajcn.2010.29490
- Cheuvront, S. N., Kenefick, R. W., Charkoudian, N., & Sawka, M. N. (2013). Physiologic basis for understanding quantitative dehydration assessment. *American Journal of Clinical Nutrition*, 97(3), 455–462. https://doi.org/10.3945/ajcn.112.044172
- Cheuvront, S. N., Kenefick, R. W., & Zambraski, E. J. (2015). Spot Urine concentrations should not be used for hydration assessment: A methodology review. *International Journal of Sport Nutrition and Exercise Metabolism*, 25(3), 293–297. https://doi.org/10.1123/ijsnem.2014-0138
- Cleary, Michelle A.; Hetzler, Ronald K.; Wasson, Darcy; Wages, Jennifer J.; Stickley, C. (2014). Hydration behaviors before and after an education and prescribed hydration intervention in adolescent athletes. *Journal of Sports Science and Medicine*, 14(1), 23–28. https://doi.org/10.4085/1062-6050-47.3.05
- Coso, J. Del, Estevez, E., Baquero, R. A., & Mora-Rodriguez, R. (2008). Anaerobic performance when rehydrating with water or commercially available sports drinks

during prolonged exercise in the heat. *Applied Physiology, Nutrition, and Metabolism, 33*(2), 290–298. https://doi.org/10.1139/H07-188

- Cunningham, J.J. (1990). Calculation of energy expenditure from indirect calorimetry: assessment of the Weir equation. *Nutrition*, 6(3): 222–3.
- Da Silva, R. P., Mündel, T., Natali, A. J., Bara Filho, M. G., Alfenas, R. C. G., Lima, J. R. P., ... Marins, J. C. B. (2012). Pre-game hydration status, sweat loss, and fluid intake in elite Brazilian young male soccer players during competition. *Journal of Sports Sciences*, 30(1), 37–42. https://doi.org/10.1080/02640414.2011.623711
- Da Silva, R. P., Mündel, T., Natali, A. J., Bara Filho, M. G., Lima, J. R. P., Alfenas, R. C. G., ... Marins, J. C. B. (2011). Fluid balance of elite Brazilian youth soccer players during consecutive days of training. *Journal of Sports Sciences*, 29(7), 725–732. https://doi.org/10.1080/02640414.2011.552189
- Davis, B. A., Thigpen, L. K., Hornsby, J. H., Green, J. M., Coates, T. E., & O'Neal, E. K. (2014). Hydration kinetics and 10-km outdoor running performance following 75% versus 150% between bout fluid replacement. *European Journal of Sport Science*, 14(7), 703–710. https://doi.org/10.1080/17461391.2014.894578
- Davis, J.-K., Laurent, M. C., Allen, K. E., Green, M. J., Stolworthy, N. I., Welch, T. R., & Nevett, M. E. (2015). Influence of dehydration on intermittent sprints, 29(9), 2586–2593.
- Dion, T., Savoie, F. A., Asselin, A., Gariepy, C., & Goulet, E. D. B. (2013). Halfmarathon running performance is not improved by a rate of fluid intake above that dictated by thirst sensation in trained distance runners. *European Journal of Applied Physiology*, 113(12), 3011–3020. https://doi.org/10.1007/s00421-013-2730-8
- Edwards, A. M., Mann, M. E., Marfell-Jones, M. J., Rankin, D. M., Noakes, T. D., & Shillington, D. P. (2007). Influence of moderate dehydration on soccer performance: Physiological responses to 45 min of outdoor match-play and the immediate subsequent performance of sport-specific and mental concentration tests. *British Journal of Sports Medicine*, *41*(6), 385–391. https://doi.org/10.1136/bjsm.2006.033860
- Fernandez-Elias, V. E., Martinez-Abellan, A., Lopez-Gullon, J. M., Moran-Navarro, R., Pallares, J. G., De La Cruz-Sanchez, E., & Mora-Rodriguez, R. (2014). Validity of hydration non-invasive indices during the weightcutting and official weigh-in for olympic combat sports. *PLoS ONE*, 9(4). https://doi.org/10.1371/journal.pone.0095336

Furber, M. J. W., Roberts, J. D., & Roberts, M. G. (2017). A valid and reliable nutrition

knowledge questionnaire for track and field athletes. *BMC Nutrition*, *3*(1), 36. https://doi.org/10.1186/s40795-017-0156-0

- Godek, S. F., Peduzzi, C., Burkholder, R., Condon, S., Dorshimer, G., & Bartolozzi, A. R. (2010). Sweat rates, sweat sodium concentrations, and sodium losses in 3 groups of professional football players. *Journal of Athletic Training*, 45(4), 364–371. https://doi.org/10.4085/1062-6050-45.4.364
- Goulet, E. D. (2012). Dehydration and endurance performance in competitive athletes. *Nutrition Reviews*, 70(SUPPL/2). https://doi.org/10.1111/j.1753-4887.2012.00530.x
- Goulet, E. D. B. (2013). Effect of exercise-induced dehydration on endurance performance: evaluating the impact of exercise protocols on outcomes using a metaanalytic procedure. *British Journal of Sports Medicine*, 47(11), 679–686. https://doi.org/10.1136/bjsports-2012-090958
- Green, M. S., Corona, B. T., Doyle, J. A., & Ingalls, C. P. (2008). Carbohydrate-protein drinks do not enhance recovery from exercise-induced muscle injury. *International Journal of Sport Nutrition and Exercise Metabolism*, 18(1), 1–18. https://doi.org/10.1123/ijsnem.18.1.1
- Greenleaf, J. E. (1992). Problem: thirst, drinking behavior, and involuntary dehydration. *Medicine and Science in Sports and Exercise*.
- Hamouti, N., Del Coso, J., Ávila, A., & Mora-Rodriguez, R. (2010). Effects of athletes' muscle mass on urinary markers of hydration status. *European Journal of Applied Physiology*, 109(2), 213–219. https://doi.org/10.1007/s00421-009-1333-x
- Hamouti, N., Del Coso, J., & Mora-Rodriguez, R. (2013). Comparison between blood and urinary fluid balance indices during dehydrating exercise and the subsequent hypohydration when fluid is not restored. *European Journal of Applied Physiology*, *113*(3), 611–620. https://doi.org/10.1007/s00421-012-2467-9
- Heavens, K. R., Charkoudian, N., O'Brien, C., Kenefick, R. W., & Cheuvront, S. N. (2016). Noninvasive assessment of extracellular and intracellular dehydration in healthy humans using the resistance-reactance-score graph method. *American Journal of Clinical Nutrition*, 103(3), 724–729. https://doi.org/10.3945/ajcn.115.115352
- Holway, F. E., & Spriet, L. L. (2011). Sport-specific nutrition: Practical strategies for team sports. *Journal of Sports Sciences*, 29(SUPPL. 1). https://doi.org/10.1080/02640414.2011.605459

Hooper, L., Bunn, D. K., Abdelhamid, A., Gillings, R., Jennings, A., Maas, K., ...

Fairweather-Tait, S. J. (2016). Water-loss (intracellular) dehydration assessed using urinary tests: How well do they work? Diagnostic accuracy in older people. *American Journal of Clinical Nutrition*, *104*(1), 121–131. https://doi.org/10.3945/ajcn.115.119925

- J.D. Adams, Stavros A. Kavouras, Evan C. Johnson, Lisa T. Jansen, Catalina Capitan, Joseph I. Robillard, A. M. (2017). The Effect of Storing Temperature and Duration on Urinary Hydration Markers J.D., 27(1). https://doi.org/10.1123/ijsnem.2016-0098
- James, L. J., Mears, S. A., & Shirreffs, S. M. (2015). Electrolyte supplementation during severe energy restriction increases exercise capacity in the heat. *European Journal* of Applied Physiology, 115(12), 2621–2629. https://doi.org/10.1007/s00421-015-3254-1
- James, L. J., Moss, J., Henry, J., Papadopoulou, C., & Mears, S. A. (2017). Hypohydration impairs endurance performance : a blinded study, 5, 1–10. https://doi.org/10.14814/phy2.13315
- Jette, M., Sidney, K., & Blumchent, G. (1990). Metabolic Equivalents (METS) in Exercise Testing, Exercise Prescription, and Evaluation of Functional Capacity, 565, 555–565. https://doi.org/10.1186/1471-2474-12-97
- Jetton, A. M., Lawrence, M. M., Meucci, M., Haines, T. L., Collier, S. R., Morris, D. M., & Utter, A. C. (2013). Dehydration and Acute Weight Gain in Mixed Martial Arts Fighters Before Competition. *Journal of Strength and Conditioning Research*, 27(5), 1322–1326. https://doi.org/10.1519/JSC.0b013e31828a1e91
- Judge, L. W., Kumley, R. F., Bellar, D. M., Pike, K. L., Pierson, E. E., Weidner, T., ... Friesen, C. A. (2016). HYDRATION AND FLUID REPLACEMENT KNOWLEDGE, ATTITUDES, BARRIERS, AND BEHAVIORS OF NCAA DIVISION 1AMERICAN FOOTBALL PLAYERS. *Journal of Strength and Conditioning Research*, 30(11), 2972–2978. https://doi.org/10.1519/JSC.00000000001397
- Kavouras, S. A., Johnson, E. C., Bougatsas, D., Arnaoutis, G., Panagiotakos, D. B., Perrier, E., & Klein, A. (2015). Validation of a urine color scale for assessment of urine osmolality in healthy children. *European Journal of Nutrition*, (April). https://doi.org/10.1007/s00394-015-0905-2
- Kenefick, R. W. (2018). Drinking Strategies: Planned Drinking Versus Drinking to Thirst. Sports Medicine. https://doi.org/10.1007/s40279-017-0844-6
- Levine, J. A. (2015). Measurement of energy expenditure. *Translational Research Methods for Diabetes, Obesity and Cardiometabolic Drug Development*, 8, 169–

187. https://doi.org/10.1007/978-1-4471-4920-0_7

- Logan-Sprenger, H. M., Heigenhauser, G. J. F., Killian, K. J., & Spriet, L. L. (2012). Effects of dehydration during cycling on skeletal muscle metabolism in females. *Medicine and Science in Sports and Exercise*, 44(10), 1949–1957. https://doi.org/10.1249/MSS.0b013e31825abc7c
- Loucks, A. B. (2004). Energy balance and body composition in sports and exercise. *Journal of Sports Sciences*, 22(1), 1–14. https://doi.org/10.1080/0264041031000140518
- Magal, M., Cain, R. J., Long, J. C., & Thomas, K. S. (2015). Pre-Practice Hydration Status and the Effects of Hydration Regimen on Colle- giate Division III Male Athletes, (August 2014), 23–28.
- McDermott, B. P., Casa, D. J., Yeargin, S. W., Ganio, M. S., Lopez, R. M., & Mooradian, E. a. (2009). Hydration status, sweat rates, and rehydration education of youth football campers. *Journal of Sport Rehabilitation*, 18(4), 535–552. https://doi.org/10.1123/jsr.18.4.535
- Mears, S. A., Watson, P., & Shirreffs, S. M. (2016). Thirst responses following high intensity intermittent exercise when access to ad libitum water intake was permitted, not permitted or delayed. *Physiology and Behavior*, 157, 47–54. https://doi.org/10.1016/j.physbeh.2016.01.016
- Minton, D. M., O'Neal, E. K., & Torres-McGehee, T. M. (2015). Agreement of urine specific gravity measurements between manual and digital refractometers. *Journal* of Athletic Training, 50(1), 59–64. https://doi.org/10.4085/1062-6050-49.3.47
- NCAA. (2016). NCAA Weight Management Program. Retrieved from http://www.trackwrestling.com/tw/tickets/CreateTicket.jsp
- Nuccio, R. P., Barnes, K. A., Carter, J. M., & Baker, L. B. (2017). Fluid Balance in Team Sport Athletes and the Effect of Hypohydration on Cognitive, Technical, and Physical Performance. *Sports Medicine*, 1–32. https://doi.org/10.1007/s40279-017-0738-7
- O'Neal, E. K., Davis, B. A., Thigpen, L. K., Caufield, C. R., Horton, A. D., & McIntosh, J. R. (2012). Runners greatly underestimate sweat losses before and after a 1-hr summer run. *International Journal of Sport Nutrition and Exercise Metabolism*, 22(5), 353–362.
- Otani, H., Kaya, M., Tamaki, A., Watson, P., & Maughan, R. J. (2016). Effects of solar radiation on endurance exercise capacity in a hot environment. *European Journal of*

Applied Physiology, 116(4), 769–779. https://doi.org/10.1007/s00421-016-3335-9
Pérez-Idárraga, A., & Aragón-Vargas, L. F. (2014). Postexercise rehydration: potassium-rich drinks versus water and a sports drink. Applied Physiology, Nutrition, and Metabolism, 39(10), 1167–1174. https://doi.org/10.1139/apnm-2013-0434

- Pettersson, S., & Berg, C. M. (2014). Hydration status in elite wrestlers, judokas, boxers, and taekwondo athletes on competition day. *International Journal of Sport Nutrition and Exercise Metabolism*, 24(3), 267–275. https://doi.org/10.1123/ijsnem.2013-0100
- Pettersson, S., Ekström, M. P., & Berg, C. M. (2013). Practices of weight regulation among elite athletes in combat sports: A matter of mental advantage? *Journal of Athletic Training*, 48(1), 99–108. https://doi.org/10.4085/1062-6050-48.1.04
- Phillips, S. M., Sykes, D., & Gibson, N. (2014). Hydration status and fluid balance of elite European youth soccer players during consecutive training sessions. *Journal of Sports Science and Medicine*, 13(4), 817–822.
- Reljic, D., Hässler, E., Jost, J., & Friedmann-Bette, B. (2013). Rapid weight loss and the body fluid balance and hemoglobin mass of elite amateur boxers. *Journal of Athletic Training*, 48(1), 109–117. https://doi.org/10.4085/1062-6050-48.1.05
- Rosenberger H. E., Goff, D. C., Isom, S., Blackwell, C., Whitt-Glover, M. C., & Katula, J. A. (2013). Relationship of weekly activity minutes to metabolic syndrome in prediabetes: the healthy living partnerships to prevent diabetes. *Journal of Physical Activity & Health*, 10(5), 690–698. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23036940%0Ahttp://www.pubmedcentral.nih. gov/articlerender.fcgi?artid=PMC3765007
- Rosinger, A. (2015). Heat and hydration status: Predictors of repeated measures of urine specific gravity among Tsimane' adults in the Bolivian Amazon. *American Journal* of Physical Anthropology, 158(4), 696–707. https://doi.org/10.1002/ajpa.22813
- Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007). Exercise and fluid replacement. *Medicine and Science in Sports and Exercise*, 39(2), 377–390. https://doi.org/10.1249/mss.0b013e31802ca597
- Sawka, M. N., Montain, S. J., M.N., S., Sawka, M. N., Montain, S. J., & M.N., S. (2000). Fluid and electrolyte supplementation for exercise heat stress. *American Journal of Clinical Nutrition*, 72(2 SUPPL.), 564S--72S. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed5&NEWS= N&AN=2000300163

- Schrier, R., & Bansal, S. (2008). Diagnosis and management of hyponatremia in acute illness, *14*(6), 627–634. https://doi.org/10.1016/j.dcn.2011.01.002.
- Segar, W. E., & Moore, W. W. (1968). The regulation of antidiuretic hormone release in man. *Journal of Clinical Investigation*, 47(9), 2143–2151. https://doi.org/10.1172/JCI105900
- Sobana, R. M., & Many, J. N. (2014). Effect of Hydration Education on College Athlete ' s Hydration Status and KAP, *3*(7), 1654–1658.
- Sobana, R. M., & Nirmala, M. J. (2014). Analysis of Knowledge, Attitudes and Practices Between Male and Female College Athletes on Hydration and Fluid Replacement. *International Journal of Sports Sciences & Fitness*, 4(2), 144–156. Retrieved from http://ezyproxy.wintec.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direc t=true&db=s3h&AN=103390955&site=eds-live&scope=site
- Sommerfield, L. M., McAnulty, S. R., McBride, J. M., Zwetsloot, J. J., Austin, M. D., Mehlhorn, J. D., ... Utter, A. C. (2015). Validity of urine specific gravity when compared with plasma osmolality as a measure of hydration status in male and female NCAA collegiate athletes, 30(8), 2219–2225.
- Spencer, M., Gupta, A., Van Dam, L., Shannon, C., Menees, S., & Chey, W. D. (2016). Artificial sweeteners: A systematic review and primer for gastroenterologists. *Journal of Neurogastroenterology and Motility*, 22(2), 168–180. https://doi.org/10.5056/jnm15206
- Supriya, V. (2013). Knowledge, Attitude And Dietary Practices Of Track And Field Athletic Men And Women Aged 18-22 Years. *IntJ of Innov Research & Devt.*, 2(11), 399–404.
- Thigpen, L. K., Green, J. M., & O'Neal, E. K. (2014). Hydration profile and sweat loss perception of male and female division II basketball players during prcatice. *Journal* of Strengh and Conditioning Research, 28(12), 3425–3431. https://doi.org/10.1519/JSC.00000000000549
- Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. *Journal of the Academy of Nutrition* and Dietetics, 116(3), 501–528. https://doi.org/10.1016/j.jand.2015.12.006
- Tyrwhitt-drake, R., Ferragud, M. A., Andrés, R. U. De, En, A., Unido, R., & España, F. Y. (2015). Knowledge and perceptions of hydration : a survey among adults in the United Kingdom, France and Spain, 20(4), 128–136. https://doi.org/10.14642/RENC.2014.20.4.5026

- Utter, A. C., McAnulty, S. R., Riha, B. F., Pratt, B. a, & Grose, J. M. (2012). The Validity of Multifrequency Bioelectrical Impedance Measures to Detect Changes in the Hydration Status of Wrestlers During Acute Dehydration and Rehydration. *Journal of Strength and Conditioning Research*, 26(1), 9–15. https://doi.org/10.1519/JSC.0b013e318238ea51
- Vandermark, L. W. (2016). Relationship of Perceived Thirst to Measures of Hydration During and Following Exercise.
- Wall, B. A., Watson, G., Peiffer, J. J., Abbiss, C. R., Siegel, R., & Laursen, P. B. (2015). Current hydration guidelines are erroneous: Dehydration does not impair exercise performance in the heat. *British Journal of Sports Medicine*, 49(16). https://doi.org/10.1136/bjsports-2013-092417
- Wells, J. C. K. (2012). Ecogeographical associations between climate and human body composition: Analyses based on anthropometry and skinfolds. *American Journal of Physical Anthropology*, 147(2), 169–186. https://doi.org/10.1002/ajpa.21591
- Wingo, J. E., Ganio, M. S., & Cureton, K. J. (2012). Cardiovascular drift during heat stress: implications for exercise prescription. *Exercise and Sport Sciences Reviews*, 40(2), 88–94. https://doi.org/10.1097/JES.0b013e31824c43af
- Zubac, D., Antelj, T., Olujic, D., Ivancev, V., & Morrison, S. A. (2017). Fluid balance and hydration assessment during the weight-stable preparation phase in elite youth boxers. *Journal of Sports Sciences*, 35(8), 719–726. https://doi.org/10.1080/02640414.2016.1184302
APPENDIX A

VERBAL SCRIPT

I am a graduate student under the direction of Professor Floris Wardenaar in the School of Nutrition and Health Promotion at Arizona State University. I am conducting a research study to determine the effects of sun exposure on the hydration status and fluid balance of ASU student athletes.

I am recruiting individuals to measure their hydration status and fluid balance using urine specific gravity (USG) measurements, fluid intake and output, and comparing the differences in hydration between exercising indoors and outdoors. This will take approximately 3-4 weeks.

Your participation in this study is voluntary. If you have any questions concerning the research study, please call me at (484) 941 - 3318.

Stephanie Olzinski MS Human Nutrition Student

APPENDIX B

CONSENT FORM

Effects of Environment on Hypohydration in Student Collegiate Athletes: An Exploratory Study

Stephanie Olzinski, Supervised by Dr. Floris Wardenaar

Why am I being invited to take part in a research study?

We invite you to take part in a research study because you are a healthy, uninjured student athlete and are willing to participate in giving urine samples periodically throughout your practices/games.

Why is this research being done?

The purpose of this study is to determine the effects of sunlight on dehydration. Understanding the extent to which athletes become dehydrated outdoors compared to indoors will allow a better recommendation to be made for rehydration after events in different environments.

How long will the research last?

We expect that individuals will spend 3-4 weeks participating in the proposed activities.

How many people will be studied?

We expect about 16 people here will participate in this research study.

What happens if I say yes, I want to be in this research?

It is up to you to decide whether or not to participate. You can expect no changes to be made to your daily habits throughout the study – only your fluid balance and hydration status will be analyzed.

- The study will be completed in 3-4 visits, with an additional 30-60 minutes before and after practices
- A urine specific gravity (USG) meter and color chart will be used to test urine
- The research will consist of recording amounts of fluids consumed and hydration status through urine samples
- The research will be performed at the site of practices by Stephanie Olzinski (graduate student), Dr. Floris Wardenaar, and Amber Yudell.

What happens if I say yes, but I change my mind later?

You can leave the research at any time it will not be held against you.

If you decide to leave the research, there will be no consequences nor adverse effects. If you decide to leave the research, contact the investigator so that the investigator can discuss your reasons for deciding to leave and ensure your safety and understanding of the research.

Will being in this study help me any way?

We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits include determining an individualized rehydration protocol to properly keep you hydrated during all practices and matches, no matter what environmental condition you are exercising.

What happens to the information collected for the research?

Efforts will be made to limit the use and disclosure of your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization.

By signing this form, you consent to data or specimens that you provided being retained after the study for further research. They will be stored in the Arizona Biomedical Center Laboratory for a maximum of one year from the end date of the study. Only laboratory technicians and the afore mentioned researchers will have access to the data and specimen.

What else do I need to know? Who can I talk to?

You will be informed of the results of the research through your coach.

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at solzinsk@asu.edu.

This research has been reviewed and approved by the Bioscience IRB ("IRB"). You may talk to them at (480) 965-6788 or research.integrity@asu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
 - You cannot reach the research team.
 - You want to talk to someone besides the research team.
 - You have questions about your rights as a research participant.
 - You want to get information or provide input about this research.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

Signature of participant	Date
Printed name of participant	
Signature of person obtaining consent	Date

Printed name of person obtaining consent

APPENDIX C SCREENER SURVEY

Cool the Fork

This is a pre-screening questionnaire for the Cool the Fork study. Please answer all questions by. It helps us to decide if you are eligible to be included in the study. Thank you on behalf of the research team! Dr. Floris Wardenaar Principal Investigator

Q1 What is your gender?	
\bigcirc	Male
0	Female
Q2 What is your current he	eight? (feet and inches)
Q3 What is your current w	eight? (pounds)
Q4 Are you allowed to particip	pate in normal practice activities?
\bigcirc	Yes
\bigcirc	No
Skip To: End of Survey If Are you	allowed to participate in normal practice activities? = No

Q5 Do you currently take a multi-vitamin supplement?

\bigcirc	Yes
\bigcirc	No
Skip To: Q7 If Do you	currently take a multi-vitamin supplement? = No
Q6 How often do	you take a multi-vitamin supplement per month?
Q7 Do you curre	ntly take a vitamin B supplement or a beta-carotene supplement?
\bigcirc	Yes
\bigcirc	No
Skip To: Q9 If Do you	currently take a vitamin B supplement or a beta-carotene supplement? = No
Q8 How often do month?	you take a vitamin B supplement and/or a beta-carotene supplement per
Q9 Do you curre	ntly use tobacco products?
\bigcirc	Yes
\bigcirc	No

Q10 Do you take any medications?

\bigcirc	Yes
\bigcirc	No

Skip To: Q12 If Do you take any medications? = No

Q11 Please list all medications you use, as specific as possible (type and brand).

Q12 What is your preferred beverage during training practice?				
\bigcirc	Water			
\bigcirc	0 calorie sports drink			
\bigcirc	Both water and sports drink			
Q13 Do you norr	nally have to go to the bathroom during training practice?			
\bigcirc	Always			
\bigcirc	Sometimes			

End of Block: Default Question Block

Never

 \bigcirc

APPENDIX D

URINE COLOR CHART



The Urine Color Chart[®] shown here will assess your hydration status (level of dehydration) in extreme environments. To use this chart, match the color of your urine to a color on the chart. If your urine color matches #1,#2 or #3 on the chart, you are well hydrated. If your urine color is #7 or darker, you are dehydrated and should consume fluids.

The scientific validation of this color chart may be found in the *International Journal of Sport Nutrition*, Volume 4, 1994, pages 265-279 and Volume 8, 1998, pages 345-355. Adapted by permission from Larry Amrstrong 2000, *Performing in Extreme Environments* (Champaign, R., Human Kinetics).

APPENDIX E

PROTOCOL AND OPERATING PROCEDURES

111 Standard Operating Procedures - Test Days

Each researcher or research assistant will have completed all proper training before participating in the study. On test days, everyone will adhere to the following protocols to ensure validity of measurements and adherence to protocol.

-Lab coats and gloves will be worn at all times when handling urine samples and proper hand washing protocols will be followed.

-Each assistant will be assigned to one specific job and will remain in that position for each study day. The positions and protocols are as follows:

-Anthropometric Station: the assistant will ensure that proper calibration of the Seca scale and stadiometer have been completed.

• The assistant will record the height of each athlete on either the day of the information session or the first study day. For each testing day, the assistant will record the weight of the athlete two times. The assistant will ensure each athlete is wearing minimal, dry clothing before stepping on the scale. The same methods will be employed for post-exercise measurement of body weight. The assistant will fill in the proper online data sheet with these three numbers and direct them to the next station, Urine Collection.

-Urine Collection Station: the assistant will be wearing a lab coat and gloves at all times. They will ensure that all necessary equipment is accounted for and properly labeled after the athletes complete their samples.

- The assistant will inform each athlete to visit the designated restroom facility and to completely empty their bladders into the urine specimen hats. They will remind the athlete to not touch the sample or to empty their bladders directly in the toilet. The athletes can dispose of toilet paper in assigned trash bags and may wash their hands. The assistant will transfer the urine from the specimen hats to the large urine bottles, ensure that each bottle is labeled with the athlete's ID number and Pre-Exercise designation, and place it in a designated area for all pre-exercise samples. The assistant will replace the dirty hat with a clean hat for the next athlete to use. The assistant will also sanitize the dirty hat to be used again. The same methods will be employed for post-exercise urine collection. After the assistant checks off on the athlete's ID number on their hand for easy identification during practice and direct them to the next station, Drinking Bottles.
- The assistant will use a calibrated precision scale to measure the weight of the empty urine collection bottles, and again when the bottles are filled with the urine samples.
- The assistant will transfer the urine into different sized, pre-labeled test tubes for later analysis.

-Distribution and Collection of Drinking Bottles and Tracking Devices: Each athlete will receive a personalized drinking bottle with their choice of beverage: water, a sports drink, or one bottle of water and one bottle of sports drink.

- The assistant will give each athlete their personal bottle, which will be labeled with their study ID and another character identifying the bottle number and fluid (see Station Checklist in Appendix E). They will be weighed and filled with the beverage of choice. The bottle will be weighed before and after the fluid is added using a calibrated precision scale, and the weights will be recorded on the athlete's online data sheet. The assistant will remind the athlete that they can drink freely from their bottle during practice but must not use the contents to rinse their mouth or face.
- The assistant will give each athlete a Zephyr and assist them with securing it in the proper position.
- If the athlete has no further questions, then she will be instructed to change into their practice clothes and then enter onto the practice field.

-During Training: The athletes will complete their training as normal as instructed by the coaches. During the practice, the environmental temperatures will be recorded using the Heat Stress Trackers. The time of the practice will be recorded, as well as the activities performed by the athletes and the number and duration of drink breaks they are allowed. If an athlete must use the restroom during practice, they will be instructed to visit the Urine Collection Station and empty their bladders as previously instructed. Additionally, if the athlete requires more drinking fluids during practice, their bottles will be replaced with another preweighed bottle. These extra calculations will be factored into their total fluid balance (input of beverages and output of urine).

-Distribution and Collection of First-morning Urine Cups:

- After each athlete completes training, the will return to the Urine Collection Station to again empty their bladder for the Post-Exercise Collection. They will change back into their dry clothes and go to the Anthropometric Station to have their post-exercise weight measured. The assistant at this station will ensure the athlete's online data sheet is filled in with the following:
 - Pre- and Post-Exercise weights
 - Drinking Bottle weight(s)
 - During-practice urine collection or refilling of drinking bottle (if necessary)
- If everything is checked off, the assistant will provide the athlete with the small urine cup for the morning after the study. The assistant will remind the athlete that they are to use this cup during the first time their empty their bladder the next morning. The athlete does not need to provide a full sample, but rather is to collect urine from the midstream in the cup. The

athlete will ensure the cup is tightly sealed and will return in that day to the Tempe campus for the researchers to collect.

-Addendum: As some athletes were using the restroom before arriving at practice, we offered to send the athletes home with urine collection containers the night before the second and third study days so that we could collect and accurate pre-exercise urine sample.

-A checklist for the athletes and assistants to follow is as follows:

- 1. Arrive at practice
- 2. Pre-exercise urine collection
- 3. (Height), Pre-exercise body weight in dry clothes
- 4. Obtain water bottle and Zephyr
- 5. Practice may use the bathroom or get more fluids if needed
- 6. Post-exercise urine collection
- 7. Post-exercise body weight in dry clothes
- 8. Obtain first-morning sample cup
- 9. Return first-morning sample cup the next day (collect from the midstream urine)

<u>Standard Operating Procedures – Sample Analysis</u>

The researchers or assistants will perform analysis on the urine samples, and complete all necessary calculations to determine hydration status and fluid balance of each athlete after each study day is completed; and after all three study days are completed and all first-morning samples are returned. Two analyses will be performed on the pre- and post-exercise and first-morning urine samples: color and USG value. The analyses will be performed on the urine samples in the test tubes.

- A validated Urine Color Chart will be used to analyze the color of each urine sample. The colors are indicative of hydration status, with darker colors implying higher levels of dehydration and light-clear colors implying levels of proper hydration. Held against a white piece of paper, the samples will be compared to the color chart to determine what number the sample most closely matches between 1 and 8. The number will be recorded on that athlete's online data sheet for the specified study day.
- An analog refractometer and a digital refractometer as well as a hydrometer will be used to analyze the urine specific gravity (USG) of each urine sample after proper calibration. The USG value will be measured when the urine samples are at room temperature (about 20 °C) which has been previously determined to provide the most accurate measurement. The values will be recorded twice, and to three-four decimal places (depending on the accuracy of the tool) on that athlete's online data sheet for the specified study day.

Accounting for Additional Fluid Losses: As urine excretion is not the only source of fluid losses from the body, each athlete's data will take into account additional losses from sweat, feces and solid food pre- and post- exercise after weighing the athlete. Following all calculations being completed for each study day, the data will be statistically analyzed.

Station Checklists

Beverage Station
Tape
Sharpie
26 (13 players, 1a 1b 2a 2b, etc, + 13 extra bottles (X1, X2, etc, all to be filled with water) + 4 extra bottles (YA, YB, YC, YD to be potential 4th bottle or if any spill)
13 Zephyrs
Table
Safety Pins
Ice (Soccer Stadium)
Powerade (Soccer Stadium)

•Water (Soccer Stadium)

Urine Station

•Table

•Scale with batteries

•30 mL vials

•Small vials along with square box to hold the small vials

•Gloves

•Goggles

•Lab coats

•Urine pads

•Urine specimen hats

•Big containers for pre and post urines

•Pipettes

•DI water

•Cleaning supplies

APPENDIX F

EXPLANATION OF RESULTS TO ATHLETES

Explaining the Results

Fluid Balance

<u>Percentage weight difference (weight scale)</u>: When it comes to fluid balance and hydration, you want to stay within a 2% range of body weight fluctuations.

<u>Fluid intake (sport bottle)</u>: Since your body sweats to cool off, you want to ensure you consume sufficient fluids by drinking 1-2 cups per 30 minutes. Post-physical activity, you typically want to consume 150% of fluids lost to replenish fluid levels. However, if you find that you tend to gain weight during exercise, (even if it is only a 1% increase) you probably hydrate well and do not need to be as concerned about replenishing fluid levels.

<u>Sweat rate (sport towel)</u>: In typical dry Arizona heat your body tends to get rid of sweat quite efficiently (without you even noticing). Therefore, it is important to focus on the uptake of fluids to maintain hydration levels.

Exercise

<u>Energy expenditure (muscle)</u>: This outcome is based on a measurement done with a wearable device (Zephyr). The Zephyr registers heart rate, activity, movement, and breathing rate. These measurements can be used in a formula and accurately estimate energy expenditure. Normal heart rate monitors that estimate energy expenditure often use a simple formula, but for this study we also factored your activity and breathing rate into the equation.

<u>Average heart rate frequency (heart)</u>: Maximum heart rate is the age-related number of beats per minute of the heart when working at its highest capacity. To calculate maximum heart rate, you would take 220 – age. Maximum heart rate can be obtained by participating in vigorous exercise. Training at a high intensity with minimal rest can help an athlete reach their maximum heart rate. This value can be compared to the team's overall average heart rate frequency to see compare-and-contrast values.

Distance covered in meters (shoe): Every 1,600 meters ran at practice is equivalent to 1 mile.

<u>Time on the field (stopwatch)</u>: This was the total amount of time you and your teammates spent on the field.

Hydration Status

Urine Specific Gravity (USG) values are a great indicator of whether someone is hydrated or dehydrated. It is important to look at the USG values throughout the whole process of pre-practice, post-practice, and the morning samples to get a better overall picture of total hydration status. Seeing when and how much the USG values vary can provide insight on when an athlete should focus more on hydrating. A super happy smiley indicates a very good hydration status with a value between 1.000-1.021. The happy smiley indicates normal hydration with values ranging from 1.022-1.026. A non-smiling smiley indicate light dehydration with values 1.027-1.028. A sad smiley is scored based on USG values from 1.029 and beyond. It is suggested that student-athletes should consider talking to sports dietitian Amber Yudell if they have one or more smileys that do not 'smile'. She can help with developing better hydration strategies for both on and off the field.

Environment

<u>Temperature (thermometer)</u>: During the study environmental temperature was measuring in the shade. Temperature is the degree or intensity of heat present in a substance or object, especially as expressed according to a comparative scale and shown by a thermometer or perceived by touch. The normal core body temperature is about 98.6 degrees Fahrenheit (°F). Activities and high environmental temperature can increase body temperature.

<u>Humidity (water drop)</u>: This represent the amount of water vapor in the air. This value was measured to accurately determine the environmental status. When it is dry outside it can cause the athlete to sweat a lot without noticing it. Due to this dry environment it can cause the sweat to evaporate quicker. If it is humid outside it can cause the athlete to produce more visible sweat on the skin because the sweat does not evaporate as quickly as in a dry environment.

<u>WBGT (sun and cloud)</u>: The wet-bulb globe temperature is a type of apparent temperature used to estimate the effect of temperature, humidity, wind speed, wind chill, and visible/infrared radiation on humans. The WBGT heat stress is calculated using a method where climatic variables are used in a rational thermodynamic heat exchange model. Based on NATA guidelines wet-bulb temperatures <82.0°F are safe for athletes to perform at their optimal level. If wet-bulb temperature is higher than 92.1°F there should not be any outdoor workouts or practices performed.

APPENDIX G

EXAMPLE OF INDIVIDUAL REPORT



Athleat Field Status Report

