THE EFFECT OF HEAT STRESS ON HYDRATION STATUS AND RENAL BIOMARKERS IN NCAA D1 FEMALE SOCCER PLAYERS IN SOUTH TEXAS

A Thesis

by

JORDAN BROOKE WAINWRIGHT

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Recent research suggests that recurrent heat-associated hypohydration and strenuous physical exertion may be associated with the development of acute and potentially chronic renal dysfunction. South Texas pre-season conditions in which collegiate soccer is performed in August, may warrant concerns for promoting acute kidney injury (AKI). **PURPOSE**: The purpose of this study is to investigate hydration status and renal biomarkers in NCAA Division I female soccer players in South Texas. **METHODS**: (Mean±SEM; n=21; age: 19.3±0.25 y; ht: 169.6±1.3 cm; wt: 68.4±2.4 kg; LBM: 45.9±1.1 kg). Each subject participated in baseline and ending body composition measures via DXA (iDXA, Lunar Prodigy; GE Healthcare, Madison, WI), and provided 14-urine samples throughout the preseason for the analysis of Urine Specific Gravity (USG), Urine Color Analysis (UCA), Cystatin-C (uCys-C), and Creatinine (uCr). Urine samples were collected at start of preseason (PRE-PS), fitness testing days (FT1, FT2), regular practices (MidW1, MidW2, POST-PS) and exhibition games (PRE-BU, POST-BU,12HR-BU, 24HR-BU, PRE-UT, POST-UT, 12HR-UT, 24HR-UT). Heat index (37.4± 0.8) was assessed at practice sessions and exhibition matches (Kestrel 5000 environmental meter; Kestrel Meters, Boothwyn, PA). **RESULTS**: UCA and USG showed an effect of time ($p < 0.0001$) between days with PRE-BU and PRE-UT values being lower compared to multiple days. A difference in USG ($p = 0.0009$) was found comparing PRE-BU (1.012 ± 0.001) vs POST-BU (1.018 ± 0.001) and PRE-UT (1.010 ± 0.001) vs POST-UT (1.021 ± 0.001) ($p = 0.00001$). uCr showed an effect of time ($p < 0.0001$) between days with PRE-BU and PRE-UT being lower than multiple days. Significant increases in uCys-C were present at 12H-BU, 24H-BU, MidW1, PRE-UT, POST-UT, and 12H-UT ($p = \leq 0.0001$). **CONCLUSION**: Our findings suggest that subjects arrived at exhibition games (BU, UT) in a euhydrated state with hypohydration occurring 12-hours post-

exhibition game, prior to fitness assessments (FT1, FT2), and regular morning practice (MidW1). Values of uCr increased above normative post-exhibition game and may be an indicator of exercise-induced muscular injury. Concentrations of uCys-C remained elevated following 12H-BU and did not return to normative values until 12H-UT. Our results indicate that NCAA D1 female soccer players in South Texas are at increased risk of both hypohydration and potential renal injury.

Key Words: Acute Kidney Injury, Strenuous Exercise, Heat Index, Humidity, Renal Function, Cystatin-C, Creatinine, Urine Specific Gravity, Urine Color Analysis

DEDICATION

In dedication to my mother, Debra Ann. A single mother who worked tirelessly at the chance for all of her children to be enrolled in the sports they were most passionate about. Both competitive soccer and the support of my mother, illuminated my strength and tenacity that not only got me through the struggles of my own kidney disease, but to a place where my passion is now devoted to the research and awareness of kidney injury—helping other athletes like myself, in the future.

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor and chair, Dr. Daniel Newmire. Without his continual dedication to the development of students, this thesis would not exist. Not only did he see my potential as an undergraduate student, but he continually challenged me in my master's to reach new heights. In absence of his guidance, I would not be the student and/or person I am today. Secondly, I would like to thank my co-chair, Dr. Heather Webb. Without her willingness to assist students, my development as a young professional in academia would have been indefinitely hindered. Not only has she spent countless hours teaching my research team and I biosafety in the lab, but she is always available to offer additional advice when needed. Without a doubt, a special thank you is warranted to Dr. Darryn Willoughby. Although I originally reached out to him for personal curiosity about the physiology related to my renal disease, that relationship quickly grew into something I will continue to cherish. Now as an advisor on this thesis, he has undoubtedly helped in my development as a student while expanding my knowledge for renal physiology. Lastly, I would like to thank my research team Julia Wallen, Noe DeAnda, and Anthony Sanchez. Success is truly a team effort and I am forever grateful for these individuals in not only assisting, but embodying the passion behind this thesis.

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CHAPTER I

Introduction

Soccer is a worldwide popular sport that necessitates physiological training to reach optimal levels of performance (Broodryk, Pienaar, Edwards & Sparks, 2017). The physiological demands entail soccer players to utilize both aerobic and anaerobic systems for muscular strength, endurance, power, and agility to optimally perform in games that can last 90-120-minutes (Peart, Nicks, Mangum & Tyo, 2017; Svensson & Drust, 2005). Furthermore, the National Collegiate Athletic Association (NCAA) only allows 21 practice units (including exhibition games) before the first scheduled date of competition (National Collegiate Athletic Association, 2018). With a relatively short-timeframe in the pre-season, the coaching staff is responsible for preparing student-athletes for the competitive season. Soccer and strength and conditioning coaches may provide off-season conditioning plans however, athletes arriving to pre-season unconditioned may still occur and warrant increased training loads. During the pre-season, collegiate soccer athletes may participate in two or three multiple training sessions including conditioning, strength training, technical training, tactical training, as well as participating in exhibition games. Notably, field training times may surpass two to three hours on occasion (Minett, Binkley, Weidauer & Specker, 2017; Sayers, Sayers & Binkley, 2008). Additionally, preseason for Division One female soccer players is within the month of August. In South Texas, athletes may be exposed to higher temperatures in which the risk of dehydration (i.e. loss of body water through perspiration in conjunction with inadequate fluid intake) is increased (Schlader et al., 2019). According to the National Weather Service, South Texas experienced an average temperature of 94.6 °F, relative humidity of 80%, and an average heat index of 120 °F in August of 2018 (National Weather Service Corporate Image Web Team, 2019). Such temperatures not only increase the risk of becoming hypohydrated but may lead to

severe health concerns caused via heat strain (i.e. increased core body temperature. Not only do increased temperatures exacerbate the potential of becoming hypohydrated but may lead to increased risk of developing acute renal dysfunction (Casa, Armstrong, Hillman et al., 2000; Schlader et al., 2019)

Consequently, recent research has indicated that Acute Kidney Injury (AKI) may occur in result of strenuous physical exertion, lack of hydration, and the development of heat illness (e.g. heat stroke) due to heat strain (i.e. elevated core body temperature) (Junglee et al., 2013; Mansour et al., 2017). AKI has recently replaced the terminology of Acute Renal Failure (ARF) and has been defined as an abrupt (within hours) decrease in renal function, which further encompasses both injury (structural damage) and impairment (loss of function). Specifically, AKI is present for less than three months with longer occurrences of renal abnormalities warranting the diagnoses of Chronic Kidney Disease (CKD) (Thadhani, Pascual, & Bonventre, 1996; Vaidya, Ferguson, & Bonventre, 2008; Lippi et al., 2012; Mehta et al., 2007). Furthermore, strenuous physical activity and recurrent heat-associated dehydration has been associated with the development of permanent kidney damage (CKD) via repeated occurrences of AKI in manual laborers of Coastal Central America. This form of CKD is commonly known as “Mesoamerican nephropathy” and the physiological mechanisms responsible for this unique form of CKD are believed to be associated with heightened physical exertion and hot working environments (Mansour et al., 2017; Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015; Schlader et al., 2019). Although not universal, the association between renal dysfunction and the development of heat illnesses appears to exacerbate the risk of developing AKI regardless of manual laborers apparent acclimation to heat (Junglee et al., 2013). Thus,

recurrent exposure to heightened physical demands in hot environments may not only lead to dehydration but may increase the risk of renal injury in susceptible athletic populations.

Heat Stress, Exercise and Renal Function

During exercise, renal hemodynamics change to fulfill musculature needs through the redirection of blood flow. Relative to the intensity of exercise, renal blood flow (RBF) may decrease to less than 25% of resting value when strenuous exercise is performed (Mansour et al., 2017; Poortmans, 1984). At rest, the kidneys receive approximately 25% of cardiac output (5 L/min) equating to 1.2-1.3 L/min. As exercise intensity increases, blood flow to abdominal viscera and kidneys are significantly reduced and the vast amount of cardiac output is directed to cardiac and skeletal muscle (Joyner & Casey, 2015; Schlader et al., 2019; Poortmans, 1984). Furthermore, the reduction of RBF is due to the stimulation of the sympathetic nervous system and the release of catecholamines in response to exercise (Lippi et al., 2012). Additionally, during high ambient temperatures (>30 °C), the body redirects blood flow away from internal organs via vasoconstriction and towards the subcutaneous level (Roelands et al., 2011). This redirection facilitates heat transfer through the process of subcutaneous heat diffusion (i.e. sweating). In environments with high humidity, the body is unable to cool itself due to the drastic decrease of sweat evaporation from the skin. This inhibits the body's ability to cool itself and allows body temperature to increase. Concurrent with exercise-induced increases, metabolic heat production promotes increased perspiration rates and may potentiate hypohydration (Bouchama, 2002; Bongers et al., 2018). Specifically, fluid deficits greater than 2% of body weight may compromise aerobic exercise performance and cognitive function, and may lead to severe health concerns, especially in hot environments (Shirreffs & Sawka, 2011; Thomas, Erdman & Burke, 2016). The maintenance of total body water (TBW) is crucial to an athlete's health as it assist

with metabolism, biochemical reactions, circulatory functions, transportation of substrates across cellular membranes, regulation of body temperature, as well as many other physiological processes (Armstrong, 2007; Oppliger & Bartok, 2002). Dehydration includes both exercise-induced dehydration (i.e. develops during exercise) and hypohydration (i.e. dehydration induced prior to exercise) (Barr, 1999). Furthermore, dehydration is defined as "excessive loss of water from a living organism" while hypohydration is "the state of water deficit" with an increase in fluid (rehydration) allowing euhydration to occur (i.e. normal hydration) (Kavouras, 2019). Additionally, dehydration increases serum osmolality and can potentially lead to decreased plasma volume (i.e. hypovolemia) if fluid intake is inadequate. (Thomas, Erdman & Burke, 2016). Increases in serum osmolality (i.e. due to reductions in total plasma volume) and sodium (Na^+) concentrations stimulate the secretion of the antidiuretic hormone arginine vasopressin (AVP) in attempt to increase water reabsorption and maintain homeostasis (Verbalis, 2007).

Specific to renal function, recurrent dehydration independently has been associated with the development of permanent kidney damage through several potential mechanisms such as; increased concentrations of circulating AVP, aldose reductase-fructokinase pathway activation, and chronic hyperuricemia (Azzawi & Shirley, 1983; Mansour et al., 2017; Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015). Notably, the combination of heat stress and strenuous physical activity will provoke an increased response of these mechanisms and further the development of AKI (see Figure 1.) (Schlader et al., 2019). Circulating AVP is related to the stimulation of fructokinase (i.e. enzyme involved in metabolism of fructose) which is responsible for diminished adenosine triphosphate (ATP) availability within the renal tubules and the generation of uric acid (Roncal-Jimenez et al., 2013). Although circulating AVP is related to the progression of CKD in animal models, its impact on human pathophysiology in

relation to CKD progression is postulated. Notably, hyperosmolality can also activate aldose reductase which catalyzes glucose into sorbitol as a protective mechanism against a hyperosmolar renal environment (Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015; Schlader et al., 2019). The activation of aldose reductase may further stimulate the enzyme fructokinase to metabolize sorbitol into fructose. Importantly, one of the major sites fructokinase is expressed is the proximal tubule (Roncal-Jimenez et al., 2013). Fructose is then metabolized in a process that consumes intracellular ATP, depletes intracellular phosphate, activates adenosine monophosphate (AMP) deaminase as its metabolism is energetically expensive. This depletion of ATP may further lead to the generation of uric acid, inflammatory mediators (e.g. cytokines, prostaglandins), and oxidants (Roncal-Jimenez et al., 2013). Furthermore, tubular injury and further fibrosis (i.e. accumulation and deposition of extracellular matrix [ECM] components) has previously been shown to occur in result of the oxidative stress via the aldose reductase-fructokinase pathway (Cho, 2010; Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015). Hyperuricemia may occur due to increases in the production of uric acid due to subclinical rhabdomyolysis (i.e. mild muscular injury) from strenuous exertion. Additionally, hyperuricemia has also been associated with a reduction in urate excretion due to renal vasoconstriction (Fathallah-Shaykh & Cramer, 2014; Hahn, Kanbay, Lanaspa, Johnson & Ejaz, 2016; Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015; Schlader et al., 2019). In addition to the activation of these pathways, the reduction in RBF has also been suggested to cause localized ischemia due to heterogenous blood flow distribution within the kidneys (Schlader et al., 2019). It is hypothesized that this reduction in renal perfusion reduces oxygen delivery to the kidneys diminishing ATP availability (Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015; Schlader et al., 2019). Thus, mechanisms caused via

heat associated dehydration in assistance of strenuous exercise may lead to factors that inhibit renal function and further the development of AKI.

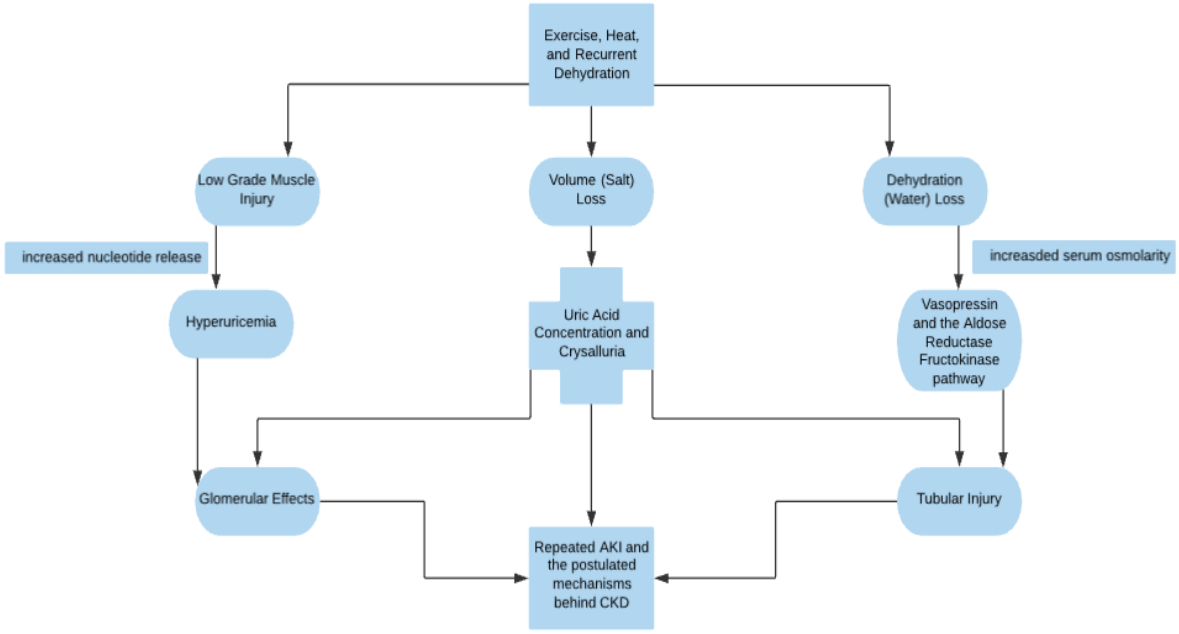

Figure 1. Proposed Mechanisms of Acute Kidney Injury. Figure adapted from Roncal-Jimenez, C., et al., (2016). Heat stress nephropathy from exercise-induced uric acid crystalluria: a perspective on Mesoamerican nephropathy. *American journal of kidney diseases*, *67*(1), 20-30.

Strikingly, one traditional method of detecting AKI utilizes serum creatinine (sCr) (i.e. end product of creatine phosphate in muscle). However, sCr fails to account for potential variation due to physiological muscle mass, dietary habits, and/or clinical issues with skeletal muscle (i.e. atrophy). Importantly, the usage of creatinine may be inaccurate in individuals who follow a low and/or high protein diet, are malnourished, or individuals building muscle such as athletes who may also have some level of muscular injury during training (Bonventre & Sabbisetti, 2010). Although creatinine levels are utilized to calculate kidney function via glomerular filtration rate (GFR), it is not a biomarker specific to tubular and/or glomerular injury (Stevens, Schmid, Greene, Li, Beck, Joffe & Levey, 2009). According to Coca, Yalavarthy, Concato, and Parikh (2008) the American Society of Nephrology has appointed potential

biomarkers of identifying AKI as a research priority. Current biomarkers that have been utilized in the identification of AKI include both Cystatin C (Cys-C) and Creatinine (Cr) (Coca, Yalavarthy, Concato & Parikh, 2008; Zhang, Lu, Sheng & Jin, 2011). Cys-C is commonly used as a reference of GFR as more than 99% of Cys-C is filtered by the glomeruli and catabolized (but not secreted) by the renal tubules. Thus, even the slightest presence of Cys-C in urine may indicate renal impairment. (see Figure 2.) (Coca, Yalavarthy, Concato & Parikh, 2008; Junglee et al., 2013; Zhang, Lu, Sheng & Jin, 2011). (Fathallah-Shaykh & Cramer, 2014; Hahn, Kanbay, Lanaspa, Johnson & Ejaz, 2016; Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015). Although creatinine is influenced by age, muscle mass, and dietary intake, Cys-C has proven to be independent of those factors (Nejat et al., 2010). Although hydration status is highly researched within the athletic population, there are limited resources available regarding athletic performance in recurrent high temperature environments and its potential effects on renal function. Additionally, the relationship between potential recurrent dehydration and renal injury in response to repeated bouts of strenuous exercise in hot environments has yet to be evaluated.

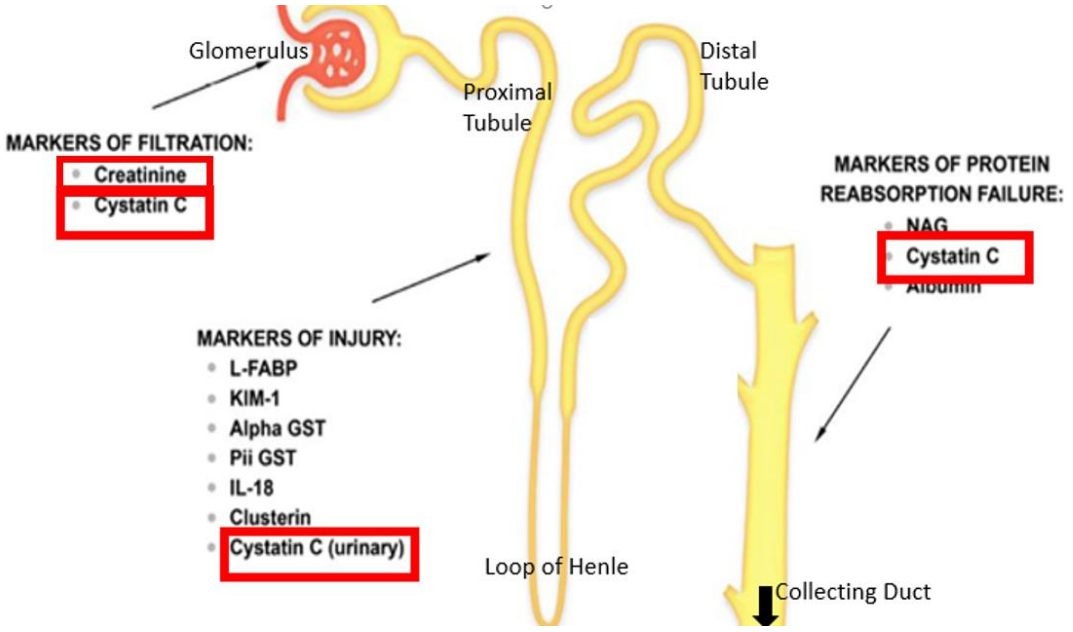

Figure 2. Physiological Description of Renal Injury Biomarkers. Figure adapted from Malyszko, J., Lukaszyk, E., Glowinska, I., & Durlik, M. (2015). Biomarkers of delayed graft function as a form of acute kidney injury in kidney transplantation. *Scientific reports*, *5*, 11684.

Purpose of this Study

The purpose of this study is to investigate the effects of heat stress during pre-season training in South Texas on NCAA Division I female soccer players hydration status and urine biomarkers of renal function related to acute kidney injury.

Hypotheses

H1- Athletes will present increases in urinary Creatinine and Cys-C concentrations following Fitness Testing Day 1 (FT1), Fitness Testing Day 2 (FT2), Post-Exhibition Game 1 (Post-EG1), Post 12hr- Exhibition Game 1 (Post 12hr-EG1), Post-Exhibition Game 2 (Post-EG2), and Post 12hr- Exhibition Game 2 (Post 12hr-EG2).

H2- Athletes will present levels of dehydration (USG) following Fitness Testing Day 1 (FT1), Fitness Testing Day 2 (FT2), Post-Exhibition Game 1 (Post-EG1), Post 12hr- Exhibition Game 1 (Post 12hr-EG1), Post-Exhibition Game 2 (Post-EG2), and Post 12hr- Exhibition Game 2 (Post 12hr-EG2).

H3- There will be an effect of time on increased concentrations of urinary creatinine and Cys-C values during pre-season practices and exhibition games

H4- Urinary biomarkers Cys-C and creatinine will increase regardless of a maintained euhydrated state (i.e. normal hydration).

Significance of the Study

To the authors knowledge, no previous research has evaluated the effects of preseason on renal function within a competitive sports team. This research has the potential to serve as a reference in the growing interest of renal function parameters. Furthermore, this research will not only impact soccer teams within South Texas but may serve as a reference for other athletic teams, manual laborers, and others that may face similar environmental situations. Additionally,

our findings may assist athletic trainers, coaches, and players in assessing hydration status and furthermore renal function via noninvasive urinary biomarkers, uCys-C and uCr

Assumptions of the Study

The basic assumptions for this study:

1. Subjects complied with protocols associated.

2. Coaches complied with protocols associated.

3. Weather will remain stable and consistent throughout the pre-season training protocol.

4. Subject behavior and compliancy will remain consistent throughout the preseason training protocol.

Limitations

1. The observational prospective study design limits controllability.

2. We did not control athlete's potential alcohol and/or dietary intake.

3. We did not control the variability of exercise stimuli and adaptations of athlete's recovery habits.

4. Data collection was impacted by preseason travel schedule.

CHAPTER II

Review of Literature

The development of AKI may be detected through numerous serum and urinary biomarkers. This literature review will discuss the potential mechanisms in which AKI occurs. Following, the description of urinary biomarkers Cystatin-C (uCys-C), and Creatinine (uCr) will be discussed. In compliance with the urinary biomarkers, this literature review will also focus on hydration status and associated measures. Furthermore, evidence related to heat stress, strenuous exercise, and its effect on urinary biomarkers and hydration status will be discussed.

The Kidneys and Acute Kidney Injury

The renal system is responsible for the excretion of metabolic waste and foreign substances, regulation of water and electrolyte balance, extracellular fluid volume, plasma osmolality, red blood cell production, vascular resistance, acid-base balance, vitamin D production, and gluconeogenesis (Eaton & Pooler, 2013). Within these responsibilities, exercise is known to disrupt acid-base balance when workload surpasses anaerobic threshold and the accumulation of hydrogen ions (H^+) exceeds buffering capacity (Cerretelli & Samaja, 2003). In addition, exercise induces changes in protein excretion due to potential muscle injury and/or breakdown (e.g. myoglobin release) relative to exercise intensity. Notably, post-exercise proteinuria (i.e. protein in urine) is commonly present following strenuous exercise (60% of VO2max) and has also been associated with increased glomerular permeability (Saeed, Devaki, Mahendrakar, & Holley, 2012; Poortmans & Vanderstraeten, 1994). Exercise is also known to disrupt water and electrolyte balance as well as plasma osmolality through increased metabolic heat production and perspiration rates (i.e. sweating) (Bongers et al., 2018). Thus, the responsibilities of the renal system are increased following exercise in order to maintain internal

homeostasis. Consequently, the level of work placed on the renal system in result of strenuous exercise in heat may provoke enhanced renal dysfunction.

Acute kidney injury (AKI) has previously been described as functional or structural abnormalities or markers of kidney damage including abnormalities in blood, urine, or tissue tests or imaging studies that are present for less than three months (Mehta et al., 2007). Furthermore, clinical studies suggest that AKI occurs in result of a decreased intrarenal and renal perfusion (i.e. reduced RBF), a reduced filtering capacity of the glomerulus, damage to the renal tubules via toxins or an obstructive insult (e.g. crystallization of UA), or edema and tubulointerstitial inflammation (Thadhani, Pascual & Bonventre,1996; Vaidya, Ferguson & Bonventre, 2008). Although there are many theories on the development of AKI, mechanisms in which injury to the kidneys may occur include increased levels of AVP and its effects on the kidneys, chronic hyperuricemia, and activation of the aldose reductase-fructokinase pathway **(**see Figure 3.) (Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015; Schlader et al., 2019).

In response to hyperosmolality, the release of AVP from the posterior pituitary gland is stimulated to increase water reabsorption and maintain homeostasis. Although circulating AVP has been associated with the progression of CKD in animal models, its role in the development of AKI in relation to dehydration in humans is only postulated. However, the stimulation of the fructokinase pathway (i.e. metabolism of fructose) has previously been associated with actions of AVP release (Roncal-Jimenez et al., 2014; Schlader et al., 2019; Song et al., 2016) Furthermore, an increase in serum osmolarity stimulates the activation of aldose reductase in attempts to generate sorbitol as it assists in the protection of tubular cells from the high osmolarity in the extracellular environment (i.e. renal medulla). Although stimulation of aldose reductase may

help with the protection of tubular cells, the proximal tubule may be negatively affected by its activation. Specifically, further degradation of sorbitol (i.e. sorbitol dehydrogenase→fructose) into fructose may lead to the stimulation of the fructokinase pathway. This stimulation results in depletion of adenosine triphosphate (ATP) as the metabolism of fructose is energetically expensive. Additionally, the stimulation of the fructokinase pathway generates inflammatory mediators, oxidants, and uric acid. Consequently, the generation of uric acid may lead to hyperuricemia (i.e. abnormally elevated levels of serum uric acid) in which renal injury within the glomeruli and tubules is heightened due to potential crystallization of uric acid. In absence of dehydration (i.e. euhydrated state), increased nucleotide release via low-grade muscular injury has also shown to contribute to the development of hyperuricemia (Roncal-Jimenez et al., 2014; Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015). Similarly, to the metabolism of fructose, the reabsorption of sodium is energetically expensive as it is reliant on the Na^+/K^+-ATPase pump. Sodium reabsorption is stimulated by aldosterone, renal sympathetic activation, and angiotensin II all which are heightened during dehydration in attempts to maintain homeostasis of fluids (Schlader et al., 2019).

According to Lippi et al., (2012), strenuous physical exertion independently may increase the occurrences of renal impairment. Moreover, acute renal impairments were related to the reduction in RBF, inflammation, increased protein excretion due to muscular damage (e.g. myoglobin; proteinuria; nucleotide release), and oxidative cellular damage (i.e. via ATP depletion) (Lippi et al., 2012). The reduction of renal perfusion is due to stimulation of renal sympathetic nerve activity, circulating AVP levels, and has also been associated with the stimulation of the renin-angiotensin-aldosterone system (RAAS). Specifically, angiotensin II acts as a vasoconstrictor in the kidneys and may also temper renal function during exercise in heat

(Schlader et al., 2019). Furthermore, a popular hypothesis is that the reduction in RBF may induce hypoxemia due to reduced oxygen delivery to the renal cortex. Consequently, it is believed that the reduction of oxygen delivery may provoke a localized reduction in ATP as well as ischemia (Bongers et al., 2018; Schlader et al., 2019). Although reductions in RBF have shown to usually resolve with recovery, Hope and Tyssebotn (1983) demonstrated the effects of long-lasting dehydration on total and local RBF in rodent models. Following eight days of water deprivation, RBF decreased by 65% and renal vascular resistance increased by 110% (i.e. attributed to increased blood viscosity). Additionally, localized ischemia in cortical regions and intermittent blood flow occurred within one rat model (Hope & Tyssebotn, 1983). Nelimarkka, Halkola, and Niinikoski (1982) assessed renal perfusion in the renal cortical and medullary tissues of dogs. Similarly, they observed differences in renal perfusion via reductions in cortical blood flow in absence of change in medullary blood flow (Nelimarkka, Halkola & Niinikoski, 1982). These findings indicate that there is an increased heterogeneity of filtration and intrarenal flow within the kidneys of both rodent and dog models. Meaning, dehydration alone may compromise oxygen delivery due to reduced renal perfusion. Notably, dehydration, exercise, and heat stress independently decrease RBF and reductions are heightened when these conditions are in conjunction with one another (Hope & Tyssebotn, 1983; Nelimarkka, Halkola & Niinikoski, 1982; Schlader et al., 2019). In theory, the reduction of oxygen supply will reduce available ATP and increase the risk of developing AKI via increased inflammation and oxidative stress in humans as well. Thus, athletes who are exposed to repeated bouts of strenuous exercise in hot environments are not only at risk of becoming dehydrated but may also be at risk for developing AKI.

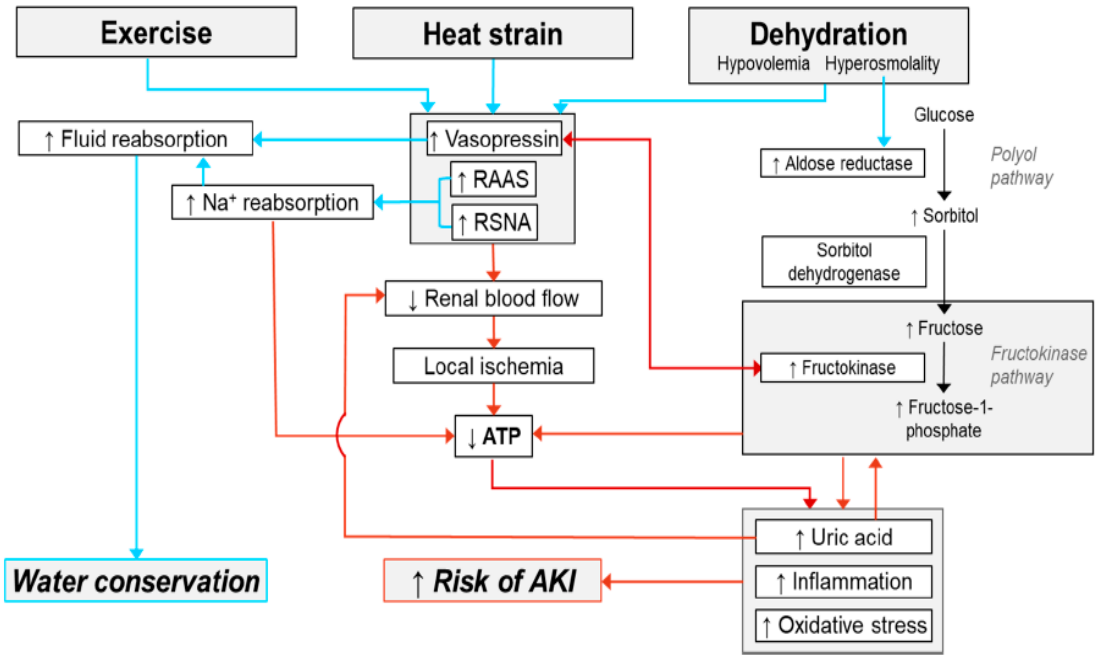

Figure 3. Exercise, Heat Strain, & Dehydration. Figure from Schlader, Z. J., Hostler, D., Parker, M. D., Pryor, R. R., Lohr, J. W., Johnson, B. D., & Chapman, C. L. (2019). The Potential for Renal Injury Elicited by Physical Work in the Heat. *Nutrients*, *11*(9), 2087.

Renal Function Parameters and Associated Biomarkers

In traditional form, AKI could be detected by urinary (fractional excretion of Na^+, urinary cast) and blood (creatinine, blood urea nitrogen) markers. Furthermore, serum creatinine is utilized within two different classification systems of AKI. Specifically, the RIFLE (i.e. Risk, Injury, Failure, Loss, End-stage renal disease) criteria and AKIN (i.e. Acute Kidney Injury Network) are clinical traditional classification systems for diagnosing AKI. However, while measurements of creatinine may be useful for populations with Chronic Kidney Disease (CKD), traditional methods are insensitive to the early detection of the onset of AKI (Han, Waikar, Johnson, Betensky, Dent, Devarajan & Bonventre, 2008). Measurements of glomerular filtration rate (GFR) are critical in the detection, management, and evaluation of kidney function. However, when GFR is calculated, variables included in the calculation (i.e. age, sex, race,

creatinine) do not account for potential variation in creatinine due to factors such as, physiological muscle mass, dietary habits, and/or protein-wasting issues with skeletal muscle (i.e. atrophy). Thus, estimations of glomerular filtration rate in healthy populations may be inaccurate in individuals who utilize low and/or high protein diets, are malnourished, or are attempting to build muscle (Stevens, Schmid, Greene, Li, Beck, Joffe & Levey, 2009).

Recent findings suggest that the urinary biomarker Cys-C is a strong non-invasive indicator of AKI and it has been utilized to detect the early onset of AKI development (Mehta et al., 2007; Vaidya, Ferguson & Bonventre, 2008; Uchida & Gotoh, 2002). Further, Cys-C in relation to creatinine appears to be less affected my skeletal muscle mass. Thus, Cys-C may serve as a valuable biomarker in athletic populations for the assessment of glomerular filtration rate and thus renal function (Stevens, Schmid, Greene, Li, Beck, Joffe & Levey, 2009).

Urinary Cystatin-C

Cystatin C (Cys-C) is a 13-kDa protein that is freely filtered by the glomerulus in which is reabsorbed and catabolized (but not secreted) by the renal tubules. It is believed that Cys-C is a crucial extracellular inhibitor of cysteine proteases (enzyme that degrades proteins). In a healthy kidney, urinary Cystatin-C is reabsorbed by the proximal tubule and there are no present concentrations in final urine (Vaidya, Ferguson & Bonventre, 2008). However, when renal injury occurs, Cys-C can be present in urine as reabsorption is decreased. As mentioned, concentrations of Cys-C seem to be independent of skeletal muscle mass, sex and age which in return makes this a sensitive marker of GFR more so than creatinine (Vaidya, Ferguson & Bonventre, 2008). Nejat and colleagues (2010) investigated the relationship between Cys-C and AKI in intensive care (ICU) patients and found that urinary Cys-C independently indicates AKI. Following the assessment of 125 patients diagnosed with AKI, urinary concentration ≥0.45 mg/L (0.09-2.54) indicate tubular dysfunction, and further AKI. Non-AKI concentrations of uCys-C were defined

as ≤0.07mg/L (0.03-0.28). Notably, elevated levels of urinary Cys-C predicted death within 30 days of admission into ICU with the risk of death being doubled in patients with uCys-C concentrations >0.1mg/dL (Nejat et al., 2010). Conti and colleagues (2006) assessed uCys-C concentrations in several patients with kidney disease amongst a control group. Patients with tubular related renal disease (4.31 ± 3.85 mg/L) presented significantly higher values than the control group (0.096± 0.044 mg/L). Additionally, patients with glomerular specific renal disease displayed increased levels of uCys-C (0.106± 0.133 mg/L). Consequently, increased uCys-C levels have proven to be an accurate method of detecting of tubular dysfunction (Conti et al., 2006). Furthermore, uCys-C has shown to be an accurate, noninvasive, and rather precise method of assessing potential renal dysfunction.

Urinary Creatinine

Creatinine is a small (113 Da) nitrogenous end product of muscle creatine catabolism. Specifically, creatinine is formed by irreversible dehydration of body creatine phosphate from muscle metabolism (Barr et al., 2005; Hosten et al., 1990). The formation of creatinine initiates with the transamidination from arginine to glycine to form guanidoacetic acid (GAA) or glycocyamine. These reactions occur in the small intestine's mucosa, the pancreas, yet primarily, the kidneys. After GAA formation, it is then transferred to the liver where the formation of creatine is created via S-adenosyl methionine (SAM). Creatine then enters circulation where 94-98% is stored within skeletal muscle for future usage. Creatine Phosphokinase (CPK) then catalyzes the reaction of muscle creatine being phosphorylated to creatine phosphate. Lastly, approximately 2% of these stores are converted irreversibly to nonenzymatic creatinine. Consequently, it is evident that creatinine production reflects the amount of lean body mass an individual may have. Creatinine has also shown to be dependent of age (reduced muscle mass), dietary consumption (specifically protein), and is further affected in populations with potential

skeletal-muscular dysfunctions (Barr et al., 2005; Hosten, 1990). Within normal subjects, the kidneys excrete creatinine with minimal extrarenal disposal or demonstrable metabolism. Due to its low molecule weight, creatinine is freely filtered within the glomerulus and is not affected by urine flow rate. Notably, creatinine excretion has shown to increase following exercise without significant changes in serum concentrations (Barr et al., 2005; Hosten, 1990; Calles-Escanson et al., 1984).

Several organizations have created standards for spot urinary sampling. The World Health Organization (WHO) indicates urinary samples as too diluted if urinary creatinine concentrations are <30 mg/dL and too concentrated is samples are >300 mg/dL (Barr et al., 2005; WHO, 1996). In a study conducted by Barr and colleagues, ~2,000 subjects had their urinary creatinine assessed over time. Within this population of 20-29-year-old females, the 10th percentile averaged 37.24 mg/dL, 50th percentile averaged 132.8 mg/dL, and the 90th percentile averaged 246.6 mg/dL of urinary creatinine. Thus, suggesting that the 90th percentile had increased levels of urinary creatinine and further kidney dysfunction. Authors also found that body mass index (BMI) and fat-free mass (FFM) was significantly related to urinary concentration levels (Barr et al., 2005). Nejat and colleagues (2010) also assessed uCr concentrations in patients diagnosed with AKI. Urinary creatinine concentrations of ≥7.40 mmol/L (133.3 mg/dL) were present within ICU patients diagnosed with AKI. Non-AKI levels of uCr were defined as ≤ 4.60 mmol/L (82.88 mg/dL) (Nejat et al., 2010).

Hydration Status

Total body water (TBW) accounts for about 50-60% of body mass and occupies both intracellular (ICF) and extracellular (ECF) indices (*i.e.* TBW= ICF + ECF) (Maughan, 2003). Intracellular fluid consists of approximately two-thirds of TBW (65% TBW). Extracellular fluid accounts for one-third of TBW (35% TBW) including plasma (Armstrong, 2007; Oppliger &

Bartok, 2002; Shirreffs & Sawka, 2011). Fluid deficits greater than 2-3% body weight may compromise aerobic exercise performance and cognitive function, especially in hot environments (Shirreffs & Sawka, 2011; Thomas, Erdman & Burke, 2016). Injuries related to dehydration include, but are not limited to cramps, fatigue, heat stroke, heat exhaustion, and the potential development of AKI (Oppliger & Bartok, 2002).

Furthermore, dehydration disturbs concentrations of body water and sodium equilibrium. As mentioned previously, this signals the posterior pituitary gland to release vasopressin. Vasopressin then promotes water retention and stimulates the sensation of thirst. As explained previously, without rehydration a cascade of events may occur in which health concerns may arise (i.e. heat illness/injury, renal stress). Notably, heat-associated dehydration has also been suggested to cause hyperuricemia (i.e. elevated uric acid) as one may not be able to replenish what fluids were lost efficiently (Roncal-Jimenez, Lanaspa, Jensen, Sanchez- Lozada & Johnson, 2015). Additionally, athletes continually exposed to both heat and humidity are more susceptible to falling into a vicious cycle of dehydration. Notably, when an athlete has not been previously exposed to such environmental conditions, the athlete(s) may experience elevated sweat rates and further sodium losses. Furthermore, heat acclimatization takes approximately 10-14 days to occur and days prior to such acclimation may promote dehydration (Casa, Armstrong, Hillman et al., 2000; Kitam et al., 2018; Kutlu & Guler 2006). Notably, several mechanisms such as urine specific gravity (USG) and percentage of body weight change have shown to be accurate, fast, practical, and noninvasive means of assessing hydration status in athletes (Baker, Lang & Kenney, 2009; Sommerfield et al., 2016). Furthermore, the American College of Sports Medicine (ACSM) recommends athletes estimate sweating rates according to pre-exercise and post-exercise body mass changes (Sawka et al. 2007).

Renal Function Biomarker Responses to Exercise

Although hydration status in response to exercise is highly researched, information regarding renal function in apparent healthy athletic populations is limited. However, although literature and the understanding of recurrent strenuous exercise is lacking, there are numerous studies that have indicated the development of renal impairment after a single bout of strenuous exercise and one previous article observed both acute and prolonged effects of exercise.

Urinary Cystatin-C

Bongers and colleagues (2018) assessed urinary Cystatin-C (uCys-C) as an indicator of glomerular filtration rate (GFR). Interestingly, estimated GFRcystatin-C (eGFRcystatin-C) did not change following the acute (118 ± 11 mL/min/1.73 m^2 vs. 116 ± 12 mL/min/1.73 m^2, P = 0.12) bout of exercise, however a significant decrease was found following the prolonged (103 ± 16 mL/min/1.73 m^2, P < 0.001, Fig. 1) exercise bout. Furthermore, urinary Cys-C concentrations increased following both acute (0.03 mg/L [0.01-0.08]) and prolonged (0.15 mg/L [0.09-0.26]) exercise bouts. Specifically, concentrations of Cys-C were significantly different ($p<0.001$) following prolonged exercise. Furthermore, it is evident that the kidneys can maintain function following such an acute bout of exercise. However, in the addition of dehydration, heat, and prolonged strenuous exercise it is also evident that concentration on uCys-C increased while eGFRcystatin-C decreases. However, the authors failed to correlate the decreased values of eGFRcystatin-C with hydration status. Notably, > 99% of Cys-C is filtered through the glomeruli and should also be reabsorbed. The increases in uCys-C concentration may indicate injury within the kidney if urinary concentrations are increased above normal ranges. However, a concentration of 0.15 mg/L does not indicate AKI. This may indicate permeability due to potential injury, but the increases do not indicate AKI. Herget-Rosenthal et al., (2007) found that increases in uCys-C reflected structural and functional renal tubular impairment independently

and may be used as an independent marker of tubular dysfunction in a clinical setting (Conti et al., 2006; Nejat et al., 2010). However, uCys-C in considerably healthy athletic populations is lacking evidence within the literature and it is rather unknown what repeated bouts of exercise may impose on renal health.

Urinary Creatinine

Calles-Escandon et al., (1984) assesses the effects of exercise on urinary creatinine with subjects cycling at 45% of their VO_{2Max} for 90 minutes. Authors found that creatinine increased by 50% post-exercise. Notably, subjects were fed a vegetarian based diet prior to initiating training to ensure that dietary consumption of protein did not influence acute responses in creatinine. Thus, it is evident that the dehydration of creatine stores via exercise is responsible for increase urinary concentrations of creatinine.

Morales et al., (2017) assessed cardiopulmonary performance in soccer players with alterations in renal function. The authors found a significant relationship between subjects with a decreased GFR (dGFR: 55.0 vs. nGFR: 97.4 mL/min/1.73m^2) and urinary protein excretion (dGFR: 14.74 vs. nGFR: 11.26 mg/dL). Consequently, this suggest that the physical stress of training may induce acute alterations in renal function. The normal GFR (nGFR) group appeared to have a higher urinary creatinine concentration (224.1 mg/dL) than the dGFR group (112.4 mg/dL). However, the authors failed to explain this difference, this may also be an indicator of training status of the athletes as no differences in serum creatinine was present. Meaning, during high-intensity training, phosphocreatine (PCr) is available for adenosine triphosphate (ATP) regeneration. Although PCr stores are limited during high intensity exercise and depleted within ~10 seconds, trained athletes can regenerate stores at a faster capacity than those untrained (Tomlin & Wenger, 2001). Thus, the nGFR group may have synthesized and further utilized more creatine stores than the dGFR group explaining the differences in urinary creatinine.

Furthermore, the authors did not account for lean body mass in relation to creatinine concentrations. Thus, it is not evident whether the nGFR groups lean body mass influences the elevated UCr measures (Morales et al., 2017; Wyss & Daouk, 2000).

In addition to analyzing Cys-C, Bongers and colleagues (2018) also assessed changes in uCr following an acute and prolonged bout of cycling. Evident and significant changes were found after both acute (9.2 mmol/L) and prolonged exercise (26.3 mmol/L) in uCr concentrations. Specifically, from baseline measures (5.0 mmol/L), prolonged exercise increased uCr more so than an acute bout of exercise. However, the authors failed to account for the lean body mass of subjects in present creatinine concentrations. Although exercise has shown to increase uCr due to dehydration of creatine stores, uCr has proven to be dependent on muscle mass (Bongers et al., 2018; Wyss & Daouk, 2000).

Turgut et al., (2003) assessed the influence of acute exercise on urinary creatinine in both female and male participants. Notably, the exercise stimuli consisted of "heating, running, and standard volleyball" for 2 hours. The authors found significant increases in uCr from before exercise (93.15 mg/dL) and after exercise (187.60 mg/dL). Specific to females, significant differences were also found between before exercise (98.74 mg/dL) and afternoon exercise (201.55 mg/dL). Although differences appear to be more significant in the male group, that can be attributed to males having a greater muscle mass than their counterparts. The authors attributed the increased levels of uCr to increased glomerular filtration permeability and satiation of filtered proteins via proximal tubular reabsorption (Turgut et al., 2003).

Hydration Status

Several mechanisms such as urine specific gravity (USG) and percentage of body weight change have shown to be accurate, fast, and noninvasive means of assessing hydration status in athletes (Baker, Lang & Kenney, 2009; Sommerfield et al., 2016). Furthermore, values of USG

for the detection of dehydration is widely accepted at ≥1.020 (Kiitam et al., 2018). Furthermore, the American College of Sports Medicine (ACSM) recommends athletes estimate sweating rates according to pre-exercise and post-exercise body mass changes (Sawka et al. 2007).

Castro-Sepulveda, Astudillo, Letelier and Zbinden-Foncea (2016) found that only 2% of elite female soccer players were in a euhydrated (normal [USG <1.020]) state when attending exhibition games and official games. The authors also found that 100% of the female athletes were hypohydrated prior to training sessions (USG >1.020). Notably, the cautionary hypohydrated state was attributed to weather conditions (i.e. 28 °C or 82.4 °F) (Castro-Sepulveda, Astudillo, Letelier & Zbinden-Foncea, 2016). Exercise in hot environments not only potentiates hypohydration, but may impose serious heat illnesses (Thomas, Erdman & Burke, 2016). In addition, Phillips, Sykes and Gibson (2014) evaluated hydration status of soccer players on three consecutive mornings prior to strenuous trainings. The authors found that 77% of athletes were hypohydrated (USG >1.020) prior to morning training sessions (Phillips, Sykes & Gibson, 2014). Furthermore, Sommerfield and colleagues (2016) observed a 3% reduction in body weight following the participation of a supervised 2-hour standard exercise regime. Edwards et al., (2007) observed dehydration via an increase in USG (>1.020) and a 2.14-2.4% reduction of body mass following a 45-minute outdoor soccer match in weather conditions of 39.28 °C. Additionally, Bongers et al., displayed a 3% reduction in body mass following 150 minutes of cycling at 25 °C.

Notably, the research reviewed attributes hypohydrated state to the warm weather conditions present. Alarmingly, South Texas presents higher temperatures of ~34.8 °C (94.6 °F), a relative humidity of 80%, and a heat index of 48.9 °C (120 °F) during the preseason training (National Weather Service Corporate Image Web Team, 2019). Thus, South Texas soccer

players and athletes are at higher risk of recurrent dehydration and thus the development or heat illness and further serious health concerns.

CHAPTER III.

Methods

Participants

A convenience sample of 21 Texas A&M University- Corpus Christi female soccer players competing in the National Collegiate Athletic Association (NCAA) Division One soccer league were recruited for participation. Subjects were medically cleared to participate by the team physician and athletic training staff to participate in this study. Due to the observational nature of this study, all individuals who were medically cleared to participate in pre-season were recruited to participate in the study. Subjects were excluded from the study if injured and/or they missed more than one week of the preseason training protocol. Recruited participants signed consent forms prior to participation in this study and following the approval by Texas A&M University-Corpus Christi's Institutional Review Board (IRB) and the Institutional Biosafety Committee (IBC).

Anthropometrics and Body Composition

Height and weight were assessed via portable SECA scale and stadiometer (Seca model 769). Whole body composition, regional body composition, and bone mineral density were assessed using the GE Lunar Dual X-Ray Absorptiometry (iDXA) technology (iDXA, Lunar Prodigy; GE Healthcare, Madison, WI). The iDXA is a relatively quick, safe, and noninvasive method for body composition measures (Prado & Heymsfield, 2014). Internal consistency and stability have shown to be highest in the GE iDXA. The GE iDXA is frequently referenced as the Golden Standard as it has been validated against a four and six-compartment models (Ballard, Fafara & Vukovich, 2004; Bailey, LeCheminany, Hope, Bell & Tucker, 2018).

Hydration Status

Hydration Status was evaluated via USG techniques utilizing the Clinical Refractometer model 300005 (Sper Scientific, Scottsdale, AZ). The USG technique utilized included measuring urine

density/concentration via refractometer. Urinary density concentration is measured by how much beam is refracted, whilst a beam passes through the urine (Oppliger & Bartok, 2002). Notably, accuracy of the Clinical Refractometer is between ± 0.002 and the refractive index changes proportionally to urine concentrations. For the purposes of this study, hypohydration was defined as a USG > 1.020 (Kiitam et al., 2018). Urine color analysis (UCA) was also conducted to assess hydration status. Urinary color was rated according to the previously published Urine Color Chart (scale colors ranging from 1 [lightest] to 8 [darkest]) (see Appendices 1) (Armstrong et al., 1994). When assessing urinary color, samples were against a white background in a well-lit room as recommended via previous researchers (Armstrong et al., 1994; McKenzie, Munoz & Armstrong, 2015). To ensure reliability, three members of our research team assessed sample color individually and were blinded to other ratings. Following the completion of individual ratings of each sample, the mean average was then computed between all three researchers and was documented for future analysis.

Environmental Analysis

Temperature and humidity were assessed via both the Kestrel 5000 environmental meter (Kestrel Meters, Boothwyn, PA) and a Kestrel Drop2 meter. The average height of players was determined, and environmental measures were collected at ~6 inches above the turf via the Kestrel Drop2, and at the average one-half height among participants (~Anterior Superior Iliac Spine [ASIS]). Utilizing the Kestrel 5000 environmental meter, data was continually collected starting at 30 minutes prior to each outdoor practice and exhibition match. Environmental sampling and storage of data automatically occurred every 15 seconds.

Sampling Procedures

Participants were asked to provide 14-urine samples through the course of preseason training, which included two-exhibition games. Prior to arriving, subjects were informed of

training protocols and were provided consent forms via email. Subjects were further consented following their arrival to Texas A&M University-Corpus Christi. After consenting to participation, subjects were then scheduled between 0500-0700 on PRE-PS day. Subjects were then asked to report to the Exercise Physiology Lab Blood Lab (EPBL) for baseline assessments of anthropometrics and urine collection.

On the day of baseline measures (PRE-PS), height and weight were measured through use of a portable SECA scale and stadiometer (Seca model 769), and then body composition analysis was conducted utilizing Dual X-Ray Absorptiometry (DXA) technology (iDXA, Lunar Prodigy; GE Healthcare, Madison, WI). For this test, participants were asked to lie on the padded DXA table and remain motionless as the scanning arm passes over them. Furthermore, subjects were given a urine specimen cup and directed to the restroom closest to EPBL. Subjects then returned with their urine sample and placed them in the respective assigned container.

Following baseline collections (PRE-PS), subjects began preseason training at ~0700 the following morning in which fitness assessments were initiated. Fitness testing took place at the beginning of preseason for two days (FT1 & FT2). Urine collections occurred between 0500-0630 throughout the preseason practices. Due to the physiological demands and potential lack of heat acclimation upon the start of the preseason, urine collections occurred every morning the first week of preseason to closely monitor hydration status and renal function (following FT1 & FT2). To assess the impact of exhibition games (BU & UT) on renal function, urine samples were collected prior to the initiation of game warm-ups (PRE-BU/UT), post-game (POST-BU/UT), 12-hours post (12HR-BU/UT), and 24-hours post (24HR-BU/UT). Morning urine collections continued at 0500-0630 prior to regular preseason training (MidW1 & MidW2). Ending measurements were collected at 0500-0630 prior to the athletes traveling out of state

(POST-PS) (see Figure 4.). Subjects were asked to report to EPBL for ending body composition measures and urine collections.

Urinary Collection and Analysis

Urine collection took place between 0500-0630 on designated practice days prior to the initiation of morning practices (FT1, FT2, MidW1, and MidW2). Subjects were asked to provide a mid-stream sample after using a cleansing towelette. Specimen cups were placed in the corresponding athlete locker each morning of designated urine collection and/or handed to the athletes the night prior if they could not wait until arrival to the locker room to provide their sample. Athletes arrived at the locker room and provided a morning urine sample and/or brought their morning urine sample to practice with their corresponding cup and placed it inside of a designated cooler for transportation to EPBL for analysis and storage.

Urine collection took place immediately prior to warm-up of exhibition games and immediately following the completion of the game (within 30 minutes). Specifically, BU pre-game urine was collected at ~1500 hours (PRE-BU) and ~1900 following the completion of the game (POST-BU). On UT game day, PRE-UT urine was collected at ~1700 hours and ~2100 hours POST-UT. Subjects were given a labeled urine specimen cup upon entering the locker rooms and were asked to place their sample in a designated cooler. Following the completion of the game, the athletes were provided a labeled urine specimen upon re-entering the locker room. Subjects were asked again to place their sample in a designated cooler for transportation back to EPBL for analysis and storage.

Following the BU and UT games, athletes were granted rest day(s) by their coaching staff and were given labeled urinary specimen cups for 12HR-POST and 24HR-POST collections. Upon leaving the locker rooms, athletes were given required times for collection and thorough instructions. Notably, athletes were messaged ~1 hour prior to designated collection times to

ensure the appropriate urinary collection schedule. Following the BU game, subjects were asked to provide samples at ~12 hours-BU (0700) POST-BU urine collection and again ~24 hours-BU (1900) hours POST-BU collection. Following the UT game, subjects were asked to provide urine samples~ 12 hours-UT (0900) POST-UT urine collection and again ~24 hours-UT (2100) POST-UT urine collection. Subjects were then asked to bring their 12HR and 24HR samples to practice the following morning(s).

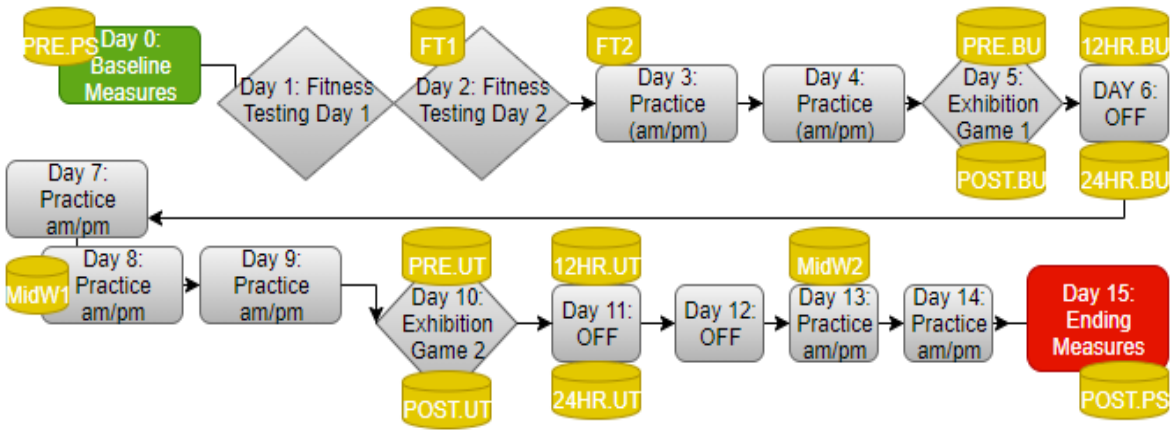

Figure 4. Urine Collection: General Study Design

Urine Color Analysis (UCA)

Urine color analysis was assessed throughout the preseason training protocol with all 14 urine samples collected via the Urine Color Scale (Armstrong et al., 1994). Following urine collection at PRE-PS, FT1, FT2, MidW1, MidW2, and POST-PS, samples were immediately transferred back to EPBL for urine color analysis and further storage into microcentrifuge tubes (MCT) at -80°C. Due to traveling to Baylor for this first exhibition match, urine samples were stored over night at -20°C and remained refrigerated upon traveling back to Corpus Christi. Urinary color was assessed immediately upon arrival to EPBL and stored for later analysis of other renal biomarkers. To ensure the same protocol was utilized between exhibition games, UT game samples were refrigerated overnight, and urinary color and storage took place the

following day. Urine color analysis took place in EPBL which is a well-lit laboratory. Samples were held against a white background and were rated from 1 (lightest) to 8 (darkest) (Armstrong et al., 1994). To ensure reliability, three members of the research team assessed urine color independently with previous ratings hidden to limit bias ratings. Following the completion of all urinary samples being rated, urine samples were then transferred to MCTs for future analysis of urine specific gravity and potential renal injury.

Urine Specific Gravity (USG)

Urine Specific gravity was assessed via a Clinical Refractometer. Urine samples designated for USG were stored at -20 °C throughout the preseason data collection and were analyzed upon completion of data collection. Samples were removed from -20 °C and brought to room temperature (20-25 °C) prior to analysis of USG. On baseline (PRE-PS) and ending day (POST-PS), samples were collected in the nearest restroom by EPBL and USG was assessed following the completion of data collection. On practice days (FT1, FT2, MidW1, and MidW2), urine was collected in the locker-room at the Dr. Jack Dugan soccer and track field stadium. Following collection, urine samples were transported back to EPBL and stored for later analysis. The research team traveled to Baylor University and collected urine prior to the exhibition game (PRE-BU) and following the game (POST-BU). Labeled specimen cups were then supplied to the athletes for 12HR and 24HR collection the following day. PRE-BU and POST-BU samples were refrigerated (-20 °C) overnight at BU and kept in a cooler as the research team traveled back to Corpus Christi. Samples were assessed via urinary color and then were stored immediately following the return to EPBL for later analysis. The second exhibition match took place at the Dr. Jack Dugan track and field stadium in Corpus Christi. Urine samples were collected prior to the game (PRE-UT) at the Dr. Jack Dugan Soccer Stadium and following the completion of the game (POST-UT). Urine samples were collected in the locker-room and

transferred back to EPBL for refrigeration overnight and later storage the following day. Labeled specimen cups were provided for 12HR and 24HR collections. Athletes were asked to bring their recovery samples (12HR & 24HR) the following morning where samples were brought to EPBL for storage.

Urinary Cystatin-C and Creatinine

Following the collection of urine samples, they were transferred to MCTs and stored at -80 °C until the completion of data collection and later uCr and uCys-C analysis. Prior to analysis, samples were thawed to room temperature (20-25 °C) and were analyzed with corresponding ELISA kits. Urinary Cys-C was analyzed utilizing the Human Cystatin-C Platinum ELISA (Invitrogen: BMS2279) in which present urinary Cys-C binds to HRP-conjugated anti-human Cys-C antibody allowing quantification of Cys-C. Our Intra-assay CV% was 10.3% following the analysis of Cystatin-C. Urinary creatinine was assessed via Colorimetric Urinary Creatinine Assay Kit (Cell BioLabs, INC.: STA-378). This assay kit detects urinary creatinine by the Jaffe reaction, in which a reaction between creatinine and alkaline picrate produces an orange-red complex. Our intra-assay CV% following the analysis of Creatinine was 10.96%. Both uCys-C and uCr 96-well plates were read in duplicate with the Bio-Rad iMark Microplate Reader (Bio-Rad, Hercules, CA) at the recommended 450nm.

Statistical Analysis

To analyze data, GraphPad Prism version 8.0.0 (San Diego, California USA) was utilized. The statistical analysis of UCA, USG, uCr, and uCys-C were conducted using a repeated measure One-way ANOVA (RMANOVA) with Geisser-Greenhouse corrections to correct for a lack of sphericity. Additionally, a mixed-effect model was utilized to account for missing data points. A post hoc analysis was performed with Tukey for multiple comparison tests and Dunnett for comparing to PRE-PS values (baseline). Furthermore, paired t-test were utilized to assess

differences between pre- and post-exhibition games. Normally distributed data were presented as mean ± standard error of mean (SEM) with the level of significance set at $P < 0.05$.

CHAPTER IV.

Results

This chapter presents the results of the statistical analyses conducted on the data collected for this investigation. Analyses regarding hydration status and renal function will be discussed following a description of subject demographics and environmental considerations. A repeated-measures, One-way ANOVA was utilized to assess the effect of time on hydration status and renal function parameters. Additionally, paired t-tests were utilized to depict differences between pre-game and post-game (BU and UT) markers of hydration status and renal function.

Demographics

Twenty-one NCAA D1 female soccer players in South Texas volunteered to participate in this study. This sample population self-identified among various ethnic backgrounds (Caucasian [18], Hispanic [2], Asian [1]). Each subject consented for participation (n=21) was medically cleared by the associated licensed practitioner and athletic training staff prior to participation in this study. Several subjects were excluded from this study due to injuries and/or illness that resulted in ≥ 1-week of missed pre-season trainings. Injuries and/or illness included a kidney infection (1), ankle injury (1), foot injury (2), knee injury (1), and a fractured arm (1). Of the remaining 15 subjects, the top 9 athletes that recorded the highest workload (≥40.10 m/min) displacement and average heart rate (≥128.3 HR_{Avg}) throughout the preseason training protocol were selected for potential renal biomarker analysis. All subjects participated in eight morning practices (0700), nine evening (1900) practices, and two-exhibition games (1500-BU and 1700-UT). Temperature (°C), relative humidity (%), and heat index (°C) were recorded at every training session and exhibition match. Additional demographic data for subjects and preseason environmental considerations are listed in *Table 1*.

Table 1. Demographics.

Descriptive Statistics

	Mean ± SEM	Min-Max	n =
Age (y)	19.3 ± 0.25	18-22.5	21
Height (cm)	169.6 ± 1.3	154.9-181.6	21
Weight (kg)	68.4 ± 2.4	54.09-99.5	21
LBM (kg)	45.9 ± 1.1	36.41-56.01	21
Fat Mass (kg)	22.5 ± 1.6	13.91-43.58	21
Body Fat %	32.2 ± 1.3	24.2-44.2	21
Temperature (°C)	30.3 ± 0.44	25.4-36.6	19
Relative Humidity (%)	72.2 ± 1.7	54.3-85.9	19
Heat Index (°C)	37.4 ± 0.8	27.5-47.9	19
Work Displacment (m/min)	40.67 ± 0.92	35.7-47.7	15
HR_{Avg} (bpm)	129.3 ± 0.16	128.2-130.2	15
uCys-C (mg/L)	0.035 ± 0.003	0.023-0.050	9
Creatinine (mg/dL)	177.6 ± 16.1	91.59-265.6	9
USG	1.02 ± 0.001	1.005-1.038	20
Urine Color Analysis	4.63 ± 0.4	1.5-7.75	21

Lean body mass (LBM); Heart rate (HR); Urinary Cystatin-C (uCys-C); Urine specific gravity (USG);

Hydration Status Analysis

Urine Color Analysis

Following statistical analyses of urine color (UCA), significant differences were found amongst numerous timepoints (see Figure 5). Comparing UCA over the span of the preseason training protocol (PRE-PS, FT1, FT2, PRE-BU, POST-BU, 12H-BU, 24H-BU, MidW1, PRE-UT, POST-UT, 12H-UT, 24H-UT, MidW2, POST-PS). Urine color appeared to be rated significantly lower during pre-game assessments PRE-BU (2.50 ± 0.44) and PRE-UT (2.119 ± 0.3321) in comparison to post exhibition games and regular pre-season practices ($p = <0.0001$).

Additionally, UCA at 24H-UT was significantly lower than 12H-UT (p = .02). Lastly, the preseason average for urine color amongst athletes was 4.63 ± 0.4 (1.5-7.75).

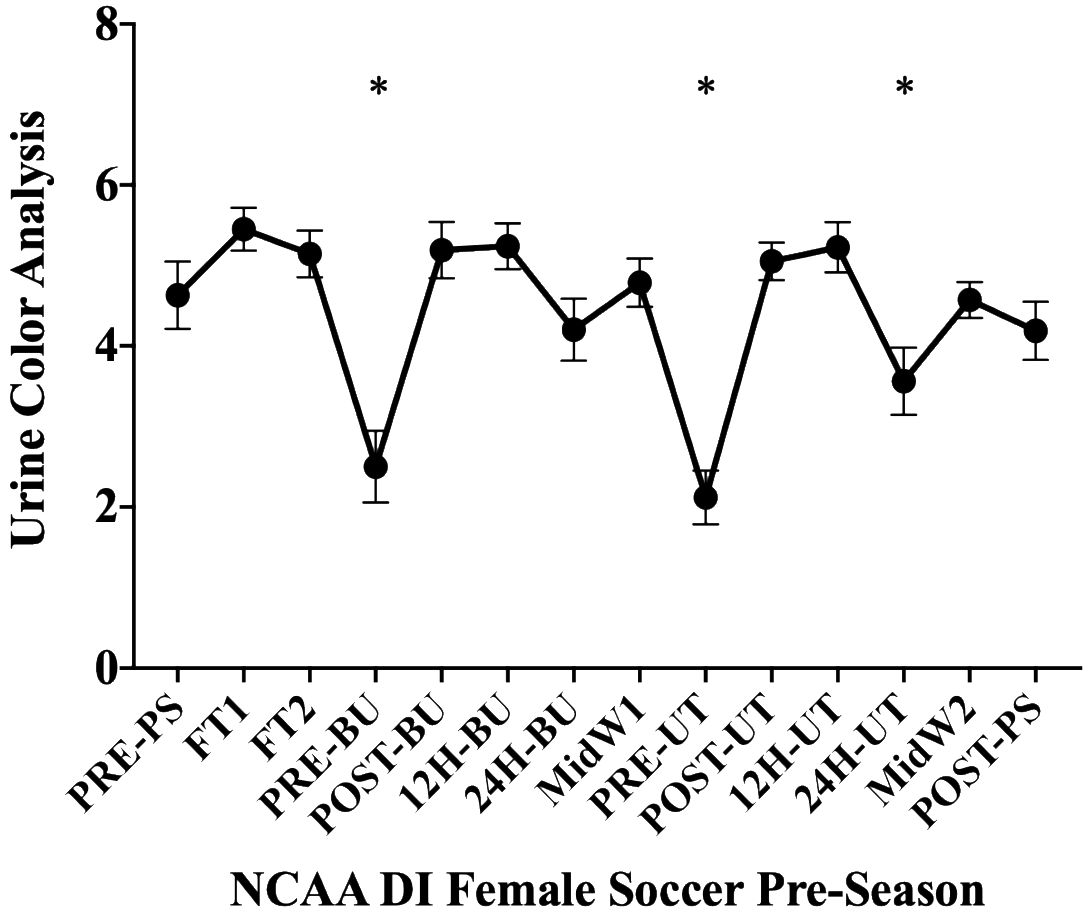

Figure 5: Urine Color Analysis (UCA) (Mean ± SEM; * denotes p = < .05) showed an effect of time with differences between days (p = <0.0001; F (6.330, 123.7) = 12.12). Multiple comparisons showed that PRE-BU (2.50 ± 0.44; 95% CI: 1.573-3.427) and PRE-UT (2.119 ± 0.3321; 95% CI: 1.426 to 2.812) lower values compared to multiple days. Additionally, 24H-UT was lower than 12H-UT (p = .02; 95% CI: -3.154 to -0.1712). No differences were found between Pre-Game UCA measures.

Urine Specific Gravity

A repeated-measured ANOVA (RMANOVA) was performed to assess USG throughout the span of the preseason training protocol (see Figure 6). Significant differences were found in urinary concentrations on numerous at differing time points. Primarily, both pre-exhibition game samples, PRE-BU (1.012 ± 0.001) and PRE-UT (1.009 ± 0.001), were lower than post-exhibition

game and regular practices. Additionally, 24H-UT (showed a significantly lower USG score (mean ± SEM) in comparison to FT2 (mean ± SEM); p = 0.0434) and MidW1 (mean ± SEM); p = 0.0055). Lastly, MidW1 has significantly higher USG scores than MidW2 (mean ± SEM); p = 0.0088).

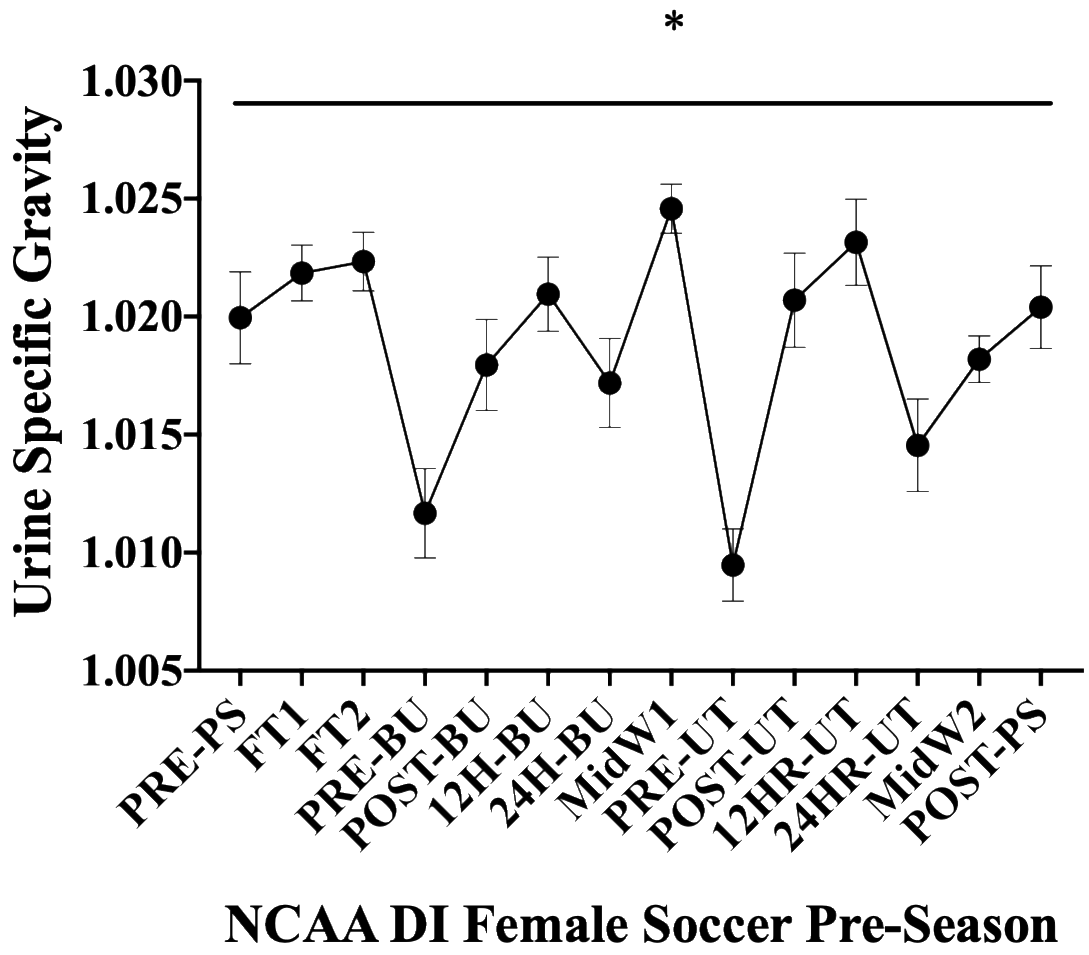

Figure 6: Urine Specific Gravity (USG) (Mean ± SEM; * denotes p = < .05) analysis showed an effect of time with differences between days (p = <0.0001; F (6.632, 130.1) = 8.529). Multiple comparison analysis showed PRE-BU (1.012 ± 0.001; 95% CI: 1.008 to 1.016) and PRE-UT (1.009 ± 0.001; 95% CI: 1.006 to 1.013) had lower USG values compared to multiple days. No differences were found between Pre-game USG measures. Additionally, differences were found between FT2 vs. 24H-UT (p = 0.0434; 95% CI: 0.0001429 to 0.01542), MidW1 vs. 24H-UT (p = 0.0055; 95% CI: 0.002204 to 0.01784), and MidW1 vs. MidW2 (p = 0.0088; 95% CI: 0.001150 to 0.01161).

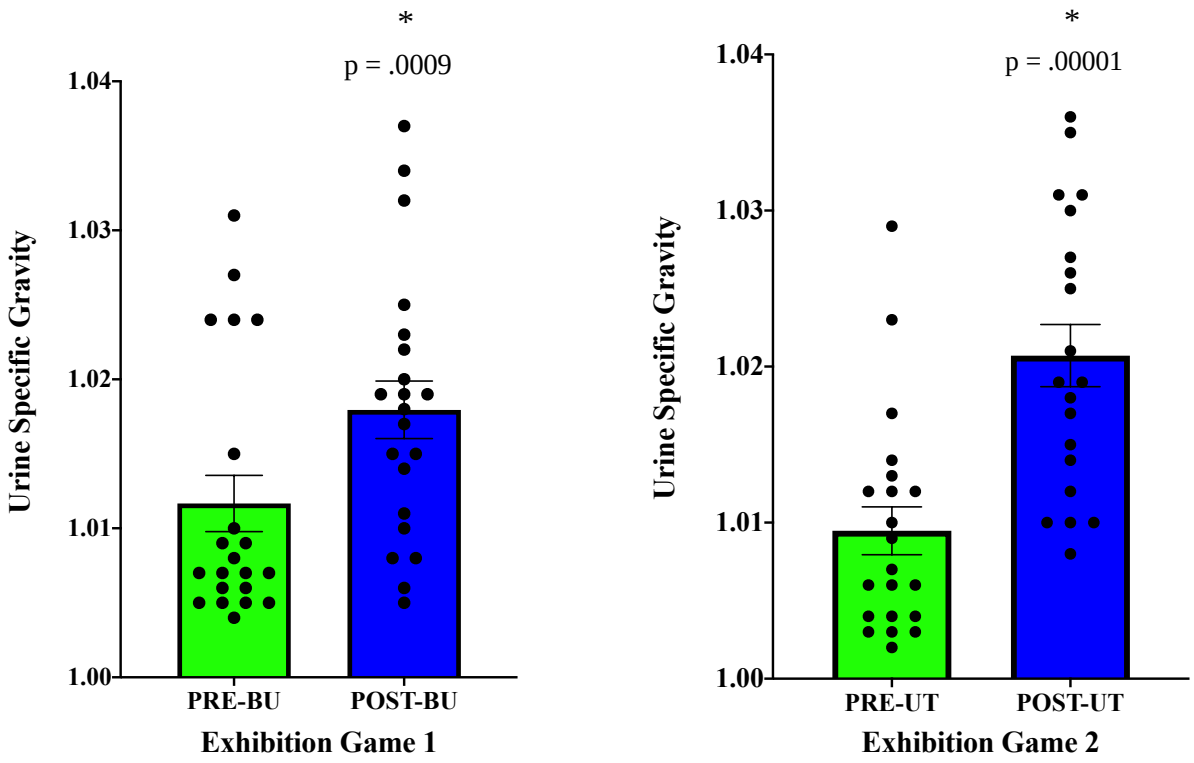

Figure 7: LEFT, Urine Specific Gravity (USG) (Mean ± SEM; * denotes p = < .05) a difference (p = 0.0009; ω_2 =0.43) was found comparing PRE-BU (1.012 ± 0.001; 95% CI: 1.008 to 1.016) and POST-BU (1.018 ± 0.001; 95% CI: 1.014 to 1.022) USG measures. **Figure 8: RIGHT,** Urine Specific Gravity (USG) (Mean ± SEM; * denotes p = < .05) a difference (p = 0.00001) was found comparing PRE-UT (1.010 ± 0.001; 95% CI: 1.006 to 1.013) and POST-UT (1.021 ± 0.001; 95% CI: 1.017 to 1.025) USG measures.

A paired sample t-tests were utilized in Figures 7 and 8 to assess differences in hydration status comparing pre-post exhibition match with USG. Following the first exhibition match (BU), a significant difference (p = 0.0009) is evident in the increased USG score POST-BU (1.018 ± 0.001) in comparison to PRE-BU (1.012 ± 0.001). Similarly, following the second exhibition match (UT), there is a significant difference (p = .00001) between PRE-UT (1.010 ± 0.001) and POST-UT (1.021 ± 0.001) with POST-UT expressing elevated USG scores.

Renal Function Analysis

Urinary Creatinine

Utilizing a RMANOVA analysis to assess urinary creatinine differences throughout the preseason training protocol (see Figure 9). Urinary creatinine was standardized per individual lean body mass measurements ($mg \cdot dL^{-1} \cdot LBM^{-1}$) to account for variability in lean body mass and its contribution and impact on urinary creatinine concentrations. Significant differences were evident at PRE-BU (2.553 ± 0.54) and PRE-UT (1.604 ± 0.42) with lower urinary creatinine present in comparison to regular practices and post-exhibition games. Additionally, significant differences are present between 24H-BU and POST-UT ($p=0.0015$) with POST-UT uCr scores being significantly higher than the previous exhibition games 24H recovery sample. Lastly, MidW1 and 12H-UT urinary creatinine levels are significantly higher than the second exhibition games 24H recover sample 24H-UT ($p = 0.0457$; $p = 0.0305$).

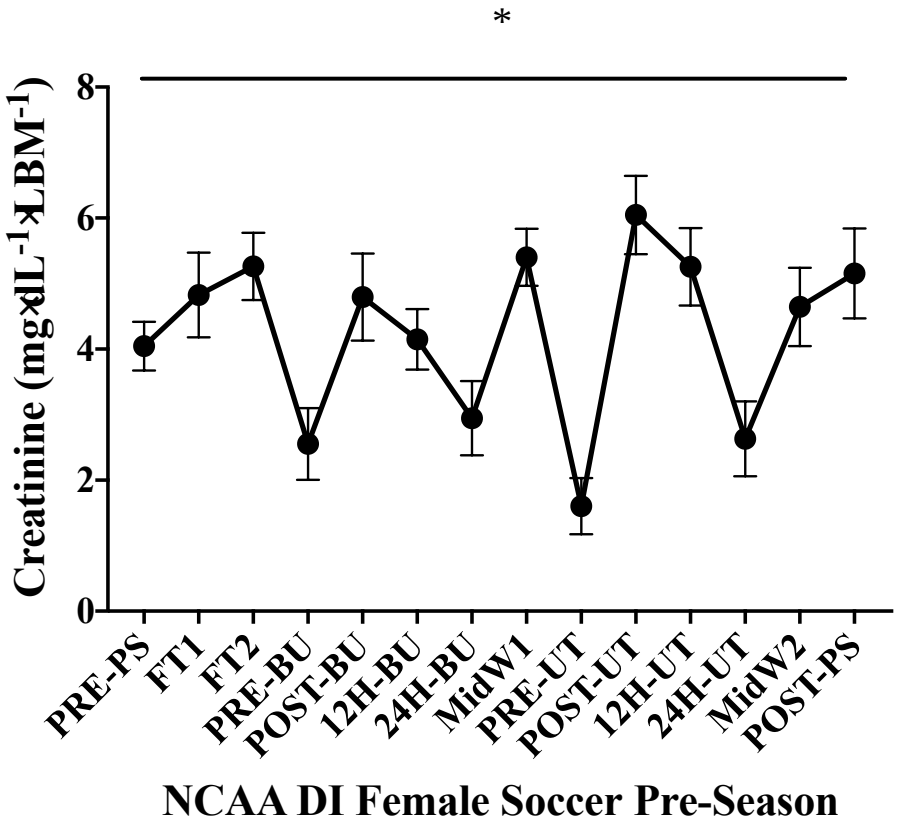

Figure 9: Creatinine ($mg \cdot dL^{-1} \cdot LBM^{-1}$) (Mean ± SEM; * denotes p = < .05) analysis showed an effect of time p = <0.0001; F (5.644, 61.65) = 6.797) with a difference found between days. Multiple comparisons showed PRE-BU (2.553 ± 0.5479; 95% CI: 1.347 to 3.759) and PRE-UT (1.604 ± 0.42; 95% CI: 0.6594 to 2.548) showed to be lower than multiple days. No difference was found between Pre-BU and PRE-UT. Additionally, differences were found between 24H-BU vs. POST-UT (p = 0.0015: 95% CI: -5.040 to -1.164), MidW1 vs. 24H-UT (p = 0.0457 95% CI: 0.03967 to 5.501), and 12H-UT vs. 24H-UT (p = 0.0305; 95% CI: 0.1954 to 5.055).

Using a One-way RMANOVA, concentrations of uCr during Exhibition Game 1 (BU) were analyzed (see Figure 10.). Over the time course of analysis of Exhibition Game 1 (PRE-BU, POST-BU, 12H-BU, & 24H-BU), a significant difference was found between urinary concentrations of creatinine from PRE-BU to 24H-BU recovery sample (p= 0.0195). Although close to significance levels, further analysis indicates that there are no significant differences in uCr concentration increases from PRE-BU to POST-BU (p= 0.05).

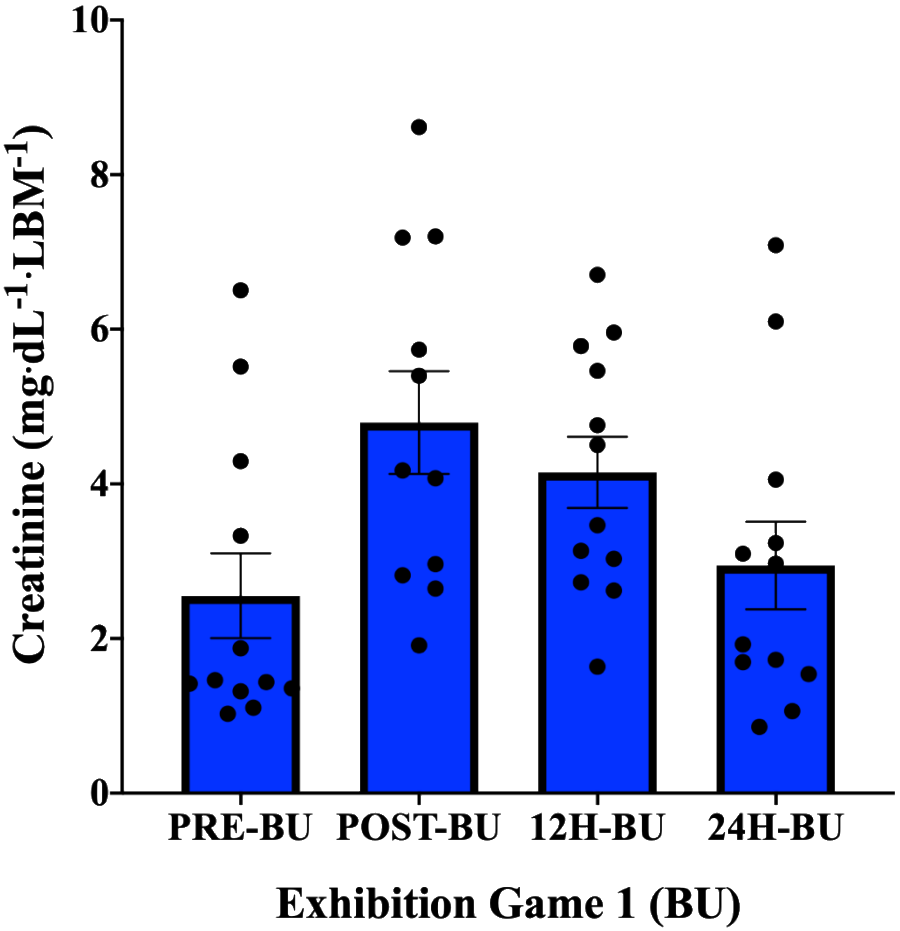

Figure 10: Creatinine ($mg \cdot dL^{-1} \cdot LBM^{-1}$) (Mean ± SEM; * denotes p = < .05) the time course analysis of Exhibition Game 1 (PRE-BU to 24H-BU) showed a difference between the concentration of Creatinine (p = 0.0195; F (1.868, 19.92) = 4.965). However, further multiple comparison analysis showed no differences between measures (PRE-BU vs. POST-BU p = 0.0527; -4.505 to 0.02411).

A paired sample t-test was utilized to assess pre-post-game differences in urinary creatinine concentrations (see Figure 11). When assessed independently from recovery samples (12H-BU & 24H-BU), a significant difference was found between PRE-BU and POST-BU (p = .01). Specifically, concentrations of uCr POST-BU (4.794 ± 0.66) are significantly higher than PRE-BU (2.662 ± 0.58).

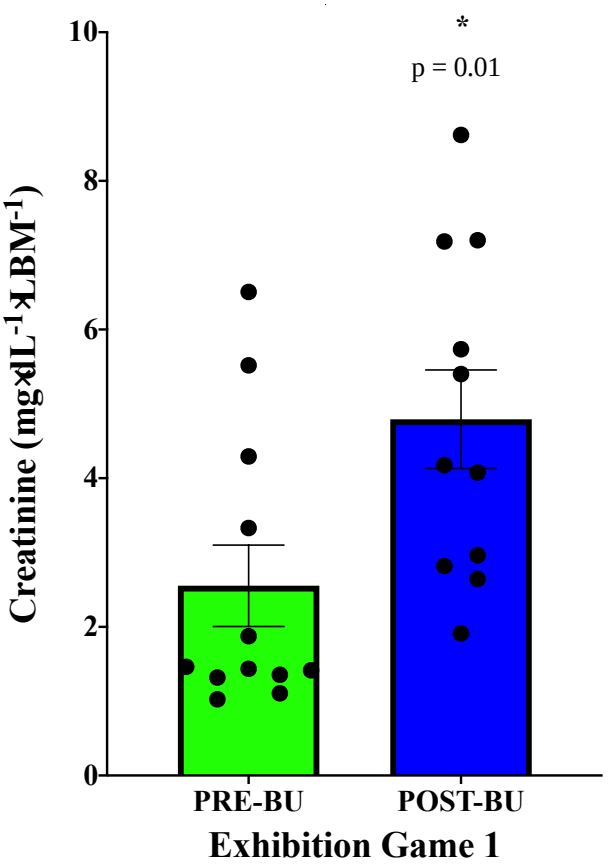

Figure 11: Creatinine ($mg \cdot dL^{-1} \cdot LBM^{-1}$) (Mean ± SEM; * denotes p = < .05) the analysis of Exhibition Game 1 showed a difference (p = .01; ω_2 = 0.4433) between PRE-BU (2.662 ± 0.58; 95% CI: 1.351 to 3.973) and POST-BU (4.794 ± 0.66; 95% CI: 3.315 to 6.273).

Like Exhibition Game 1 (BU), a One-way RMANOVA was utilized to depict differences in urinary concentrations of creatinine in Exhibition Game 2 (PRE-UT, POST-UT, 12H-UT, 24H-UT) (see Figure 12.). Unlike the first exhibition game (BU), several significant differences are present. Primarily, a significant difference is evident throughout the time course of Exhibition Game 2 (p= <0.0001). Secondly, there a significant difference between PRE-UT and POST-UT with post-game concentrations being significantly higher (p= <0.0001). Additionally, a significant difference is present between PRE-UT and the 12H recovery sample with 12H-UT being significantly higher (p=<0.0001). Lastly, there is a significant difference between 24H-UT and POST-UT (p=0.007), as well as 12H-UT (p=0.004) with 24H-UT expressing significantly lower values of uCr.

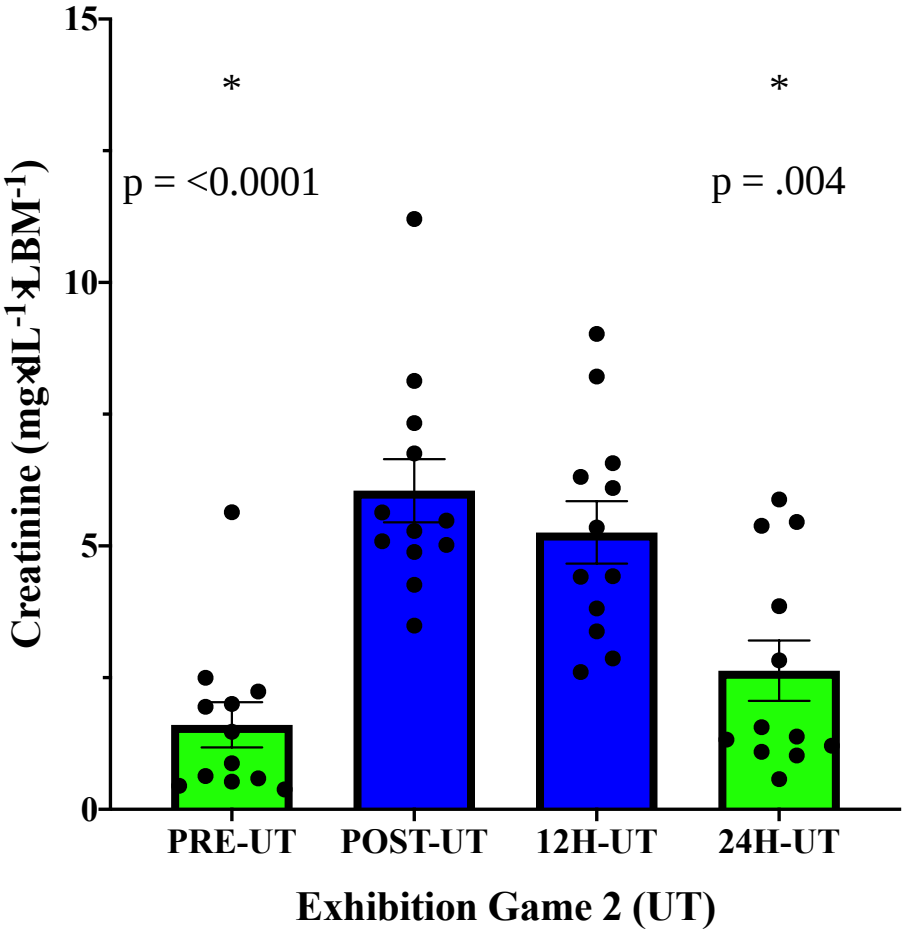

Figure 12: Creatinine ($mg \cdot dL^{-1} \cdot LBM^{-1}$) (Mean ± SEM; * denotes p = < .05) the time course analysis of Exhibition Game 2 (PRE-UT to 24H-UT) showed a difference between the concentration of Creatinine (p = <0.0001; F (2.081, 22.90) = 24.04). Subsequent multiple comparisons showed that differences between PRE-UT vs. POST-UT (p = <0.0001; 95% CI: -5.973 to -2.915) and 12H-UT (p = <0.0001; 95% CI: -4.929 to -2.376). Differences were also found between 24H-UT vs. POST-UT (p = 0.007; 0.9300 to 5.903) and 12H-UT (p = 0.004; 95% CI: 0.8728 to 4.378).

A paired sample t-test was utilized to assess pre-post-game differences in urinary creatinine concentrations (see Figure 13). When assessed independently from recovery samples (12H-UT & 24H-UT), a significant difference was found between PRE-UT and POST-UT (p = <0.0001). Specifically, concentrations of uCr POST-UT (6.04 ± 0.59) are significantly higher than PRE-UT (1.604 ± 0.42).

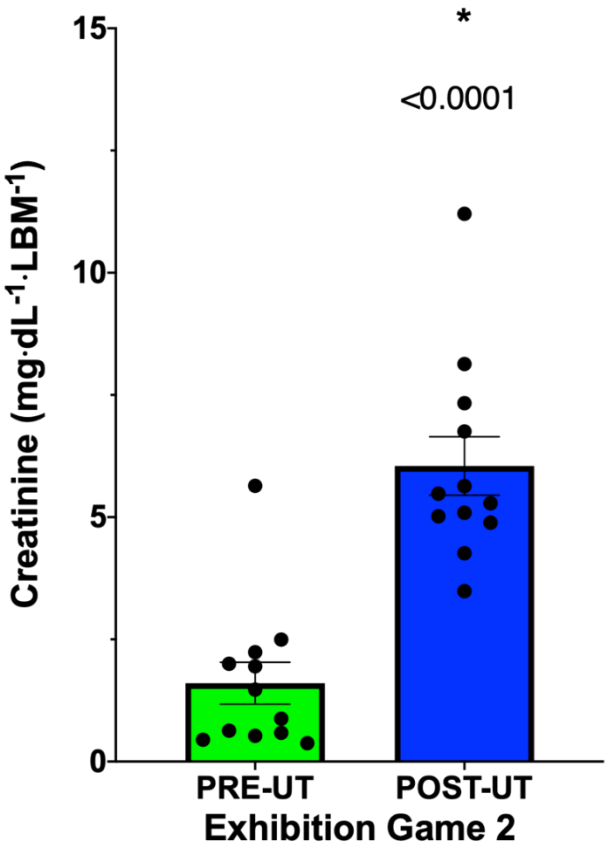

Figure 13: Creatinine ($mg \cdot dL^{-1} \cdot LBM^{-1}$) (Mean ± SEM; * denotes p = < .05) Exhibition Game 2 showed a difference (p = <0.0001) between PRE-UT measure of Creatinine (1.604 ± 0.42; 95% CI: 0.65 to 2.54) and POST-UT (6.04 ± 0.59; 95% CI: 4.73 to 7.36).

Table 2. Preseason uCreatinine (mg/dL)

uCreatinine	**(mg/dL)**		
Timepoint	Mean ± SEM	Min-Max	uCr ≥ Normative Values
PRE-PS	177.6 ± 16.10	142.2 - 213.0	*
FT1	229.9 ± 22.27	180.3 - 279.5	*
FT2	229.8 ± 20.61	184.4 - 275.2	*
PRE-BU	114.8 ± 26.87	55.70 - 174.0	
POST-BU	214.1 ± 31.90	143.0 - 285.2	*
12H-BU	183.0 ± 20.60	137.6 - 228.3	*
24H-BU	131.6 ± 26.26	73.75 - 189.4	
MidW1	235.4 ± 16.90	198.2 - 272.6	*
PRE-UT	72.96 ± 21.95	24.65 - 121.3	
POST-UT	266.3 ± 26.59	207.8 - 324.9	**
12H-UT	234.3 ± 29.49	169.4 - 299.2	*
24H-UT	115.8 ± 25.87	58.87 - 172.7	
MidW2	202.2 ± 24.29	148.7 - 255.6	*
POST-PS	231.3 ± 33.90	70.98 - 427.96	*

n= 9; * ≥ 50th % of recommended uCr concentration [132.8mg/dL];
** ≥ 90th % of recommended uCr concentration [246.6mg/dL]

Urinary Cystatin-C

A One-way RMANOVA was utilized to assess differences in urinary Cystatin- C (uCys-C) throughout the preseason training protocol (see Figure 14**).** Primarily, a significant difference was found between the time course of preseason and uCys-C concentrations (p= <0.0001). There are evident and significant increases in uCys-C concentrations at 12H-BU, 24H-BU, MidW1, PRE-UT, POST-UT, and 12H-UT (p= ≤ 0.0001). Specifically, those increases are significantly different from the initial 4 urine collections, PRE-PS (p=.0305), FT1 (p= 0.026), FT2 (p= 0.041), and PRE-BU (p= 0.024). Secondly, a significant difference (p= 0.0313) was found between MidW1 (0.448 ± 0.02) and POST-BU (0.217 ± 0.09). Additionally, POST-BU presented significantly lower (p= 0.001) concentrations of uCys-C in comparison to POST-UT (0.509 ± 0.08). Lastly, in comparison to elevated timepoints 12H-BU, 24H-BU, MidW1, PRE-UT, and

POST-UT, significant differences via lower concentrations of uCys-C are evident at 12H-UT (0.267 ± 0.12), 24H-UT (0.046 ± 0.02), MidW2 (0.162 ± 0.042), and POST-PS (0.142 ± 0.037).

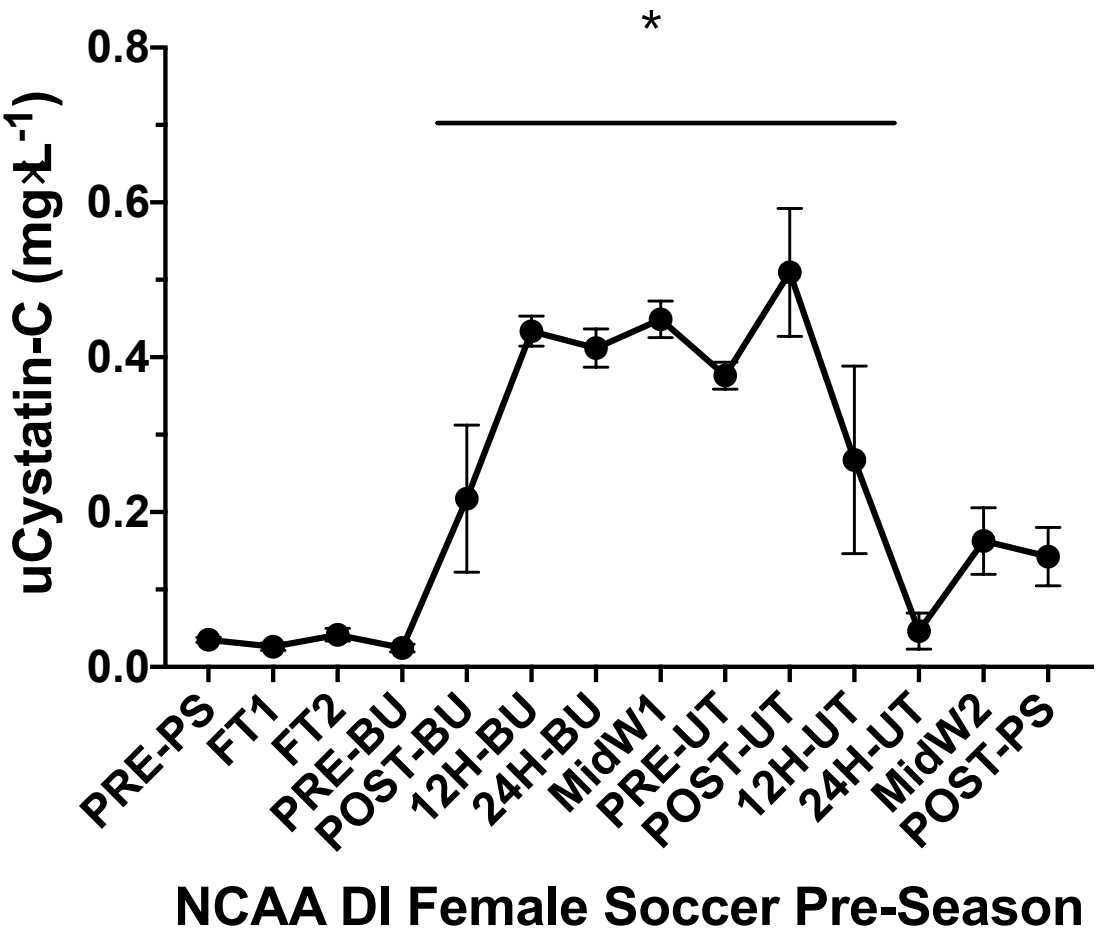

Figure 14: Cystatin-C (uCys-C) (mg·L^{-1}) (Mean ± SEM; * denotes p = < .05) the analysis of uCys-C showed an effect of time (p = <0.0001; F (13, 84) = 18.98) and difference found between days p = <0.0001; F (2.464, 14.4 = 19.71). Multiple comparison analysis showed multiple day differences in elevated uCys-C concentration at 12H-BU, 24H-BU, MidW1, PRE-UT, POST-UT, 12H-UT (p = ≤ 0.0001) compared to Pre-PS (.0305 ± 0.003; 95% CI: 0.02 to 0.04), 12H-BU, 24H-BU, MidW1, PRE-UT, POST-UT, 12H-UT (p = < 0.0001 to .002) compared to FT1 (0.026 ± 0.004; 95% CI: 0.015 to 0.037), 12H-BU, 24H-BU, MidW1, PRE-UT, POST-UT (p = <0.0001) compared to FT2 (0.041 ± 0.008; 95% CI: 0.022 to 0.06), 12H-BU, 24H-BU, MidW1, PRE-UT, POST-UT, 12H-UT (p = < 0.0001 to 0.01) compared Pre-BU (0.024 ± 0.004; 95% CI: 0.013 to 0.035); a difference was found (p = 0.0313) between MidW1 (0.448 ± 0.02; 95% CI: 0.394 to 0.503) vs POST-BU (0.217 ± 0.09; 95% CI: -0.046 to 0.481); a difference was found (p = 0.001) between POST-UT (0.509 ± 0.08; 95% CI: 0.314 to 0.704) vs. POST-BU; differences were found (p = <0.0001 to .02) 12H-UT (0.267 ± 0.12; 95% CI: -0.068 to 0.6036) , 24H-UT (0.046 ± 0.02; 95% CI: -0.25 to 0.34), MidW2 (0.162 ± 0.042; 95% CI: 0.061 to 0.264), and

POST-PS (0.142 ± 0.037; 95% CI: 0.022 to 0.262) showed lower concentrations of uCys-C than 12H-BU, 24H-BU, MidW1, PRE-UT, and POST-UT.

In *Table 3*, a post hoc analysis was performed with Tukey for multiple comparison tests and a Dunnett for comparing baseline (PRE-PS) values to the remaining preseason timepoints. Asterisks following mean averages indicate significant differences from baseline values. It is evident that PRE-BU and PRE-UT USG and UCA scores are lower than baseline values. Additionally, PRE-UT uCr concentrations are significantly lower than baseline values. Lastly, elevations in uCys-C at 12H-BU, 24H-BU, MidW1, PRE-UT, and POST-UT are significantly different from baseline values.

Table 3. Preseason Urinary Markers in Relation to Baseline Values

	Time Course of NCAA DI Female Soccer Preseason			Urinary Markers			
			Heat Index (°C)	UCA	USG	uCr ($mg \cdot dL^{-1} LBM^{-1}$)	uCys-C ($mg \cdot L^{-1}$)
1	PRE-PS	Pre-Preseason (Baseline)		4.63 ± 0.41	1.02 ± 0.002	4.04 ± 0.37	0.035 ± 0.003
2	FT1	Fitness Testing Day 1	38.32	5.45 ± 0.26	1.022 ± 0.001	4.82 ± 0.65	0.026 ± 0.005
3	FT2	Fitness Testing Day 2	35.35	5.14 ± 0.29	1.022 ± 0.001	5.26 ± 0.51	0.041 ± 0.008
4	PRE-BU	Pre-BU Exhibition Game 1	47.98	2.5 ± 0.44*	1.012 ± 0.002*	2.55 ± 0.55	0.024 ± 0.005
5	POST-BU	Post-BU Exhibition Game 1		5.19 ± 0.34	1.018 ± 0.002	4.79 ± 0.66	0.21 ± 0.095
6	12H-BU	12 h Post-BU Exhibition Game 1	38.03	5.23 ± 0.28	1.021 ± 0.002	4.15 ± 0.46	0.43 ± 0.019*
7	24H-BU	24 h Post-BU Exhibition Game 1	39.08	4.20 ± 0.38	1.017 ± 0.002	2.94 ± 0.57	0.41 ± 0.025*
8	MidW1	Regular Practice Day 1	36.05	4.78 ± 0.30	1.025 ± 0.001	5.40 ± 0.44	0.44 ± 0.024*
9	PRE-UT	Pre-UT Exhibition Game 2	36.61	2.11 ± 0.33*	1.009 ± 0.002*	1.60 ± 0.43*	0.37 ± 0.017*
10	POST-UT	Post-UT Exhibition Game 2		5.05 ± 0.23	1.021 ± 0.002	6.04 ± 0.60	0.50 ± 0.083*
11	12H-UT	12 h Post-UTSA Exhibition Game 2		5.22 ± 0.31	1.023 ± 0.002	5.25 ± 0.59	0.26 ± 0.121
12	24H-UT	24 h Post-UTSA Exhibition Game 2	37.73	3.56 ± 0.41	1.015 ± 0.002	2.63 ± 0.57	0.046 ± 0.023
13	MidW2	Regular Practice Day 2	31.94	4.57 ± 0.22	1.018 ± 0.001	4.64 ± 0.60	0.16 ± 0.043
14	POST-PS	Post-Preseason (End)		4.18 ± 0.36	1.02 ± 0.002	5.15 ± 0.69	0.14 ± 0.038

(Mean ± SEM; * denotes p = < .05 compared to Pre-Preseason (PRE-PS)) Urinary Color Analysis (UCA); Urine Specific Gravity (USG); Creatinine (uCr); Cystatin-C (uCys-C).

CHAPTER V.

Discussion

The purpose of our study was to investigate the prevalence of hypohydration and renal dysfunction in NCAA D1 female soccer players exposed to elevated temperatures, humidity, and heightened physical activity during a preseason training protocol. Our data indicates a high occurrence of hypohydration amongst female collegiate soccer players in South Texas. Most athletes arrived at morning training sessions in a hypohydrated state prior to any physical exertion in a warm environment. Secondly, we found abnormal elevations in urinary renal function and injury biomarkers, urinary creatinine and cystatin-C throughout the training protocol.

Hydration Status

Urine Color Analysis

Although a subjective tool, urine color analysis is commonly utilized to assess hydration status (McKenzie, Munoz, & Armstrong, 2015). Notably, UCA was added to help define the potential usage of this noninvasive technique when assessing athlete's hydration status. Previous literature has reported correlations (r = 0.60-0.80) between urine specific gravity and UCA as valid measures of identifying hypohydration (Olzinski et al., 2019). On the urine color scale, a rating of ≥ 4.5-5 has commonly defined hypohydration and has further indicated a ≥ 2% body mass loss (88.9% sensitivity) (McKenzie, Munoz, & Armstrong, 2015). Our data indicates that 9 out of 14 (64.3%) urine samples collected, would be defined as hypohydrated (see Figure 5.). Euhydration (≤ 3) was evident prior to both exhibition games (PRE-BU and PRE-UT) and 24-hours following the second exhibition match (24H-UT). However, such results are expected as urine color as well as other hydration markers have shown to increase following exercise.

Webb, Salandy, and Beckford (2016) assessed urine color in varied sports amongst university athletes and found that soccer players were 14% more hypohydrated in comparison to

other athletes (i.e. basketball, netball, cricket, swimming, track & field, and volleyball). Notably, 39% of athletes arrived to training in a hypohydrated state with that percentage further increasing to 75% following training. Hence, the increase in urinary color following exercise may be interpreted as having a decreased hydration status. This supports our findings as urine color significantly increased ($p = < 0.0001$) following both exhibition games. Our results also indicate that athletes arrived in a definable hypohydrated state prior to morning trainings. Further, this may also infer that athletes are unable to recover following evening trainings in time for the next morning practice(s). Similarly, Arnaoutis et al., (2015) assessed a urine color in 59 athletes participating in different sports. Pre-practice hydration evaluation indicated that 23.7% of athletes arrived at morning practices in a hypohydrated state. Following their respective trainings, 57 out of 59 (96.6%) athletes were definably hypohydrated and was attributed to outdoor temperature and humidity (27.6 ± 0.9 °C; 58 ± 8%). This further supports our findings of increased urine color following exhibition games BU and UT. Notably, pre-session hypohydration is also evident in the mentioned studies and support our findings at FT1, FT2, MidW1, and MidW2. Consequently, it has been documented that first morning urine samples may be more susceptible to heightened USG scores due to increased urinary concentrations overnight (Arnaoutis et al., 2015). However, our athletes participated in multiple training sessions per day with evening practices ending at ~2100 and following morning practice(s) beginning at 0700. Although we did not assess urine prior to evening practices, a lack of recovery is evident following the evening exhibition games as athletes were unable to recover to a euhydrated state by 12-hours post both games. This may further indicate that athletes are unable to recover to a euhydrated state following evening practices in time for the next morning practices.

Urine Specific Gravity

Like UCA, USG has also been attributed to a ≥ 2% body mass loss with cutoff ranges for hypohydration being 1.020 and 1.025 (McKenzie, Munoz, & Armstrong, 2015). Our data indicates that 7 out of 14 (50%) urine samples collected indicate hypohydration (≥ 1.020). USG scores indicate that athletes arrived at exhibition games in a euhydrated state with hypohydration occurring 12-hours post the BU game, immediately post-game (UT), and 12-hours post UT. Alarmingly, athletes only reported to one preseason practice in a euhydrated state (MidW2). However, increases in USG following practices is a common occurrence amongst athletes. Arnaoutis et al., (2015) also assessed USG and found that 89.8% (53 out of 59) of athletes were hypohydrated following their respective trainings. Notably, this hypohydrated state was evident despite fluid availability in training facilities during practices. Olzinski et al., (2019) assessed the difference between indoor and outdoor training on hydration status. Authors found that when exercising indoors, 49% of athletes were hypohydrated with that percentage increasing to 58% when the same group exercised outdoors. However, temperatures indoors (19- 25 °C) were higher than those recorded outdoors (17-23 °C). The increase in the prevalence of hypohydration was related to a reduced fluid intake when training outdoors in comparison to indoors. The authors attribute the higher fluid intake to the elevated indoor temperatures and the potential increase in circulating vasopressin, although not directly assessed. It is known that potential increases in serum osmolality and sodium (Na^+) concentrations stimulate the secretion of the antidiuretic hormone arginine vasopressin (AVP) in attempt to increase water reabsorption and maintain homeostasis through the sensation of thirst (Verbalis, 2007). However, our study did not assess circulating vasopressin and its role is solely speculated.

In comparison, our athletes were exposed to an average temperature of 30.3°C (HI: 37.4± 0.8 °C) which is remarkably higher than values reported in the mentioned studies. Our athletes

also had multiple training sessions in a day for consecutive days. Our data indicates that athletes may not be able to properly recover to a euhydrated state after evening practices and/or before the next morning practice(s). When comparing the exhibition games (BU and UT), although athletes arrived to the second exhibition match (UT) in a lower euhydrated state (1.010 ± 0.001) than the first exhibition match (1.012 ± 0.001), hypohydration was present following the UT game (1.021 ± 0.001) and not the BU game. Interestingly, athletes were exposed to 36.7 °C (HI: 47.9 °C) during the BU game and 30.2 °C (HI:36.6 °C) during the UT game. This may support an inferred role of circulating vasopressin as euhydration was maintained following the BU game (Olzinski et al. 2019). However, both games presented hypohydration during the 12-hour recovery samples collected. This aligns with our prediction that athletes are unable to recover following evening practices in time for the next morning practice(s) as USG scores remained hypohydrated 12-hours post evening exhibition games. Interestingly, the 24-hour UT sample recovered to a lower euhydrated state (~1.014) in comparison to BU (~1.017). This may infer that heat acclimation occurred and athletes were able to recover to a better state of euhydration following the second exhibition match. It is known that repeated heat exposure stimulates profuse sweating and induces important molecular adaptations that stimulate physiological heat acclimation. According to Pryor and colleagues (2018), trained athletes adapt well to heat within 5–7 days with longer lasting adaptations occurring at 10-14 days. However, heat acclimation was not directly assessed and improvements in recovery following the second exhibition match are only speculated. Although an increased in sweat rate may explain hypohydration POST-UT regardless of PRE-UT having a lower USG than BU, this is merely an assumption.

Renal Function and Injury Biomarkers

Urinary Creatinine

Creatinine concentrations are largely influenced by skeletal muscle as creatinine is created via the usage of creatine phosphate stores in muscle (Baxmann et al., 2008; Da Ponte et al., 2018; Turgut et al., 2003). To control for individual skeletal muscle mass, we standardized our urinary concentrations by the athletes lean body mass ($mg \cdot dL^{-1} \cdot LBM^{-1}$). Like hydration status, uCr concentrations were lowest prior to initiation of both exhibition games. However, increases in uCr were evident immediately following both exhibitions games and reduced at 12-hours post and further at 24-hours post. When comparing both exhibitions games, increases in uCr are in-line with USG scores. Specifically, UT presented lower uCr concentrations (1.6± 1.5 $mg \cdot dL^{-1} \cdot LBM^{-1}$) pre-game than BU (2.55 ±1.9 $mg \cdot dL^{-1} \cdot LBM^{-1}$). However, post-UT uCr increased more (6.05 ± 2.1 $mg \cdot dL^{-1} \cdot LBM^{-1}$) in comparison to BU (4.8 ± 2.2 $mg \cdot dL^{-1} \cdot LBM^{-1}$) regardless of pre-game values being remarkably higher prior to BU. This aligns heavily with our USG results and may imply that hypohydration may further exacerbate uCr concentrations. Interestingly, and like USG, athletes' uCr decreased more following 24-hours UT (2.63 ± 1.9 $mg \cdot dL^{-1} \cdot LBM^{-1}$) in comparison to BU (2.9 ± 1.9 $mg \cdot dL^{-1} \cdot LBM^{-1}$). Fluctuations in response to exercise in uCr are commonly reported in literature, however. Bongers and colleagues (2018) assessed uCr following an acute and prolonged bout of cycling. Following an acute bout of exercise, uCr increased to 9.2 mmol/L (104.07 mg/dL) while uCr increased to 26.3 mmol/L (297.5 mg/dL). Similarly, Turgut et al., (2003) assessed uCr concentrations following 2 hours of exercise. In female athletes, uCr increased from 98.74 mg/dL to 201.55 mg/dL following exercise. Notably, male participants had significantly higher uCr levels than female participants. The differences can be attributed to males having a greater muscle mass than their counterparts. Thus, it is evident that skeletal muscle mass influences uCr concentration measures. Baxmann et al., (2008)

found that subjects who participated in moderate to intense exercise had significantly higher uCr concentrations than sedentary subjects. Additionally, the non-sedentary participants had a higher lean body mass, protein intake, and lower body mass index. Authors found significant relationships between lean body mass ($p = 0.001$) and uCr concentrations. Therefore, it may be assumed that increases in uCr reflect skeletal muscle injury and dehydration of creatine phosphate stores in response to strenuous physical activity rather than renal dysfunction (Baxmann et al., 2008; Da Ponte et al., 2018). This assumption aligns with the periodical increases throughout the preseason as uCr increases were evident following physical activity and decreased following granted rest periods.

Increases in uCr have also been associated with an increased glomerular filtration permeability and satiation of filtered proteins via proximal tubular reabsorption (Turgut et al., 2003). According to Lippi et al., strenuous physical activity independently has been associated acute renal impairment due to reductions of renal blood flow, increased protein excretion due to muscular damage (i.e. nucleotide release). Although exercise-induced injury was not directly evaluated in this study, several authors have suggested uCr as a potential indicator of muscle-injury (Junglee et al.,2012). Notably, a rather constant rate of creatinine is excreted in urine as 15-20% of uCr is actively secreted from blood through renal tubules in attempts to maintain normal serum creatinine levels (Barr et al., 2004). Thus, abnormal increases in uCr may indicate an increased utilization of creatine phosphate and/or glomerular permeability if renal injury is present. When utilizing unstandardized lean body mass concentrations, our study indicated abnormal levels of uCr following exhibition games, with POST-UT (266.3 ± 26.59 mg/dL) expressing values similar to individuals assessed with renal dysfunction (see Table 2.) (Barr et al., 2004). Therefore, it is likely that increases are in response to heightened dehydration of

creatine phosphate stores, muscle-induced injury and/or glomerular filtration permeability allowing increased concentrations of uCr (Baxmann et al., 2008; Pone et al., 2018; Junglee et al., 2012; Turgut et al., 2003).

Urinary Cystatin-C

Nejat and colleagues (2010) defined AKI and tubular dysfunction as a uCys-C concentration of ≥0.45 mg/L. Our data indicated that athletes remained in normal ranges (0.03-0.3 mg/L) of uCys-C in 7 out of 14 urine (50%) urine collections (Conti et al., 2006; Uchida & Gotoh, 2002). Consequently, this also indicates that 50% of urine samples collected were above normative values. Alarmingly, POST-UT values (0.5096 mg/L) indicate potential tubular injury and display concentrations previously utilized to diagnose AKI in clinical settings. Notably, to our knowledge this study is one of the first to study uCys-C throughout an extended period. Our data reveals that uCys-C concentrations did not significantly increase until after the first exhibition match (BU) and remained elevated beyond normative values until 24-hours following the second exhibition match (UT). Interestingly, uCys-C concentrations continued to increase following 12-hours post the first exhibition match (0.4336 mg/L) and were slightly reduced at 24-hours post (0.4117 mg/L) with pregame values within normal ranges (0.02432 mg/L). Collections of 12-hour and 24-hour samples were taken on granted off-days and practice resumed the following morning. It may be assumed that athletes were unable to recover after 24-hours post BU as uCys-C concentrations remained elevated at the MidW1 practice (0.4489 mg/L). Athletes were not granted an additional off day prior to the second exhibition match and uCys-C concentrations remained elevated (0.3763 mg/L) at PRE-UT. Remarkably, POST-UT values indicate the potential of renal injury with concentrations surpassing clinical diagnosis of AKI (0.5096 mg/L). Like hydration trends and uCr, uCys-C concentrations appear to recover at a

faster rate in comparison to the BU at 12-hours post UT (0.2674 mg/L) and 24-hours post UT (0.04622 mg/L).

Within the literature excessive heat and physical exertion has been linked to increased circulating vasopressin and further renal injury (Roncal-Jimenez et al., 2015; Schlader et al., 2019). When comparing exhibition games, the BU game had a heat index of 47.98 °C while the UT game was performed later in the evening and had a heat index of 36.61°C. Although solely speculated, increases in circulating vasopressin may explain not only the maintained euhydration state following BU, but also the exacerbated increases in uCys-C. Consequently, increases in vasopressin stimulates the aldose reductase-fructokinase pathway which is responsible for diminished ATP availability within renal tubules and has been associated with the Chronic Kidney Disease epidemic known as "Mesoamerican nephropathy" (Mansour et al., 2017; Roncal-Jimenez et al., 2015; Schlader et al., 2019). Melin et al., (1997) observed significant increases ($p < 0.05$) in arginine vasopressin when hypohydration was present following "thermal dehydration" in which a 3% reduction in body mass was achieved. However, vasopressin levels were reduced when hydration was restored. Porhomayon et al., (2015) found that when ICU patients were given vasopressin as treatment, a prevalence of 20% of patients developed AKI. Authors also stated that vasopressin was an independent factor that predicted the occurrence of AKI ($p = .02$). This may further support the impact of the potential increased vasopressin as uCys-C continued to increase 12-hours post the BU game. Interestingly, this may also indicate that athletes did not properly rehydrate following the BU game. This is a likely occurrence as athletes returned to their travel bus promptly after their game where they then traveled ~5 hours back to Corpus Christi. It is unknown whether athletes prioritized rehydration on their bus ride back to Corpus Christi. The following exhibition match (UT), presented elevated uCys-C

measures at beginning of the game. It may also be assumed that elevations were exacerbated as the kidneys were unable to recover appropriately from previous (potential) injury following the BU game.

Although evidence supporting uCys-C in athletic populations is lacking, Bongers et al., (2017) assessed the impact of acute versus repetitive walking-exercise on renal injury. Prior to initiating walking, uCys-C was lower (0.05 mg/L) in comparison to following an acute bout of walking (0.09 mg/L). Further, uCys-C was reduced following the 3rd consecutive day of walking (0.06 mg/L). However, uCys-C concentrations were within normal ranges and it was concluded that the exercise intensity did not influence tubular dysfunction. The following year, Bongers and colleagues (2018) assessed uCys-C following an acute (30 minutes) and prolonged (150 minutes) bout of exercise and/or when 3% hypohydration was achieved. Authors found that increases in uCys-C were present following both acute (0.03 mg/L) and prolonged exercise (0.15 mg/L). Thus, it is evident that uCys-C increased with prolonged exercise and an increased intensity in comparison to the previous study. However, it is rather unknown how repeated bouts of strenuous exercise may impact uCys-C concentrations, and further renal injury. Our findings may indicate that uCys-C remains elevated due to repeated bouts of strenuous physical activity in heightened temperatures.

Although uCys-C was not evaluated Divine et al., (2018) assessed an American football team during their respective preseason period. Authors found that 43.4% of athletes had a reduction of 31.6% in renal function as defined by their glomerular filtration rate (GFR). This is the first study to our knowledge that has assessed an athletic team during an extended period (10 days). Notably, samples were only collected at baseline, half-way, and on the 10th day. Thus, its s

evident that our study may showcase evident renal dysfunction in athletes who were assessed continually throughout their preseason. It is further evident that more information is needed to fully understand the behavior of uCys-C under these conditions.

Limitations

There are several limitations within our observational study. First, it has been documented that first morning urine samples may be more susceptible to heightened USG scores due to increased urinary concentrations overnight (Arnaoutis et al., 2015). Secondly, to limit our influence on the preseason training protocol, water intake was not controlled, and most subjects failed to report their personal intake via dietary recalls. Further, the impact of the environment (heat stress) on measures was not directly assessed. We did not control exercise stimuli, athlete's recovery habits, and/or the allotted recovery time granted to athletes. Thus, it may also be assumed that athlete's behavioral choices may have impacted hydration status rather than environmental factors. Urinary creatinine may reflect exercise-induced muscular injury rather than renal injury. Urinary samples were not immediately placed in cyrostorage or measured, which may have impacted degradation of markers. Failure to retrieve core temperature measurements impacts our analysis of results and the potential impact of core body temperature, and further, potential heat acclimation.

Conclusions

Our study demonstrates that healthy female collegiate soccer players were in a hypohydrated state 50% of the preseason training protocol. It is evident that athletes were unable to recover into a euhydrated state 12-hours post evening practices and exhibition games. Our uCr findings may also serve as an indicator of muscle-induced injury through the utilization of creatine phosphate stores or may indicate increased glomerular permeability of proteins. Most

importantly, our results indicate that uCys-C remained elevated for ~1 week following the first exhibition match as athletes were unable to recover to baseline values before initiating the next training session. Notably, after the UT game, athletes were given two consecutive off-days and uCys-C values returned to normative values. Thus, female soccer players in South Texas may be susceptible to the development of renal dysfunction and/or AKI when strenuous exercise is performed in hot environments without adequate rest. Although vasopressin was not directly assessed, it may be responsible for increases in uCys-C concentrations and BU responses following the first exhibition match. Lastly, it is also evident that the athlete's recovery of these markers (i.e. UCA, USG, uCr, and uCys-C) improved by the second exhibition match. Improvements in recovery may be associated with short-term heat acclimation as trained athletes have shown to adapt well to heat in 5-7 days in previous studies. Lastly, it may be possible that the rise in uCys-C may be related to the typical reduction in RBF due to exercise and hypohydration. The relationships between exercise intensity, heat stress, and hydrations status on uCys-C requirement further examination.

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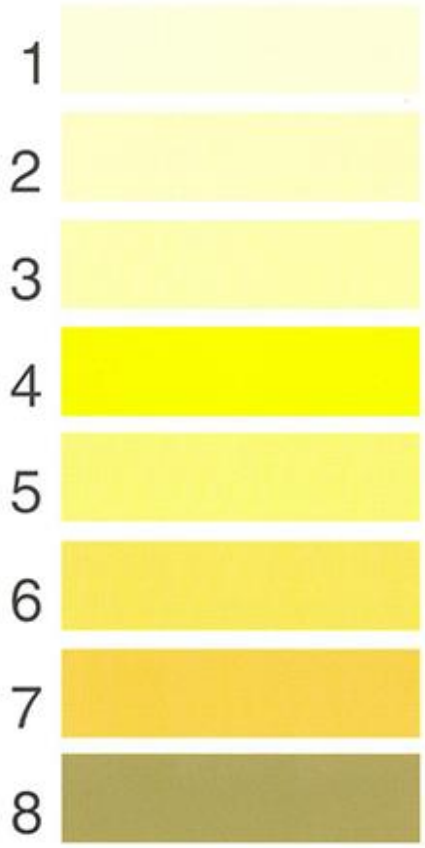
Appendices 1: Urine Color Chart



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).