

**CHANGES IN MARKERS OF HYDRATION THROUGHOUT THE COURSE OF A
COMPETITIVE SEASON AND SWEAT ELECTROLYTE COMPOSITION OF FEMALE
COLLEGIATE ATHLETES**

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ABSTRACT OF THE THESIS
CHANGES IN MARKERS OF HYDRATION THROUGHOUT THE COURSE OF A COMPETITIVE
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Hydration status has been shown to have effects on athletic performance. Maintaining proper levels of hydration is paramount for athletes. Recommendations regarding fluid and electrolyte replenishment are primarily based on sweat loss data obtained in males. The purpose of this study was to monitor hydration markers and plasma electrolytes over the course of the competitive season and evaluate whether there are chronic changes in plasma electrolytes as a function of “high” vs “low” electrolyte concentration sweat in high-level female athletes. **Methods:** Division I female athletes ($n = 42$, weight = 64.27 ± 6.69 kg, %BF = 24.09 ± 5.72 %, $VO_{2max} = 47.3 \pm 5.36$ ml/kg/min, VJ = 54.79 ± 7.35 cm, VT = 78.54 ± 3.75 % VO_{2max}) participated in blood draws every four weeks starting at preseason (T1) and continuing until 24 hours after the last game (T2, T3, T4, T5). During a practice session lasting approximately 2 hrs, regional sweat collections were made from the forearm, chest, and navel using sweat patches. Urine specific gravity (USG), Blood urea nitrogen and Creatinine ratio (BUN/Cr), blood glucose (GLU) were assessed as well as sweat and

plasma, sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), and magnesium (Mg^{2+}) concentrations were determined. Plasma osmolality (Posm) was calculated as $\text{Posm} = 1.90 [\text{Na}^+ + \text{K}^+] + \text{Glucose} + \text{BUN} + 5.0$. **RESULTS:** Soccer and field hockey were analyzed separately due to the amount of time points for hydration markers but were combined for sweat [electrolyte] analysis. There was a significant increase in Posm ($\Delta\text{Posm} = 4.0 \pm 1.3 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$) from T1 to T4 and from T4 to T5 ($\Delta\text{Posm} = 2.7 \pm 1.3 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$). USG significantly increased from $1.012 \pm .002$ at T1 to $1.018 \pm .002$ at T2 and continued to increase to $1.021 \pm .001$ at T3, where it plateaued and remained significant through T5 ($P < 0.05$) for soccer. Field hockey had a significant increase in Posm ($\Delta\text{Posm} = 2.3 \pm 0.95 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$) from T1 to T3 and from T3 to T4 ($\Delta\text{Posm} = 3.5 \pm 0.90 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$). USG significantly increased from $1.011 \pm .008$ at T1 to $1.016 \pm .007$ at T2 ($P < 0.05$) and peaked at $1.017 \pm .006$ at T3 and plateaued at T4. Plasma electrolytes changed throughout the season for both teams but remained within normal ranges. There were significant differences in sweat electrolyte content that occurred as a function of patch site ($p < .05$). The greatest $[\text{K}^+]$, $[\text{Ca}^{2+}]$, and $[\text{Mg}^{2+}]$ were found at the forearm ($[\text{K}^+] = 6.65 \pm 1.84 \text{ mM}$; $[\text{Ca}^{2+}] = 1.18 \pm 0.53 \text{ mg/dL}$; $[\text{Mg}^{2+}] = 0.36 \pm 0.19 \text{ mg/dL}$). The largest $[\text{Na}^+]$ and $[\text{Cl}^-]$ were produced at the navel ($67.02 \pm 20.25 \text{ mM}$ and $56.74 \pm 20.40 \text{ mM}$, respectively). [Electrolyte] across sites were summed to represent the total loss of each individual electrolyte. Individuals were classified as “high” or “low” concentration sweaters for each measure after calculating z-scores, using 0 as the split point. There were no differences between groups for any plasma [electrolyte] over the season ($p > .05$). **CONCLUSIONS:** The increases seen in

Posm and USG for soccer show that these athletes are in a chronic state of hypohydration. While field hockey saw similar changes, they did not meet a level of 1.020 for USG. Regional variations in sweat [electrolyte] were observed in female athletes. However acute sweat composition does not appear to effect plasma values across an athletic season. These results may suggest that these athletes adequately chronically replenish any electrolytes lost. The increases in Posm and USG may suggest that diet and fluid intake play important roles in hydration and replenishing electrolytes.

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Introduction

Proper nutrition and training are critical to help athletes perform their best. As part of proper nutrition, hydration is an often-overlooked factor to consider. Adequate hydration plays a key role in performance, as small decrements can impair performance and physiological function (Casa et al., 2010). In humans, sweat is the primary source of fluid loss during exercise. Sweat is a clear, hypotonic bio fluid produced by eccrine and procrine glands located in the epidermis, and it is composed of 99% water containing electrolytes, urea, pyruvate, and lactate (Mena-Bravo et al., 2014). It is important for high-level athletes to fully recover between training sessions to maintain performance. Although athletes might focus primarily on food after a training session, pre-, intra-, and post-exercise hydration contribute greatly to the exercise recovery paradigm.

Types of Dehydration

Euhydration defines a state of normal body water. Low total body water is known as hypohydration or dehydration (Sawka et al., 1990). Dehydration is quantified by weight loss from sweat and is referred to as a percent of body weight lost; for example, an athlete that lost 5% of body weight is 5% dehydrated. Hydration status is under homeostatic control of the hypothalamus (Sawka & Pandolf et al., 1990, Jequier et al., 2010). Antidiuretic hormone (ADH) is released from the posterior pituitary gland in response to increases in osmotic pressure of extracellular fluid (ECF) and signals the kidneys to reabsorb water to increase blood volume (Jequier et al., 2010). When this pathway is coupled with insufficient fluid intake, blood volume fails to rise, and renal

blood flow is decreased which increases the renin-angiotensin-aldosterone system activity as well as reduces glomerular filtration rate (Jequier et al., 2010). These steps lead to decreases in blood pressure. To re-establish homeostasis, the kidneys increase sodium reabsorption to bring blood pressure back to normal (Jequier et al., 2010).

There are three different types of dehydration: hypertonic, isotonic, and hypotonic. The most common form seen is hypertonic dehydration, which is characterized by blood hypernatremia (serum sodium >145 mmol/L) and hyperosmolality (serum osmolality >300 mmol/kg) (Weimberg et al., 1995), and is caused by heavy sweating. Isotonic dehydration is characterized by equal losses in both water and sodium, and is typically caused by vomiting and diarrhea. Isotonic dehydration is indicated by serum sodium of $135 - 145$ mEq/L, serum osmolality > 290 mOsm/L, and urine osmolality > 500 mOsm. This form of dehydration is often seen in aesthetic and weight class sports such as wrestling, gymnastics, and bodybuilding (Weimberg et al., 1995). The last type of dehydration is hypotonic dehydration, which is characterized by lower than normal serum sodium and osmolality, and is caused by low sodium intake and/or the use of diuretics (Weimberg et al., 1995).

Isotonic and hypotonic dehydration can be a concern for female athletes due to behavioral similarities in the causes of these types of dehydration and the female athlete triad. The triad consists of disordered eating, amenorrhea, and osteoporosis. Typically, the main cause that leads into the female athlete triad is low energy availability, meaning more calories are expended than consumed (Javed et al., 2013). This is a concern in weight class or aesthetic sports due to constant maintenance of a

certain weight or lean figure. Disordered eating is a common issue in female athletes and can be accompanied by disordered fluid intake (Bonci et al., 2008, Hart et al., 2005). Hart and colleagues reported a fluid intake range of 250-6925 mL/day in 81 patients with diagnosed eating disorders. In contrast, Armstrong and associates reported a fluid intake range of 2109-2506 mL/day in a sample of healthy females (Armstrong et al., 2012). Furthermore, it has been reported that female athletes have an average intake of 2954 mL on training days and 2245 mL on non-training days (Arabaci et al., 2015). With many variables that can affect fluid loss throughout the course of a day for athletes it is unknown if these values are enough to maintain euhydration.

Hydration and Climate

Warm and hot weather play a major role in hydration and thermoregulation. Heat exchange from the body to the environment occurs by conduction, convection, radiation, and evaporation (Howe et al., 2007). Conduction is direct transfer of heat by contact with a cooler object. Convection is the process of cooling by air passing over warmer skin. Radiation is the direct release of heat from the body into the environment (Howe et al., 2007). Evaporation is the most effective cooling mechanism, which is accomplished by vaporization of sweat into the air (Howe et al., 2007). Elevated air temperature causes the body to increase blood flow to the skin and shunt blood flow from central circulation due to increased core body temperature (Nybo et al., 2014, Howe et al., 2007). This causes an increase in sweat rate to aid in cooling the body (Maughan et al., 2004). Without fluid replacement equal to sweat loss, dehydration occurs and can be associated with injury or physiological dysregulation, including

fatigue, cramps, heat exhaustion, heat stroke, and death (Oppliger et al., 2002).

Dehydration-related illnesses are a major concern during summer months. Sports that are in preseason during the summer are at a higher risk due to typical inclusion of multiple training sessions in the same day. Athletes need to make sure they are drinking enough fluids to stay hydrated, in addition to consuming sufficient nutrients in order to replenish muscle glycogen stores (Shephard et al., 1987).

Water loss during exercise is excreted as sweat and moisture in breath, with the majority coming from sweat. Environment can affect sweat rate, sweat volume, and core body temperature. For example, in hot or humid environments, the body is put under immense physiological strain to maintain high intensity exercise resulting in high core body temperature and decrease performance (Tippet et al., 2011). High intensity exercise and high core body temperature increase the strain on many systems of the body, including the cardiovascular system, central nervous system, musculoskeletal system, respiratory system (Sawka et al., 2015).

Hydration and Performance

Dehydration of 2% body mass or more has been shown to increase physiological strain and decrease performance in a laboratory environment (Montain et al., 1992, Montain et al., 1998, Sawka et al., 1985, Buono et al., 2000). The reduction in plasma volume results in an increased heart rate to compensate for decreased stroke volume (i.e. cardiovascular drift) (Montain et al., 1992, Turkevich et al., 1988). Hyperthermia and dehydration are usually seen together and add physiological stress in the form of reduced cardiac output and blood pressure (Gonzalez-Alonso et al., 1997). Other factors

seen with dehydration include decreased work-load capacity, hyperosmolality, hypovolemia, increased rating of perceived exertion (RPE), increased stress response, and challenges to thermoregulation (Sawka et al., 1992, Casa et al., 2010). Severe dehydration and hyperthermia can also alter cognitive performance, which is important for decision-making during a sporting event (Shirrieffs et al., 2004). Cognitive performance can start to be altered at as little as 1.3% dehydrated (Lieberman et al., 2007).

Upper and lower body anaerobic power is reduced when dehydrated by 2.9% (Jones et al., 2008). One proposed mechanism for this decrease in anaerobic power is the loss of intracellular potassium, thus resulting in hyperpolarization of the muscle cell membrane, which can then cause a decrease in muscle contractibility by inhibiting calcium binding to troponin or interfere with cross-bridge formation (Sjogaard et al., 1982). Another proposed mechanism is that anaerobic metabolism and lactate flow from the muscle to the blood is impaired by dehydration (Armstrong et al., 1985).

In a field-based setting, Casa et al. looked at various conditions of trail running in the heat (Casa et al., 2010). During submaximal running when finishing time was held constant, there was a 2.6% difference in body mass loss, core body temperature was higher by .5°C, and average heart rate was 15 beats/min higher when starting in a dehydrated state compared to a euhydrated state (Casa et al., 2010). Subjects also had longer recovery times for these markers (Casa et al., 2010). Comparable results were seen for heart rate and core body temperature with maximal running as well as shorter time to finish for the hydrated group vs the dehydrated group (Casa et al., 2010). When

submaximal running was performed in a hydrated or dehydrated state with matched intensity (heart rate), subjects saw greater body mass loss, higher gastrointestinal temperature up to 30-minutes post, and slower lap times than the hydrated group (Lopez et al., 2011). Dehydration has also been found to affect the pacing ability of runners (Stearns et al., 2009).

In addition to dehydration concerns in more “pure” endurance sports, power-endurance sports are also impacted. Duffield and associates saw greater rise in core body temperature and change in hydration status with high intensity training and match play than low intensity soccer training (Duffield et al., 2012). A five percent decrease in soccer skill was shown after the Loughborough intermittent shuttle test (LIST) with no access to water; when players were allowed access during the same protocol there was no skill decrement (Mcgregor et al., 1999). Adolescent female soccer players saw no change in soccer skill during the Loughborough Soccer Passing Test (LSPT) when performed during the rest period of the LIST in a hydrated or dehydrated state but did see a significant increase in intestinal temperature, heart rate, blood lactate concentration, and RPE in the dehydrated state (Ali et al., 2009). The conflicting results in soccer skill performance may be attributed to the different tests used and the number of times the test was performed. As shown by Montain et al. (1992) and Gonzalez-Alonso et al. (1997), hydration status affects internal stress causing a greater internal load. While these effects didn’t show a decrease in soccer skill (Ali et al.), the greater internal load may not affect that specific game, but it could conceivably lead to increased recovery time for the next competition or bout. It is possible that decreases in

skill would be seen with a longer duration test or effort (as may be seen in overtime periods), or if it was repeated a day or two later to further simulate a congested match fixture schedule like that typically seen in college soccer.

Current Guidelines

Hydration guidelines established by the National Athletic Trainers' Association (NATA) recommend pre-exercise fluid intake should be 500-600 mL of water or sports drink 2-3 hours prior and 200-300 mL 10-20 minutes before the start of exercise (Casa et al., 2000). Fluid replacement during exercise should be focused on matching of sweat loss or maintenance of less than 2% body weight loss, which is roughly 200-300 mL every 10-20 minutes (Casa et al., 2000). After exercise, fluid intake must make body weight match pre-exercise body weight plus an additional amount to match urine loss during rehydration (Casa et al., 2000). During longer exercise sessions (greater than 4 hours) or in hot weather, 0.3-0.7 g/L of salt can be added to replace sweat sodium loss, which lowers the risk of hyponatremia, as well as stimulates the thirst mechanism (Casa et al., 2000). Recently NATA released a position stand on Fluid Replacement for the Physically Active (McDermott et al., 2017). The current position stand highlights on education of hydration and fluid-maintenance practices. With much of the literature reporting wide ranges of fluid replacement and fluid needs, recommendations on how much fluid have been replaced with proper education of individual fluid needs, sweat responses, hydration monitoring, and fluid replacement (McDermott et al., 2017).

The American College of Sports Medicine (ACSM) position stand on exercise and fluid replacement (2007) recommends slowly drinking beverages at least 4 hours before

exercise, aiming to drink between 5-7 mL/kg (Sawka et al., 2007). Then, depending on urine output and color, another 3-5 mL/kg 2 hours prior if urine is dark or there is no output (Sawka et al., 2007). During exercise, ACSM recommends individualized programs to prevent greater than 2% dehydration (Sawka et al., 2007). For post-exercise rehydration, normal meal and fluids will be ok if there are no time restraints for recovery. If quicker rehydration is needed, then drinking approximately 1.5 L of fluid for each kilogram of weight lost during exercise is needed (Sawka et al., 2007). Fluids or snacks should contain sodium to aid in thirst stimulation and fluid retention (Sawka et al., 2007). The main hydration goals for exercise are to start in a euhydrated state, consume enough fluids during to minimize dehydration, replenish sufficiently after exercise has completed, and include fluids containing electrolytes to replenish what is lost in sweat.

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Hydration Markers and Monitoring Methods

Hydration can be monitored and assessed in a variety of ways, with methods ranging from somewhat invasive (blood draws) to non-invasive (body weight). Hydration has no single marker but has a variety of ranges for different markers that indicate proper hydration (Armstrong et al., 2013). Many researchers have accepted that measurement of total body water and plasma osmolality used in conjunction with each other offer the “gold standard” assessment (Armstrong et al., 2013). However, this is a

laboratory test where many conditions are controlled. Since total body water is fluctuating throughout the day, using isotope dilution which requires three to five hours to equilibrate, makes it unrealistic to use during activity (Armstrong et al., 2013). The methods mentioned above are great lab based assessments. However, due to the control needed for these measurements, they are difficult to use in the field where the actual activity is performed, limiting “real-world” application.

Plasma osmolality also has limitations when used alone to measure hydration status because it does not change rapidly with fluid intake, as the body has strong neuroendocrine control to keep plasma osmolality in normal limits (Armstrong et al., 2013). When plasma osmolality is altered, there are two mechanisms that are stimulated to control homeostasis. Thirst is stimulated by cellular dehydration when osmolality of extracellular fluid is increased, and the stimulus increases linearly in proportion to increases of plasma osmolality above the threshold of 1-2% (Fink et al., 2012). The primary function of arginine vasopressin (AVP) secretion from the posterior pituitary is to increase solute-free water reabsorption of the kidneys. Secretion of AVP is in response to hyperosmolality which is caused by low total body water (Fink et al., 2012). Due to the complexity of the body’s control of fluid regulation, a variety of markers may be the best approach to determine hydration status (Armstrong et al., 2010).

Total Body Water

Total Body Water (TBW) is the fluid that occupies intracellular and extracellular spaces. Extracellular volume is all fluid outside of cells that includes interstitial fluid and

plasma water. Intracellular volume is the fluid within cell tissues (Armstrong et al., 1998, Maw et al., 1996). TBW is measured by stable isotope dilution and neutron activation analysis (Maw et al., 1996, Yasumura et al., 1983). These types of measurements require great levels of control over factors such as exercise, diet, and environment. TBW needs to be stable and in equilibrium to be measured by these methods (Armstrong et al., 2013). TBW can also be determined by bioelectrical impedance analysis (BIA) and bioelectrical impedance spectroscopy (BIS) (Oppliger et al., 2002). BIA and BIS have been shown to have r^2 values of .82 and .96 for females when compared to deuterium dilution (Matias et al., 2016). While these are both noninvasive methods and offer quick results, BIS would be the preferred method when dilution techniques are unavailable. Equipment quality determines accuracy of BIA and BIS, and cost of quality equipment may deter some from use of these types of measures.

Plasma Osmolality

Plasma osmolality (Posm) is the measure of solute concentration, defined as the number of osmotically active particles (osmoles) per kilogram of water (NLM), and is the most accurate measure of intracellular dehydration (Adolph et al., 1947). Posm is measured indirectly by osmometric methods or quantitative measurement of sodium using ion selective electrodes (Albrink et al., 1955, Nguyen et al., 2007). Posm can also be calculated using formulas made of common osmoles (Rasouli et al., 2005). In response to a TBW loss of 2% or greater, Posm increases by more than 5 mmol/kg (Cheuvront et al., 2010, Cheuvront et al., 2011). Increasing Posm results in more viscous blood which contributes to cardiovascular drift. Some limitations to Posm use for

dehydration are the ability of other osmoles to increase Posm. High intensity exercise raises lactic acid in the blood, which causes an acute increase in Posm that takes 20-30 minutes to recover (Greenleaf et al., 1979).

Blood Urea Nitrogen and Creatinine

Blood urea nitrogen (BUN) and serum creatinine (Cr) are typically used to determine renal function (Akimoto et al., 2011). When combined into a ratio (BUN:Cr), this has been used as an indicator of hydration status (Akimoto et al., 2011, Lin et al., 2014).

Athletes are susceptible to higher values of circulating Cr due to intense training (Banfi et al., 2008) as Cr is a product of the reaction between creatine and phosphocreatine and is a marker of muscle metabolism (Banfi et al., 2008). BUN has also been seen to increase with intense training but recovers and usually still falls within normal range (Yun et al., 2007). The BUN:Cr by itself may not be a useful marker for measuring dehydration in athletes due to high training intensity and high protein intake and it is recommended to be used cautiously (Robinson et al., 2004).

Urine Specific Gravity

Urine Specific gravity (USG) is the density of urine compared to the density of water and is reliant on the osmolality of the urine (Oppliger et al., 2002). USG can be measured by three different methods in the field; hygrometry, refractometry, and reagent strips. The preferred methods are refractometry, the passing of a beam of light through a urine sample and measuring how much the beam is refracted, and reagent strips, which work based on the release of hydrogen ions that cause a pH change which causes the strip to change colors depending on specific gravity (Brunzel et al., 1994). Values of USG that

indicated euhydration are often debated, with ACSM and NATA following stricter ranges of less than or equal to 1.020 for euhydration (Sawka et al., 2007, Casa et al., 2015).

This value is also supported by Opplinger et. al who related USG greater than 1.020 with significant dehydration-related weight loss (2005). Armstrong and colleagues defined more liberal ranges as USG less than 1.013 as well hydrated, 1.013-1.029 as euhydrated, and greater than 1.029 as hypohydrated (1994). The NCAA defines hydration as USG being less than 1.020 (NCAA 2003).

Urine Osmolality

Urine Osmolality (Uosm) has also been a common hydration marker to analyze. Uosm can be measured by a freezing point osmometer or by a handheld conductance meter (Shirreffs et al., 1998). Urine sodium, chloride, potassium, and urea molecules influence Uosm and can range from 100-1200 mOsmol/kg (Shirreffs et al., 1998, Kavouras et al., 2016). Current standards for hydration status using this method are: Uosm > 1052 mOsmol/kg = dehydrated, 442-1052 mOsmol/kg = euhydrated, and <442 mOsmol/kg = well hydrated (Armstrong et al., 1994).

Urine Color

Urine color (Ucol) is the easiest and least invasive observation that can be done to monitor hydration status. Although Ucol can be affected by foods, vitamin supplements, medications, illness, and exercise, it can still be used to a certain extent to monitor hydration status (L. Graff. et al., 1983, Modern Urine Chemistry et al., 1986, Ross et al., 1983). Armstrong et al. (1994) created an eight-color scale to classify urine based off correlations with Usg and Uosm ($r = +.80$ and $r = +.82$, respectively). They concluded that

well-hydrated individuals had a Ucol < 3, euhydrated 3-7 and dehydrated >7 (Armstrong et al., 1994).

Body Weight

Monitoring body weight changes is another simple technique to test hydration. When in caloric balance or on an acute basis, changes in body weight can be assumed to be due to loss of body fluid (Oppliger et al., 2002). Pre-and post-exercise body weight changes determine how much fluid needs to be replenished to achieve euhydration. Body weight monitoring can be useful for quickly assessing dehydration in team sports (Oppliger et al., 2002).

Sweat Collection

Fluid replacement guidelines offer a generalized method for rehydration. For more individualized methods, sweat needs to be examined. There are three main methods of sweat collection used by researchers. The gold standard for determining whole body sweat electrolyte loss is whole body wash-down (Shirreffs et al., 1997). The whole body wash down technique involves washing the entire body and clothes with known amount of deionized water. This method is only realistic for use in laboratory settings (Shirreffs et al., 1997). Another method used is the arm bag method. This method involves surrounding the arm with a plastic bag, so air or moisture does not escape the bag. The arm bag method does have several limitations including lack of evaporation from the skin, which causes the skin to become waterlogged and changes the normal sweat process (Shirreffs, et al., 1997). There is also no way to account for regional electrolyte composition differences. While the arm bag and whole body wash down methods are

generally used in a lab-based setting, a more practical way to collect sweat in the field is the regional absorbent-patch method, which allows an estimation of whole body sweat electrolyte concentrations (Dziedzic, et al., 2014). Unlike the whole body wash down method, using an absorbent-patch to collect sweat can be done during a wider variety of exercise modes and environmental conditions (Baker et al., 2016). An issue with the absorbent patch method, however, is that it creates a microclimate under the patch that alters sweating in that area and regional sites can over or under estimate sweat electrolyte concentrations (Kilding et al., 2009). Although the absorbent patches have their limitations, the benefits of being able to test an athlete during sport specific training and being able to collect sweat from numerous athletes quickly enhance their practicality and justification. Absorbent patch sites vary between studies, though common sites used are forearm, superior scapula, upper chest, forehead, and anterior mid-thigh (Patterson et al., 2000, Baker et al., 2011). Use of multiple sites may also give a more accurate representation or summary of regional sweat composition.

Conclusion

In summary, there are a variety of markers and methods to measure and monitor the state of hydration. With no “gold standard” assessment of hydration, it is important to choose the proper marker to match the needs of the study or the application of monitoring hydration in a team setting. Plasma osmolality and total body water together offer the most comprehensive look at hydration status, but they are also more invasive or cumbersome to perform and need a much more controlled environment than other methods. Blood Urea Nitrogen and Creatinine together can be a useful

addition to plasma osmolality but would only be beneficial for general population use due to the fact that both BUN and Cr can be increased due to high intensity training commonly seen with athletic populations. Urine specific gravity, urine osmolality, and urine color offer a less invasive method of monitoring hydration. In the field, urine markers have the ability to be measured by handheld devices to measure them and give results on the spot. Urine color assessment can also be taught to most populations and can be a feasible tool for long-term monitoring. Sweat collection by whole body wash down or regional patch determines the electrolyte content of the sweat and allows for creation of proper rehydration plans. With many different markers and varying levels of invasiveness and cost to perform tests, it is important to understand what can affect each marker and what will be needed to control for to ensure accurate measurements. The choice of markers used should also depend on the population being observed. When working athletes, especially during the season, limits the processes used. Using a comprehensive approach to monitor hydration status allows for a more complete picture.

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CHAPTER 2

**CHANGES IN MARKERS OF HYDRATION THROUGHOUT THE COURSE OF A
COMPETITIVE SEASON AND SWEAT ELECTROLYTE COMPOSITION OF FEMALE COLLEGE
ATHLETES**

By

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Introduction

Fluid intake and hydration are critical for life, as well as athletic performance (Shirreffs et al., 2004). Normal body water content is defined as euhydration, above normal is referred as hyperhydration, and below normal is referred to as hypohydration or dehydration (Armstrong et al., 2013). Dehydration greatly increases the chances of heat-related illness such as heat exhaustion and heat stroke (Casa et al., 2015). Also, dehydration that causes a 2% loss in body weight has been shown to decrease aerobic performance, cognitive performance, and sport-specific skills, with greater dehydration of 3-4% impairing anaerobic performance (Shirreffs et al., 2004, Jones et al., 2008, McGregor 1999, Casa et al., 2010, Lopez et al., 2011, Kraft et al., 2012).

Regardless of the time of year a sport is played, or whether it is played indoors or outdoors, maintaining euhydration during training and competition is paramount. A person is considered euhydrated when first morning urine specific gravity (Usg) is less than or equal to 1.020 (Sawka et al., 2007). Thigpen et al. measured Usg prior to multiple basketball practices for Division II men (n=11) and women (n=11) and found that 81% of samples were above 1.021 (Thigpen et al., 2014), indicating hypohydration. Cosgrove and colleagues studied elite rugby players who performed resistance and aerobic training on the same day. The researchers found that 89% of players started the morning session hypohydrated (Usg of $1.026 \pm .0006$) and 82% of players started the afternoon session hypohydrated, with a Usg of $1.024 \pm .008$ (Cosgrove et al., 2014). In

other professional athletes, Osterberg et al. reported 15 of 29 male basketball players had a pregame U_{sg} greater than 1.020 (Osterberg et al., 2009).

It is well known that a level of 2% hypohydration during exercise can cause endurance performance to decrease and may cause decrements to anaerobic endurance, strength and power (Casa et al., 2010, McDermott et al., 2017). Soccer and field hockey are considered power-endurance sports. Soccer has rules on substitutions and minimal stoppages which limits the players ability to rehydrate, while field hockey does not have limits or rules on substitutions offering more opportunities to rehydrate. The effects of hydration on power and strength, the other component of power-endurance sports, have been studied less. Savoie and colleagues conducted a meta-analysis examining the effects of hypohydration on muscle endurance, strength, anaerobic power and capacity, and vertical jump (Savoie et al., 2015). The average amount of hypohydration achieved from all studies analyzed was -3.0 ± 1.1 % with a range from -1.0 to -5.0 %. They found that muscle endurance decreased 8.3 ± 3.1 %, strength was decreased 5.5 ± 1.0 %, and anaerobic power and capacity decreased by 5.8 ± 2.3 % and 3.5 ± 2.3 %, respectively. Interestingly, vertical jump performance was slightly increased ($0.9 \pm .07$ %) by hypohydration, although it was not statistically significant. They concluded this to be from the slight decrease in body weight accompanying with hypohydration (Savoie et al., 2015).

The performance measures mentioned above can be related to on-field performance. Additional on-field performance variables can also be related to skill performance, which encompasses decision-making and the process of using learned

ability to organize, initiate and execute correct technique (Abernethy et al., 1996).

Severe dehydration and hyperthermia can also alter cognitive performance, which is important for decision-making during a sporting event (Shirriff et al., 2004). Cognitive performance can start to be altered at as little as 1.3% dehydrated (Lieberman et al., 2007). Macleod and associates studied the effect of 2% dehydration from a passive hyperthermia session performed on the previous day on skill performance in elite female field hockey players. Both groups were subjected to the hyperthermia session, the euhydrated group received 150% of sweat loss in fluid to rehydrate with overnight and hypohydrated group was given 300mL of fluid overnight. Subjects performed a field hockey skill performance test and then completed 50 minutes of field hockey-specific intermittent treadmill (FHITP) running. After this, they then repeated the field hockey skill performance test. Pre-testing serum osmolality was significantly different (280 ± 2 mOsmol/kg and 294 ± 1 mOsmol/kg, $P = .045$) for the euhydrated and hypohydrated groups, respectively. Skill performance time was slower in the hypohydrated trial than the euhydrated trial ($P = .029$), penalty time was significantly greater in the hypohydrated trial ($P = .024$), and decision-making time was 7% slower in the hypohydrated trial than euhydrated trial ($P = .016$) (Macleod et al., 2010). Using a similar approach with soccer players, McGregor et al. (1999) examined the effects of fluid ingestion during 90 minutes of intermittent exercise that was designed to simulate the minimum demands of a soccer game on skill performance and found a 5% performance decrease ($P < 0.05$) in the no fluid group. No loss in skill performance was seen when fluid was consumed during the protocol (McGregor et al., 1999). Therefore, proper hydration strategy should

be in place for athletes to ensure hydration prior to gameplay, during gameplay, and after in order to minimize performance decrements for next day training or competition.

Hydration biomarkers such as plasma or serum osmolality (Posm) and urine specific gravity (Usg) are among the more popular used in monitoring. Posm is considered one of the best measures in the blood to determine hydration status (Armstrong et al., 2013). However, it does have limitations due to its sensitivity to fluxes in volume of body fluid compartments. For example, consuming a high calorie meal draws water out of vasculature and into the gut to aid in digestion (Dill et al., 1974). Low intensity exercise has minimal effect on Posm and higher intensity exercise causes increase in Posm, but this change is recovered quickly after exercise cessation (20- 30 minutes) (Greenleaf et al., 1979). Chronic hydration status would thus best be measured in a fasted, rested state. Usg is a simple and easy marker to measure that requires little skill of the tester and equipment is relatively inexpensive (Oppliger et al., 2002). Oppliger and associates showed that Posm and Usg were significantly different from baseline at dehydration levels of 3% and 5%, and persisted through 60 minutes of recovery ($P < .006$) (Oppliger et al., 2005). Thus, the use of Posm and/or Usg to measure hydration will allow for accurate interpretation of hydration status.

The main source of fluid loss during exercise is sweat, which is composed of 99% water but also contains electrolytes, urea, pyruvate, and lactate (Mena-Bravo et al., 2014). Replacing electrolytes lost from sweat has been shown to enhance rehydration of total body water (Gonzalez-Alonso et al., 1992). Sweat content varies greatly among

individuals, therefore using norms for electrolyte replacement may not ensure complete replenishment for all individuals on a team (Patterson et al., 2000). Sweat electrolyte concentrations are beneficial for athletes and coaches to know to help individualize hydration strategies (Kilding, et al., 2009). The absorbent-patch method of sweat collection is one method to assess electrolyte concentrations in sweat. The patch is a small piece of cotton that is covered by a thin non-porous adhesive film and is very easy to use in a team setting and allows collection to be done during sport specific training or competitive play (Baker et al., 2016). The patch is applied to various parts of the body, typically the chest, forearm, thigh, abdomen, and back. Patch sites play a crucial role in experimental outcomes. Sites should be chosen that can avoid contact from the subject and from others while training or completing exercise protocol. If used in a team setting choosing sites that are not affected by equipment and clothing. The purpose of sweat collection should also be considered. To estimate full body concentrations using sites that have already been studied and have regression equations already determined.

Throughout a competitive season there should be one goal, maintain high-levels of performance from all athletes on a team. The competitive season causes chronic physiological changes to occur in the body which may lead to decreases in performance. Anabolic markers have been shown to change in a decrease fashion and catabolic markers have been shown to increase (Coelho et al., 2015). The effects of a competitive season have also shown decreases in mean power output as well as increased perceptual strain (Laurent et al., 2014). While much of the literature on biomarkers examine hormones and nutritional markers, there is a lack of research on hydration and electrolytes.

Two studies were conducted in order to examine issues related to hydration in female Division I power-endurance athletes. The purpose study 1 was to monitor hydration status over the course of a competitive season for female Division I athletes. The current research has identified how these markers change from pre- to post-exercise, but this study examined the effects of in-season training on hydration markers. The purpose of study 2 was to evaluate whether there are chronic changes in plasma electrolytes as a function of “high” vs “low” electrolyte concentration sweat in high-level female athletes. The electrolyte content of sweat used in conjunction with Posm and Usg should allow for a better picture of hydration status and provide useful information for optimizing hydration strategies.

Materials and Methods

Study 1

Subjects. Division I female athletes ($n = 42$, weight = 64.27 ± 6.69 kg, %BF = 24.09 ± 5.72 %, $VO_{2max} = 47.3 \pm 5.36$ ml/kg/min, VJ = 54.79 ± 7.35 cm, $VT = 78.54 \pm 3.75$ % VO_{2max}) comprised of 22 soccer and 20 field hockey players participated in the study. The athletes were recruited prior to the start of preseason. All procedures were approved by the Rutgers University Committee for the Protection of Human Subjects.

Blood and Urine Collection. Athletes reported to the Rutgers Center for Health and Human Performance (CHHP) for blood draws. Athletes were instructed to arrive at the lab between the hours of 0700 and 0900 approximately 18-36 hours following a game after an overnight fast but having had fluid *ad libitum*. This time remained consistent for the entire season to account for diurnal variation of hormones. Preseason samples were

obtained the day before the start of preseason practices (T1). Samples were collected every 4 weeks throughout the season (T2, T3, T4, T5), with the final blood draw occurring 18-36 hours after the teams' last game (field hockey = T4, soccer = T5). Upon arrival to the CHHP at each timepoint, the athletes gave a urine sample, and then had their blood drawn. Blood from an antecubital vein was drawn into an un-chilled 10 mL serum red top vacutainer tube. Blood and urine samples were immediately processed at the CHHP and analysis was performed by Quest Diagnostics using their Blueprint for Athletes diagnostic panel. Plasma sodium, potassium, chloride, magnesium, and calcium were examined along with urine specific gravity. Blood glucose and blood urea nitrogen were measured to be used to calculate plasma osmolality with the following formula, $Posm = 1.90[Na^+ + K^+] + Glucose + BUN + 5.0$ (Rasouli et al., 2005).

Statistics. All statistical analyses were completed using SPSS statistical software (IBM®, SPSS® version 23). The teams were analyzed separately due to differences in the natures of the sports and gameplay as well as different number of time points (Martinez-Lagunas et al., 2014, Gabbett et al., 2010). A within subjects repeated measure MANOVA was run for the urine and blood variables, using five-time points for soccer and four-time points for field hockey. Univariate follow-ups were then used to examine individual marker changes. If Huynh-Feldt epsilon was < 0.75, the adjusted statistic was used. Simple contrasts using T1 as comparison were conducted, and significance was set at $P < 0.05$.

Study 2

Subjects. Division I female collegiate soccer and field hockey players participated in Study 2 ($N = 29$; $M_{\text{age}} = 20 \pm 1.0$ yrs; $M_{\text{weight}} = 64.3 \pm 2.7$ kg; $M_{\text{height}} = 166.4 \pm 6.5$ cm; $M_{\text{VO}_{2\text{max}}} = 48.1 \pm 4.7$). All procedures were approved by the Rutgers University Committee for the Protection of Human Subjects and all subjects provided written informed consent.

Sweat Collection. During a practice session lasting approximately 2 hours, regional sweat collections were made from the forearm, chest, and navel using sweat patches. Sweat concentrations for sodium (Na), potassium (K), chloride (Cl), calcium (Ca), and magnesium (Mg) were determined. Players were allowed to consume water *ad libitum* during training and were instructed to be normally hydrated prior to the start of the session. All field players wore their team-provided practice clothes and protective equipment. Training consisted of a dynamic warmup, sport specific skill training, and small sided games.

Players were instructed to shave the area of the patch locations prior to reporting to training. 3M Tegaderm + pads (3M Health Care, St. Paul, MN, USA) were applied to right forearm, chest, navel. Sweat patch locations were as follows: Forearm patches were placed on the right inner forearm below elbow crease; Chest patch was placed on the center of chest below collar bone; Navel patch was placed at the center of navel above the umbilicus. Prior to application the patch sites were wiped with 3 separate alcohol swabs in a circular motion starting at the center and working toward the outside. After the site was dry, the patch was then applied to the skin making sure

there were no air bubbles or creases. After all patches were applied, the players completed their training session. Players were instructed not to touch the patches and to continue training if the patch fell off.

After the completion of the training session, patches were removed with thumb forceps (Dynarex, Orangeburg, NY, USA) then placed in airtight salivette tubes. All tubes were placed in specimen bags and stored in a refrigerator until analysis by Quest Diagnostics using their Blueprint for Athletes diagnostic panel.

Statistics. Electrolyte concentrations from each site were summed to represent total loss of each individual electrolyte. Z scores were calculated using total electrolyte loss to create a median split for high and low electrolyte sweaters. 2 (high vs low) x 4 (time) RM ANOVAs were run in SPSS, v.23. with significance set at $P < .05$.

Results

Study 1

Plasma Osmolality. The changes in Posm over the course season can be seen in Figure 1 for soccer and Figure 2 for field hockey. There was a significant increase in Posm ($\Delta\text{Posm} = 4.0 \pm 1.3 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$) from T1 to T4 and from T4 to T5 ($\Delta\text{Posm} = 2.7 \pm 1.3 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$) for soccer. Field hockey followed a similar pattern and had a significant increase in Posm ($\Delta\text{Posm} = 2.3 \pm 0.95 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$) from T1 to T3 and from T3 to T4 ($\Delta\text{Posm} = 3.5 \pm 0.90 \text{ mOsm/kg H}_2\text{O}$, $P < 0.05$).

Urine Specific Gravity. The changes in USG over the course of the season can be seen in Figure 3 for soccer and Figure 4 for field hockey. USG significantly increased from $1.012 \pm .002$ at T1 to $1.018 \pm .002$ at T2 and continued to increase to $1.021 \pm .001$ at T3, where it plateaued and remained significant through T5 ($P < 0.05$) for soccer. USG significantly increased from $1.011 \pm .008$ at T1 to $1.016 \pm .007$ at T2 ($P < 0.05$) and peaked at $1.017 \pm .006$ at T3 and plateaued at T4 for field hockey.

Plasma Sodium. Changes in Na^+ can be seen in Figure 5 and Figure 6 for soccer and field hockey, respectively. Na^+ increased from T1 to T4 and remained significant through T5 ($\Delta\text{Na}^+ = 1.81 \pm 0.67$ mmom/L, $P < 0.05$). There was a trend towards significance from T4 to T5 ($\Delta\text{Na}^+ = 1.18 \pm 0.63$ mmom/L, $P = 0.076$) for soccer. Field hockey followed a similar pattern with a significant increase from T1 to T3 and remained significant through T4 ($\Delta\text{Na}^+ = 0.90 \pm 0.42$ mmom/L, $P < 0.05$). There was also a significant increase from T3 to T4 ($\Delta\text{Na}^+ = 2.05 \pm 0.48$ mmom/L, $P < 0.05$).

Plasma Potassium. Changes in K^+ can be seen in Figure 7 and Figure 8 for soccer and field hockey respectively. K^+ increase significantly from T1 to T2 ($\Delta\text{K}^+ = 0.25 \pm 0.08$ mmom/L, $P < 0.05$) and remained significant through T3 then returned to baseline at T4 for field hockey. There were no significant changes in K^+ for soccer.

Plasma Chloride. Changes in Cl^- can be seen in Figure 9 for soccer and Figure 10 for field hockey. Cl^- significantly increased from T1 to T2 ($\Delta\text{Cl}^- = 1.6 \pm 0.6$ mmol/L, $P < 0.05$), then returned to baseline at T3. Cl^- increased again at T4 and remained significant through T5 ($\Delta\text{Cl}^- = 2.8 \pm 0.7$ mmol/L, $P < 0.05$) for soccer. Cl^- increased from T1 to T2 ($\Delta\text{Cl}^- = 1.55 \pm 0.51$

mmol/L, $P < 0.05$) and remained elevated through T3 before returning to baseline at T4 for field hockey.

Plasma Magnesium. Changes in Mg^{2+} can be seen in Figure 11 for soccer and Figure 12 for field hockey. Mg^{2+} increased from T1 to T3 ($\Delta Mg^{2+} = 0.12 \pm 0.04$ mg/dL, $P < 0.05$) for soccer. Mg^{2+} significantly decreased from T1 to T4 ($\Delta Mg^{2+} = -0.060 \pm 0.024$ mg/dL, $P < 0.05$) for field hockey.

Plasma Calcium. Changes in Ca^{2+} can be seen in Figure 13 and Figure 14 for soccer and field hockey, respectively. Ca^{2+} decreased from T1 to T2 ($\Delta Ca^{2+} = -0.16 \pm .07$ mg/dL, $P < 0.05$), then returned to baseline for soccer. Ca^{2+} trended toward a significant decrease from T1 to T4 ($\Delta Ca^{2+} = -0.195 \pm 0.103$ mg/dL, $P = 0.073$), there was a significant decrease from T3 to T4 ($\Delta Ca^{2+} = -0.18 \pm .07$ mg/dL, $P < 0.05$) for field hockey.

Study 2

Sweat Sodium. Mean concentrations for sweat sodium can be seen in Table 1.

Concentrations for the Forearm, Navel, and Chest were 39.79 ± 8.1 mMol, 67.02 ± 20.3 mMol, and 51.14 ± 15.6 mMol, respectively.

Sweat Potassium. Mean concentrations for sweat potassium can be seen in Table 1.

Concentrations for the Forearm, Navel, and Chest were 6.65 ± 1.8 mMol, 4.57 ± 1.2 mMol, and 5.34 ± 1.3 mMol, respectively.

Sweat Chloride. Mean concentrations for sweat chloride can be seen in Table 1.

Concentrations for the Forearm, Navel, and Chest were 25.83 ± 8.0 mMol, 56.74 ± 20.4 mMol, and 39.76 ± 14.2 mMol, respectively.

Sweat Calcium. Mean concentrations for sweat calcium can be seen in Table 1.

Concentrations for the Forearm, Navel, and Chest were 1.18 ± 0.5 mg/dL, 0.76 ± 0.2 mg/dL, and 0.87 ± 0.4 mg/dL, respectively.

Sweat Magnesium. Mean concentrations for sweat magnesium can be seen in Table 1.

Concentrations for the Forearm, Navel, and Chest were 0.36 ± 0.2 mg/dL, 0.15 ± 0.1 mg/dL, and 0.18 ± 0.1 mg/dL, respectively.

Sweat Classification. When comparing plasma electrolyte changes during a season in high vs low electrolyte sweaters, as defined by their sweat patch results, there were no differences in concentration changes, $P > .10$.

Discussion

The results of this study show significant changes in markers associated with hydration status. These results indicate that soccer players were in a chronic state of mild hypohydration throughout the season while field hockey players did not reach this state. This is the first study to examine hydration status over the course of the season in female athletes. Previously Thigpen et al. (2014) and Cosgrove et al. (2014) both found that athletes reported to practice in a hypohydrated state. Thigpen et al. (2014) found that 81% of the urine samples over the course of two days had $USG > 1.020$ ($n=22$), and Cosgrove et al. (2014) reported 89% of urine samples had $USG > 1.020$ at the morning training session ($n=46$). In the present study, urine analysis was done once every four weeks. Soccer players reached a team average $USG > 1.020$ from T3 to T5 while field hockey averages for USG did not reach 1.020. The different results between teams could be due to the nature of gameplay, frequent substitutions and more opportunity to

rehydrate for field hockey versus minimal substitutions and less opportunity to rehydrate for soccer. These results may suggest that athletes wake up in a hypohydrated state. Coaches and athletic trainers need to implement hydration strategies to ensure proper hydration throughout the day as well as instruct athletes on self-monitoring methods to use when they are outside of team events.

Both soccer and field hockey saw increases in plasma sodium over the course of the season. The formula used to calculate plasma osmolality is, $\text{Posm} = 1.90[\text{Na}^+ + \text{K}^+] + \text{Glucose} + \text{BUN} + 5.0$ (Rasouli et al., 2005) and plasma sodium is one of the main factors that will affect this calculation. Plasma potassium has a more minor effect due to much lower circulating concentrations; it is used in this formula to increase the correlation coefficient ($r = 0.785$ with K^+ , $r = 0.784$ without) (Rasouli et al., 2005). There are no studies that measured Posm over the course of season, but the findings are in agreement with other studies that have measured Posm acutely. Sommerfield and colleagues measured pre- and post-dehydration Posm in male wrestlers and female soccer players. Posm at pre-dehydration was 280.4 ± 2.2 mOsm/kg for males and 281.2 ± 2.2 mOsm/kg for females, after the dehydration protocol Posm increased to 288.1 ± 2.4 mOsm/kg for males and 282.1 ± 2.4 mOsm/kg for females (Sommerfield et al., 2016). The investigators concluded that the smaller change in Posm for females was due to a smaller decrease in body weight ($1.9 \pm 0.03\%$) than males ($2.9 \pm 0.09\%$) during the dehydration period (Sommerfield et al., 2016). Armstrong et al. examined female collegiate tennis players before and after an outdoor match in a hot climate and found that Posm increased from pre- ($281 - 289$ mOsm/kg) to post ($280 - 294$ mOsm/kg)

(Armstrong et al., 1994). The normal values of Posm range from 275 to 295 mOsm/kg. It is noteworthy that none of the previously reported results exceed the normal range (NLM). Since reported values are not out of clinical ranges, baseline testing for Posm is important to track changes in athletes over the course of a season.

There were fluctuations in plasma electrolyte concentrations, some of which were of statistical significance but remained within the reference ranges. Changes in electrolyte concentrations over the course of the season have not been examined previously. These results tie into sweat electrolyte concentrations. There were substantial regional variations in sweat electrolyte loss observed in female athletes. However, the acute loss of electrolytes due to sweating does not appear to impact chronic plasma values over the course of a season, even for those individuals who are classified as a high-electrolyte sweater. The availability of sports drinks during training and sodium content of foods available to college athletes may have accounted for this and could possibly explain the initial increases in plasma electrolytes observed after the start of preseason.

Conclusion

The current studies have shown that hydration monitoring is a vital tool to use when evaluating athlete health and performance. The increases seen in hydration biomarkers throughout the season resulted in some of the players being in a chronic hypohydrated state, with this effect more pronounced in the soccer players. Monitoring changes from baseline of these markers rather than using recommended ranges to determine hydration status is important because of the tight control that the body has on the

mechanisms that relate to hydration. Since no gold standard measurement of hydration status is agreed upon, monitoring multiple markers aids in the accuracy of proper interpretation of data. The use of sweat patches may have important implications for identifying those individuals considered to be high-electrolyte sweaters, and could benefit from an electrolyte-carbohydrate beverage rather than water to facilitate recovery between acute training sessions. Overall, education on hydration, methods to monitor hydration on a daily basis, and sweat replenishment should be implemented in sports for athletes to assist in maintaining performance and well-being throughout the season.

Limitations of the current study include not monitoring body weight on the days of blood draws, daily fluid intake, and menstrual regularity over the competitive season. Monitoring 24-hour urine production prior to blood draws can give a better picture of the hydration status of the athletes. Use of urine color would be useful to gain insight at daily hydration status. The limitations mentioned above may cause adherence issues with subjects due to the labor and time involved in collecting specimens and data over the course of the season. Choosing proper timing and use of all the hydration monitoring methods need to be considered. Future research should focus on more longitudinal observations of hydration. The current study showed that there are changes in hydration biomarkers over the course of the season. When monitoring sweat electrolyte concentrations with regional absorbent patches it is important to choose sites that can be used in previously researched regression equations to estimate

whole body concentrations as well as measuring body weight pre- and post- exercise to calculate amount of sweat loss.

Tables

Table 1.

	Forearm	Navel	Chest
[Na ⁺] (mM)	39.79 ± 8.1	67.02 ± 20.3*	51.14 ± 15.6
[K ⁺] (mM)	6.65 ± 1.8*	4.57 ± 1.2	5.34 ± 1.3
[Cl ⁻] (mM)	25.83 ± 8.0	56.74 ± 20.4*	39.76 ± 14.2
[Ca ²⁺] (mg/dL)	1.18 ± 0.5*	0.76 ± 0.2	0.87 ± 0.4
[Mg ²⁺] (mg/dL)	0.36 ± 0.2*	0.15 ± 0.1	0.18 ± 0.1

Table 1. Mean sweat electrolyte concentration for each patch site. The greatest [K⁺], [Ca²⁺], and [Mg²⁺] were found at the forearm and the largest [Na⁺] and [Cl⁻] were produced at the navel (p<.05).

Figures

Figure 1.

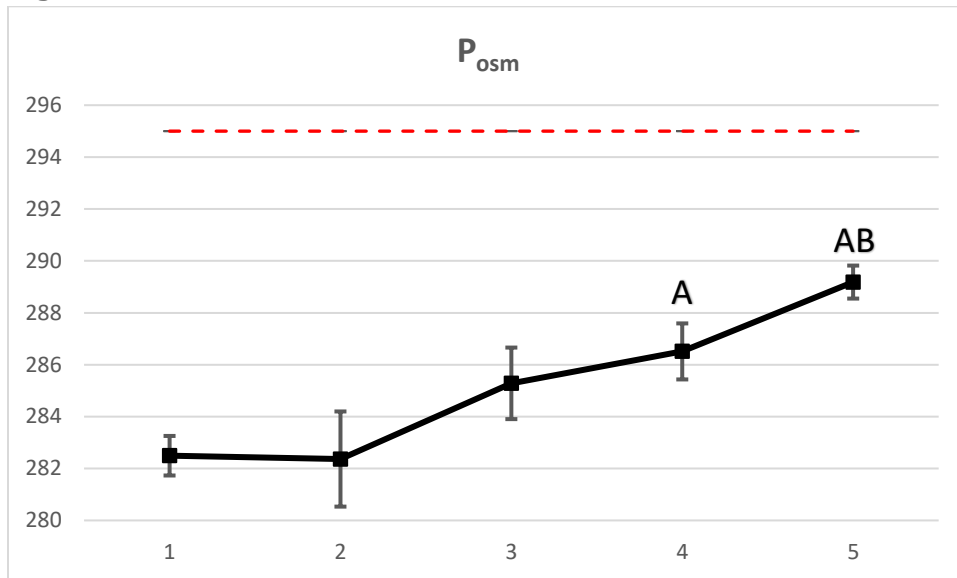


Figure 1: P_{osm} significantly increased from T1 to T4 ($\Delta P_{osm} = 4.0 \pm 1.3$ mOsm/kg H_2O , $P < 0.05$) and T4 to T5 ($\Delta P_{osm} = 2.7 \pm 1.3$ mOsm/kg H_2O , $P < 0.05$); A: denotes statistically significant increase from T1; B: denotes statistically significant increase from T4.

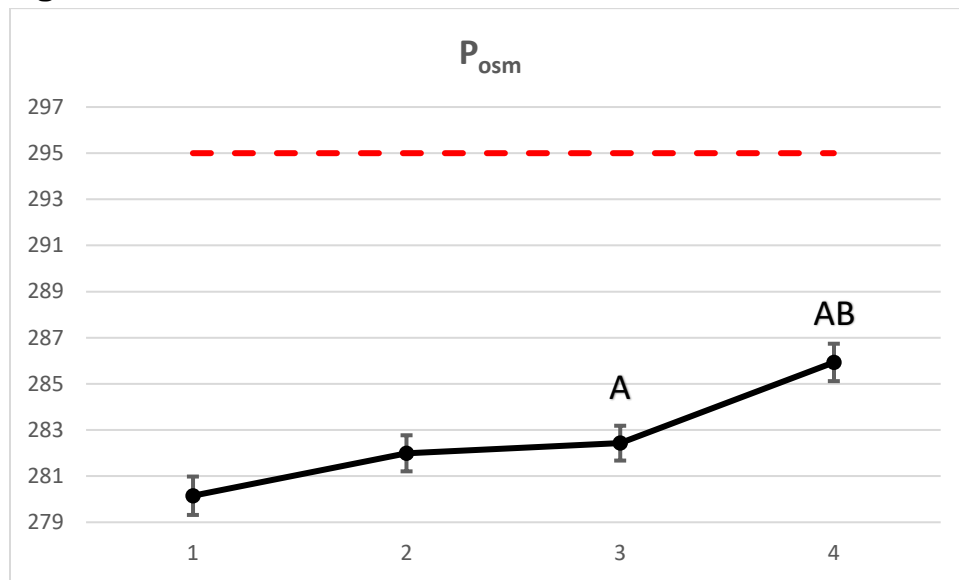
Figure 2.

Figure 2: P_{osm} significantly increased ($\Delta P_{osm} = 2.3 \pm 0.95$ mOsm/kg H_2O , $P < 0.05$) from T1 to T3 and from T3 to T4 ($\Delta P_{osm} = 3.5 \pm 0.90$ mOsm/kg H_2O , $P < 0.05$); A: denotes statistically significant increase from T1; B: denotes statistically significant increase from T3.

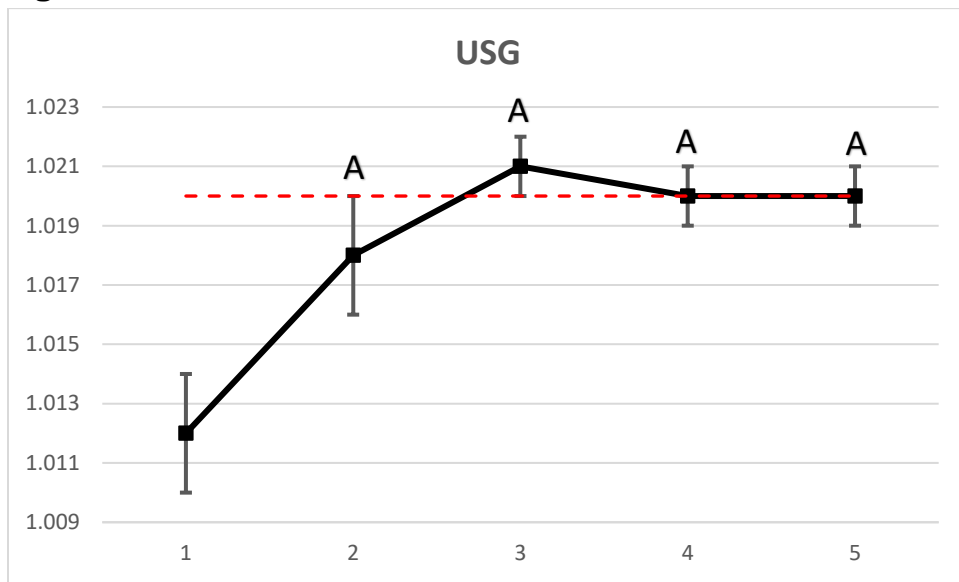
Figure 3.

Figure 3: USG significantly increased from T1 to T2 ($\Delta\text{USG} = 0.006 \pm 0.002$, $P < 0.05$); A: denotes statistically significant increase from T1

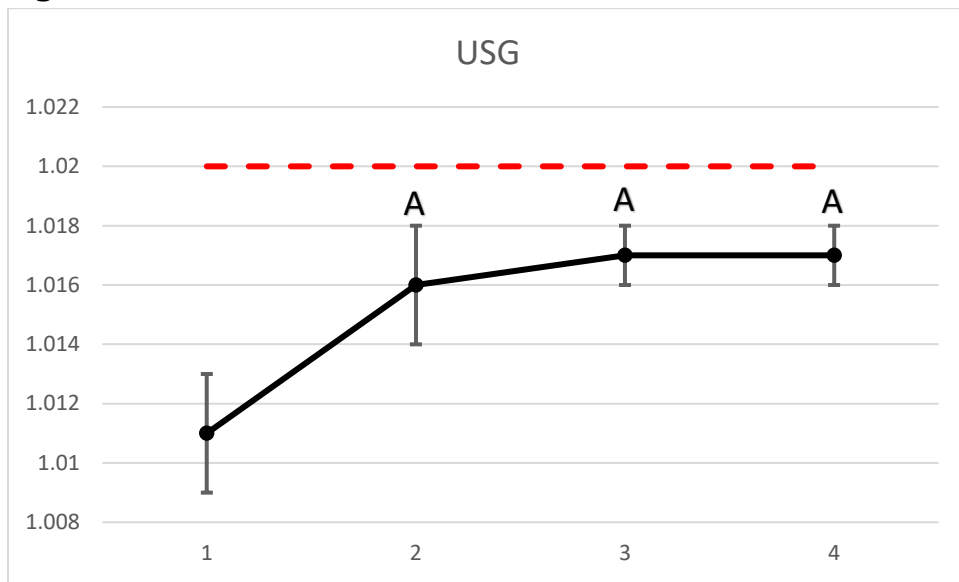
Figure 4.

Figure 4: USG significantly increased from $1.011 \pm .008$ at T1 to $1.016 \pm .007$ at T2 ($P < 0.05$) and peaked at $1.017 \pm .006$ at T3 and plateaued at T4. A: denotes statistically significant increase from T1.

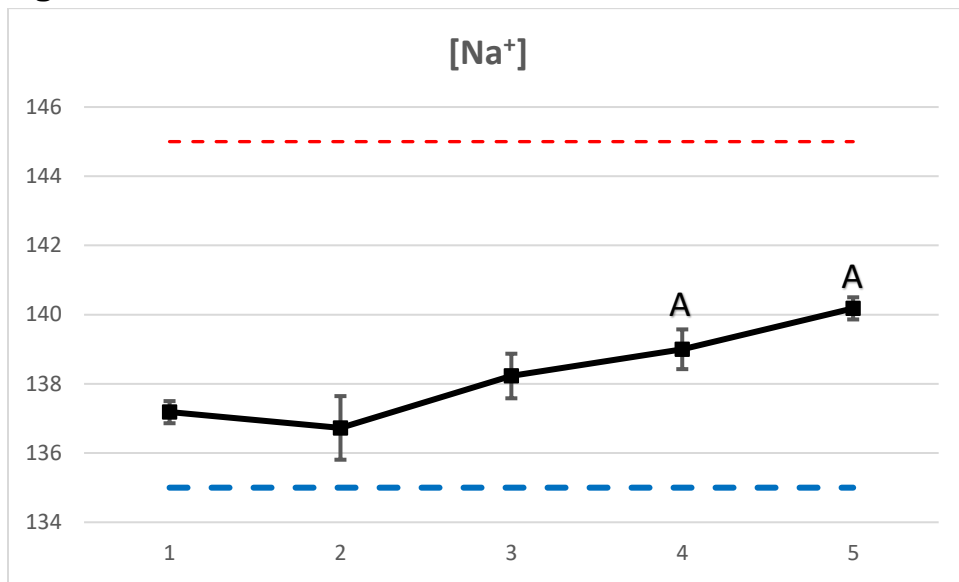
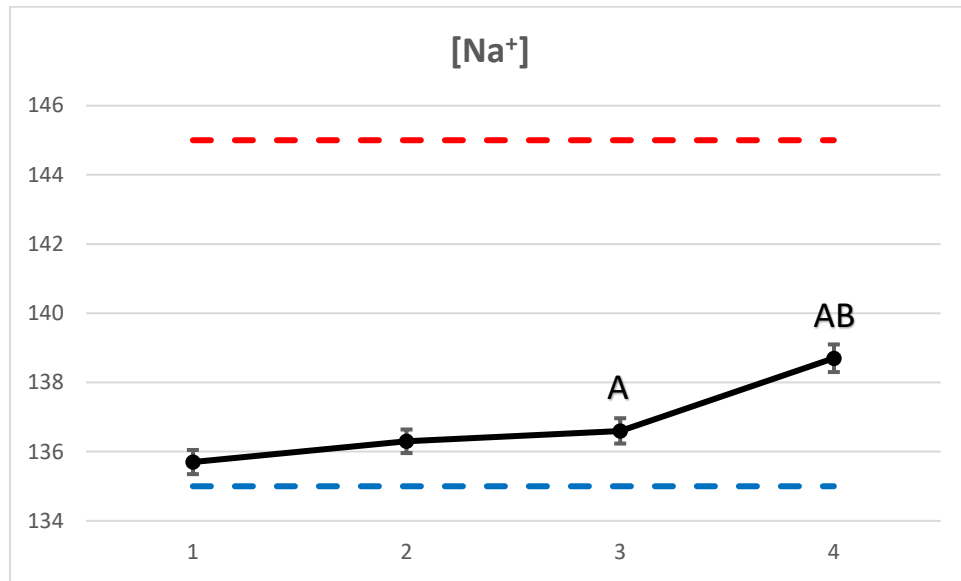
Figure 5.

Figure 5: $[Na^+]$ significantly increased from T1 to T4 ($\Delta[Na^+] = 1.81 \pm 0.67$ mmol/L, $P < 0.05$); A: denotes statistically significant increase from T1.

Figure 6.**Figure 6:** $[Na^+]$ significant increase from T1 to T3 and remained significant through T4

($\Delta[Na^+] = 0.90 \pm 0.42$ mmol/L, $P < 0.05$). There was also a significant increase from T3 to

T4 ($\Delta[Na^+] = 2.05 \pm 0.48$ mmol/L, $P < 0.05$); A: denotes statistically significant increase

from T1, B: denotes statistically significant increase from T3.

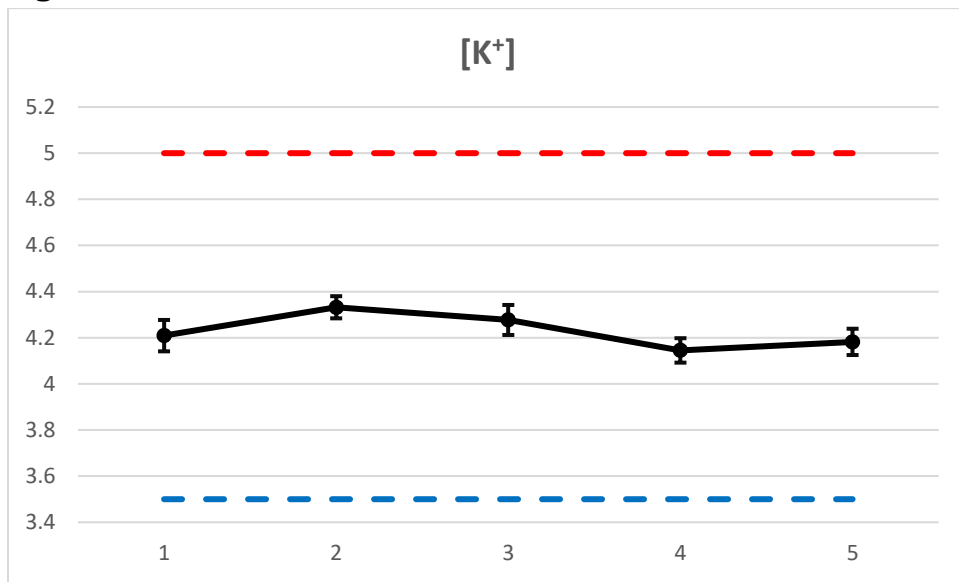
Figure 7.**Figure 7:** There was no significant changes in $[K^+]$.

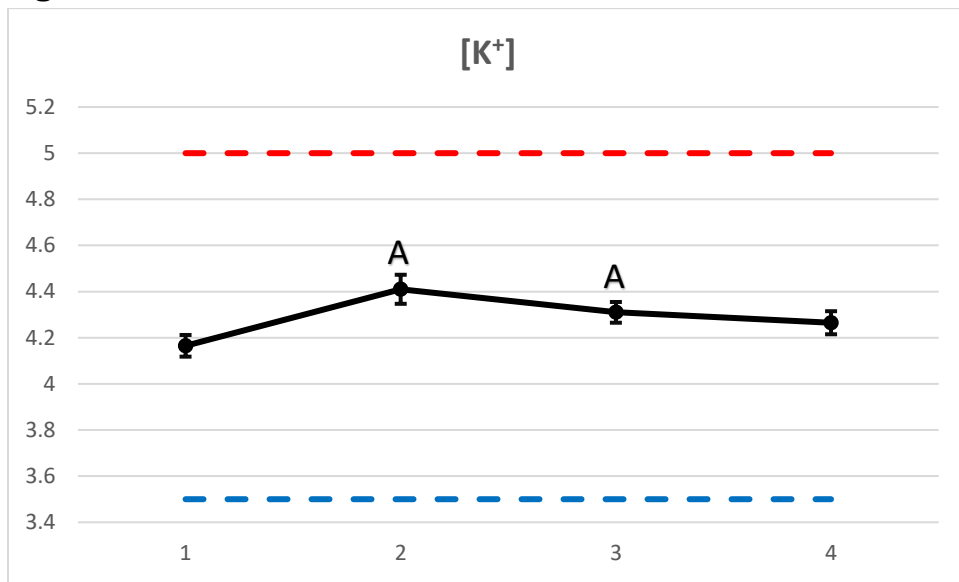
Figure 8.

Figure 8: $[K^+]$ increase significantly from T1 to T2 ($\Delta[K^+] = 0.25 \pm 0.08$ mmol/L, $P < 0.05$)

and remained significant through T3 then returned to baseline at T4. A: denotes statistically significant increase from T1.

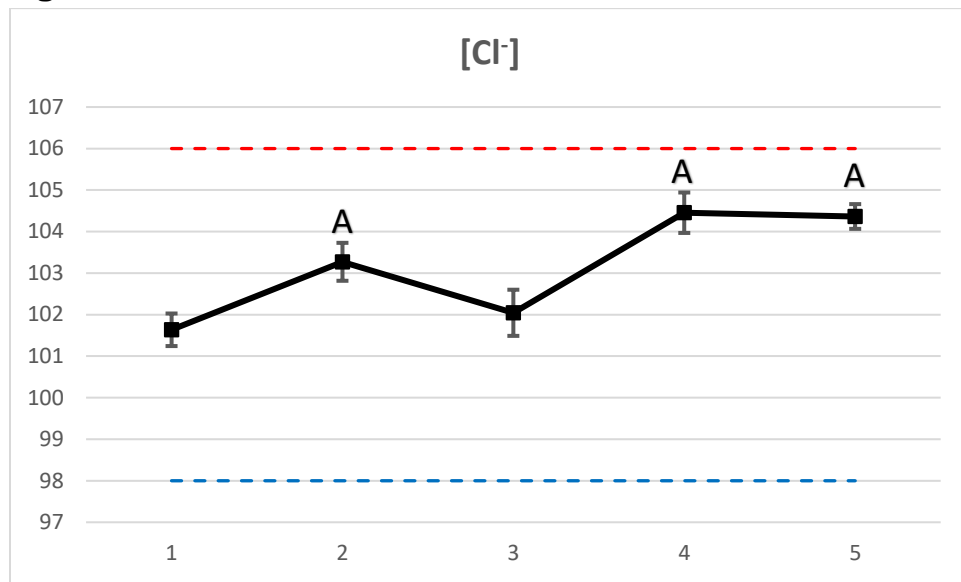
Figure 9.

Figure 9: $[Cl^-]$ significantly increased from T1 to T2 ($\Delta[Cl^-] = 1.6 \pm 0.6$ mmol/L, $P < .05$) and T1 to T4 ($\Delta[Cl^-] = 2.8 \pm 0.7$ mmol/L, $P < 0.05$); A: denotes statistically significant increase from T1.

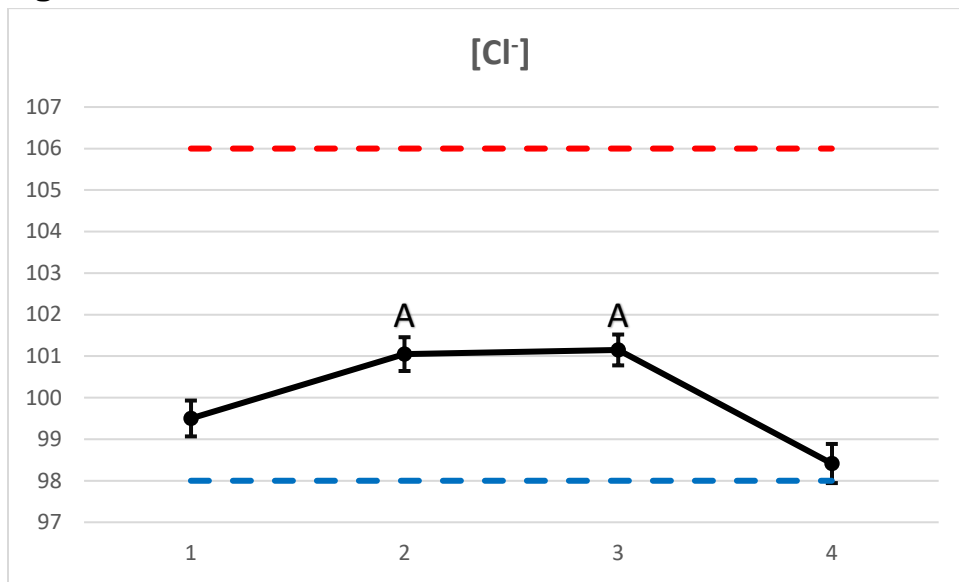
Figure 10.

Figure 10: $[\text{Cl}^-]$ increased from T1 to T2 ($\Delta[\text{Cl}^-] = 1.55 \pm 0.51$ mmol/L, $P < 0.05$) and remained elevated through T3 before returning to baseline at T4. A: denotes statistically significant increase from T1.

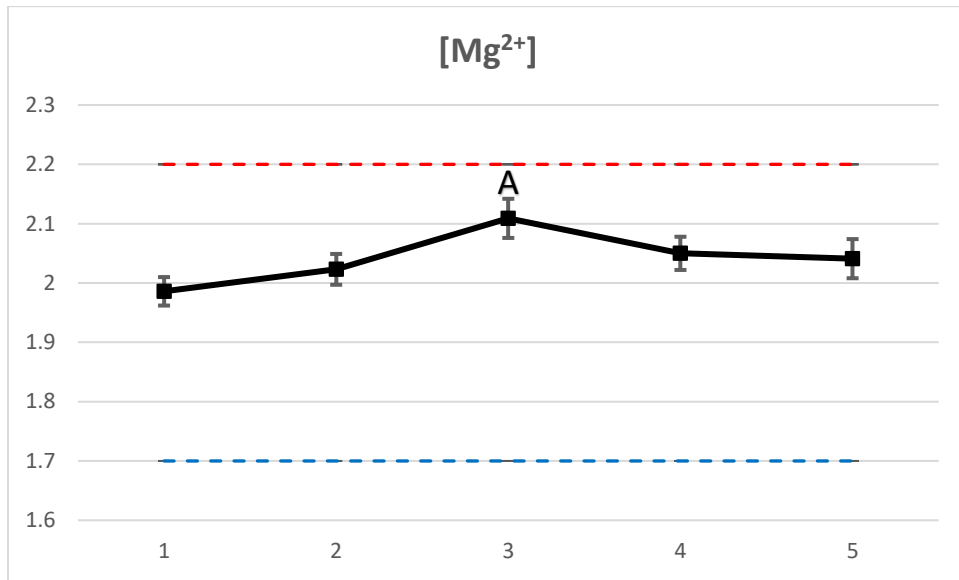
Figure 11.

Figure 11: $[Mg^{2+}]$ increased from T1 to T3 ($\Delta[Mg^{2+}] = 0.12 \pm 0.04$ mg/dL, $P < 0.05$); A: denotes statistically significant increase from T1.

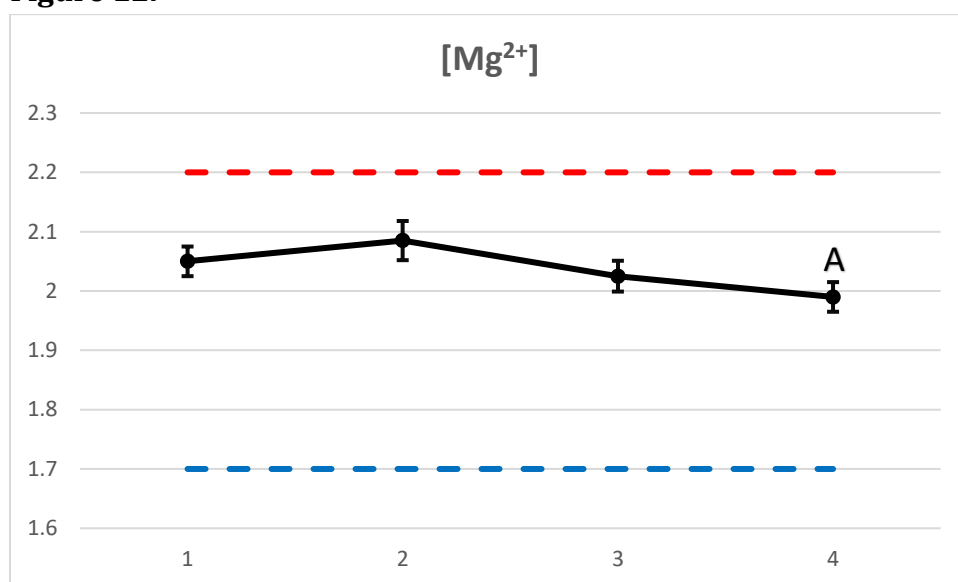
Figure 12.

Figure 12: $[Mg^{2+}]$ significantly decreased from T1 to T4 ($\Delta[Mg^{2+}] = -0.060 \pm 0.024$ mg/dL, $P < 0.05$); A: denotes statistically significant increase from T1.

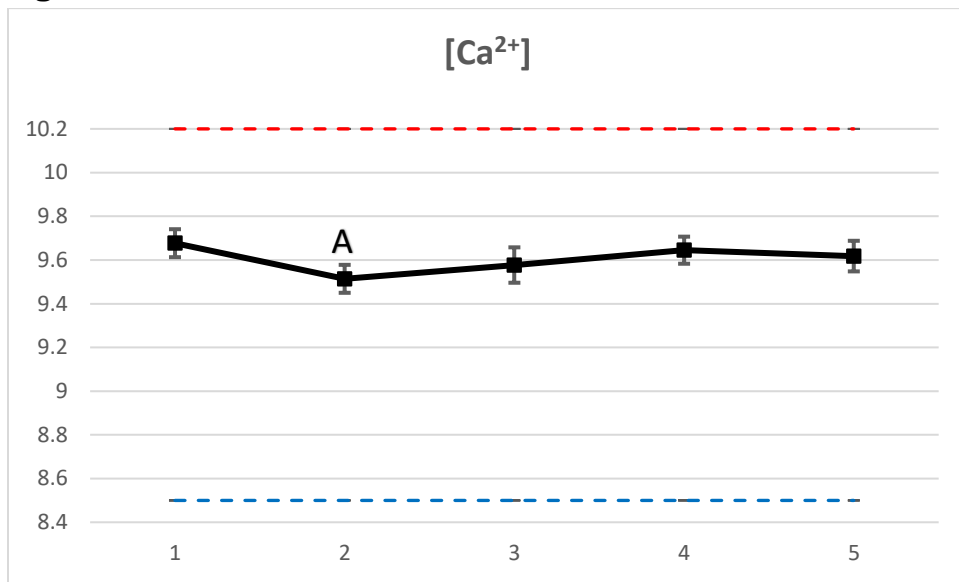
Figure 13.

Figure 13: $[Ca^{2+}]$ decreased from T1 to T2 ($\Delta[Ca^{2+}] = -.16 \pm .07$ mg/dL, $P < 0.05$); A: denotes a statistically significant decrease from T1.

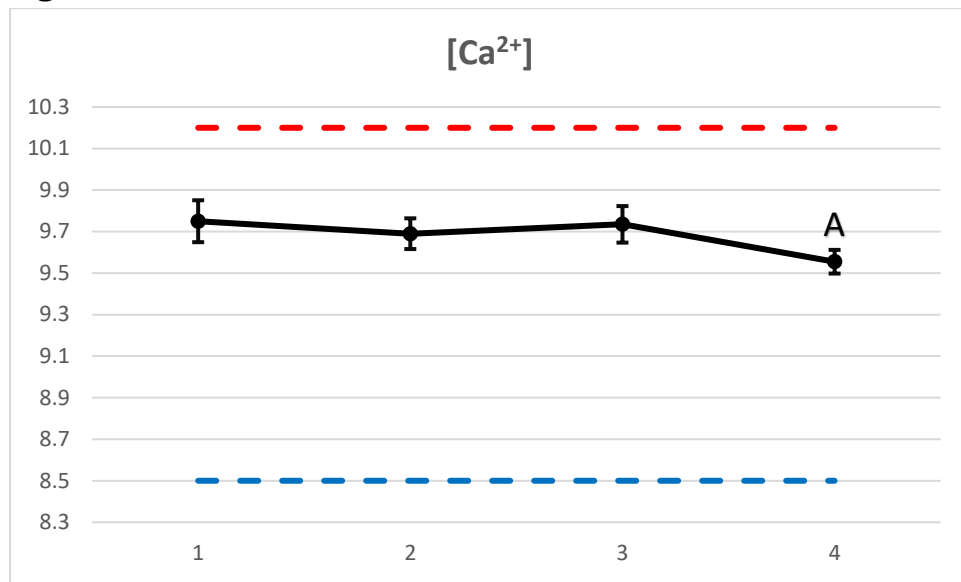
Figure 14.

Figure 14: $[Ca^{2+}]$ trended toward a significant decrease from T1 to T4 ($\Delta[Ca^{2+}] = -0.195 \pm 0.103$ mg/dL, $P=0.073$), there was a significant decrease from T3 to T4 ($\Delta[Ca^{2+}] = -0.18 \pm .07$ mg/dL, $P<0.05$); A: denotes a statistically significant decrease from T3.

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