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Pre-Practice Hydration Status of Collegiate Male American Football Players with Sickle Cell Trait

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PRE-PRACTICE HYDRATION STATUS OF COLLEGIATE MALE AMERICAN FOOTBALL PLAYERS WITH SICKLE CELL TRAIT

By

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ABSTRACT

Since 2000, 63% of deaths associated with college football were due to complications, such as sickling events; however, sickle cell trait (SCT) only affects 3-4% of participating athletes. Sickling events can be exasperated by conditions, including heat exposure, dehydration, and intense physical activity and lead to rhabdomyolysis and death. Assessing hydration status is crucial for athletes and the use of urine specific gravity (USG) has been shown to provide rapid and valid results. The purpose of this study was to assess the prevalence of dehydration among SCT carrying American college football players.

Division I collegiate football athletes participated as part of a larger study. Athletes provided a midstream urine sample, for USG assessment, each morning for three days of fall football camp during August 2021. Cut offs for USG were as follows: well-hydrated (<1.010), minimal dehydration (1.011-1.020), significant dehydration (1.021-1.030), severe hypohydration (>1.030). USG measurements evaluated a correlation between SCT and hydration status along with an association between SCT and hydration level. The data showed a strong, significant correlation for day 1 (r=0.78, p=0.001), a moderate, significant correlation for day 2 (r=0.56, p=0.045), and a moderate correlation for day 3 (r=0.41, p=0.128). The data did not show a significant association between SCT and hydration level for any of the days. These findings suggest that there is a need for increased education for both sports medicine practitioners and athletes to maintain proper hydration for SCT athletes.

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CHAPTER I

INTRODUCTION

Athletes with sickle cell trait (SCT) have an increased risk of suffering rhabdomyolysis after the onset of a sickling event. Misshapen hemoglobin that are characteristic of SCT can cause blockages in blood vessels following intense exercise (Bagley et al., 2007). The likelihood of blockages occurring increase with heat exposure and dehydration, which are common occurrences in American college football (Bonham et al., 2010). While dehydration occurs as a result of practice, it is very likely that an athlete will begin practice in a dehydrated state (Magal et al., 2015; Volpe et al., 2009; Kostelnik et al., 2021). Athletes who begin practice in a dehydrated state may be more likely to experience more severe side effects of dehydration, such as cramping or heat injury. To date, only one study has investigated the relationship between SCT and hydration status. This study found that USG measurements were lower and concentration of electrolytes in sweat of SCT athletes when compared to control athletes (Wang et al., 2021).

The purpose of this study was to evaluate the correlation between USG measurements and SCT in collegiate American football athletes. USG measurements were collected from eight SCT athletes and eight CON athletes prior to the start of practice for three days to start fall training camp. These measurements were categorized based on the hydration status of the athletes. On average, the USG measurements for the SCT athletes were lower when compared to athletes in the control group. These findings are consistent with the current standing data. With these findings, there are many opportunities for future research and education. Future research endeavors include evaluating the hydration habits of SCT athletes during exercise and at rest regardless of hydration status and investigating whether there truly is increased muscle damage in SCT athletes during exercise. With the increased risk of negative outcomes from exercise, it is imperative that practitioners continue to educate themselves and their athletes on the importance of hydration and fueling habits to ensure that the health of the athlete.

CHAPTER 2

Review of Literature

Inadequate hydration status negatively impacts athletic performance (Jones et al., 2008; MacLeod & Sunderland, 2010) In an ideal world, all athletes would begin practice in a state of euhydration, or the presence of water balance in the body. However, it is not uncommon for athletes to begin practice in a dehydrated state (Magal et al., 2015; Volpe et al., 2009). Dehydration can be defined as “the process of losing water that can occur from the hyperhydrated state to euhydration, and continuing toward hypohydration” (Greenleaf, 1992). Due to the conditions of a college athletics practice, this dehydrated state is exasperated and can lead to negative health outcomes, such as increased body temperature, hyperosmolality, reduced blood volume, skin blood flow, and sweat rate (Sherman & Lamb, 1988) increased cardiovascular strain, hypovolemia, peripheral vasodilation, tachycardia, decreased venous return and stroke volume (Nadel, 1988), and a decreased capacity to perform submaximal exercise (M. N. Sawka et al., 1984). The terms euhydrated and hypohydrated, or decreased body water content, are defined in terms of urine specific gravity (USG). A USG of < 1.013 represents a well-hydrated state, a USG between 1.013-1.029 represents a euhydrated state, and a USG of >1.029 represents a dehydrated state (L. E. Armstrong, 2007).

Hydration Status

While the physical effects of dehydration are obvious, cognitive performance begins to decline at one percent body weight loss but is significantly impaired at two percent. Cognitive tasks such as high-order cognitive procession and motor coordination are most susceptible to the effects of dehydration when compared to lower-order cognitive processes (Wittbrodt & Millard-Stafford, 2018). Similarly, sports-specific skills also decrease when an athlete experiences significant dehydration. When athletes were asked to complete a familiar sports-specific task in a euhydrated and dehydrated state, the dehydrated athletes performed worse. Not only did their time to complete the task increase, but they also obtained more penalty minutes when completing the task dehydrated (MacLeod & Sunderland, 2010). In a sport like American football, anaerobic power of an athlete plays a large role in the success of the individual. Following dehydration, both peak power and mean power decreased in both upper extremity and lower extremity strength. Along with this, motivation decreased and fatigue significantly increased among the athletes following dehydration (Jones et al., 2008). Hydration status effects all aspects of athletic performance, from speed to strength to cognitive abilities.

There are several ways to measure hydration status before and after practice. Among the most accessible are body weight, USG, and urine color. A study following female American football players during four days of practice, found that body mass decreased by 0.3% ± 0.4%, which did not change significantly over the four days. In addition, pre-practice USG and urine color were significantly lower than the post-practice measurements. This indicates that the players became less hydrated over the course of the practice. Interestingly, while thirst sensations did not show significant differences across days, the consumption of fluids did. On days with a greater rate of perceived exertion (RPE) score, the athletes consumed more water. The RPE scale (see Table 1) consists of fifteen levels, 6-20. During physical activity, an individual was asked to rate their perceived exertion from 6 (very, very light) to 20 (very, very hard). These values are closely related to heart rates, with a study done by Borg done in 1962 showing strong a correlation of (r=0.85). Perceived exertion also increases linearly with workload (Borg, 1970).

Table 1. RPE Scale

6		14	
7	Very, very light	15	Hard
8		16	
9	Very light	17	Very hard
10		18	
11	Fairly light	19	Very, very hard
12		20	
13	Somewhat Hard		

Similarly, besides day 1 of practice, water intake accompanied heart rate responses. On days that heart rates were higher, the amount of fluids the athletes consumed was greater (Lopez et al., 2021). Thirst should not be the first indication for an athlete to take in fluids. In a study conducted using eleven cyclists performing four trials (euhydration with available water, euhydration without available water, dehydration with available water, and dehydration without available water) found that thirst sensation was not an accurate indicator of hydration status. When comparing the euhydration without available water and dehydration without available water trials, the euhydrated group lost 0.4±0.5% body mass, while the dehydrated group had lost 3.2±0.6% body mass. While there was a significant difference in body mass between the two protocols, there was not a significant difference in thirst sensation (Cheung et al., 2015). Similarly, a study conducted in 2020, showed that thirst sensation did not begin until 0.8% body mass lost. However, thirst then did increase in parallel with plasma osmolality and dehydration (Armstrong et al., 2020). Impairments in performance can begin in as little as one percent body mass loss (Wittbrodt & Millard-Stafford, 2018); therefore, thirst sensation is not a reliable indication of thirst for athletes.

One study showed that 75% of male athletes arrived at practice hypohydrated or severely hypohydrated. Pre-intervention measures of USG, body weight, and height were taken prior the commencement of the study. The athletes were separated into two groups: the experimental group and the control group. The experimental group consumed an additional 16.9 fluid ounces of water during the day and an addition eight ounces of water at night, and the control group maintained normal eating and hydration habits. Seven days later, when measurements were taken again, hydration status significantly improved in the experimental group but did not in the control group (Magal et al., 2015) Similarly, a study conducted by Volpe, S., et al, in 2009 found that 66% of athletes began practice in a hypohydrated state with 13% of athletes begin in the significantly hypohydrated category based on USG measurements. Along with this, the study found that a higher percentage of male athletes were improperly hydrated when compared to female athletes at an NCAA Division I institution (Kostelnik et al., 2021; Volpe et al., 2009)

Measuring Hydration Status

Hydration status can be assessed using several methods, including measuring total body weight along in conjunction with plasma osmolality. Osmolality refers to the moles of solutes per liter of solution. The solutes most present in biological fluids include electrolytes, organic non-electrolytes, and colloids.

a. Plasma Osmolality

Plasma osmolality is determined most closely by the concentrations of sodium ions, chloride ions, bicarbonate ions, glucose, and urea (Rasouli, 2016) Plasma osmolality can be used to determine hydration status via freezing point or vapor pressure depression osmometer. To accomplish this, a sample of blood must be collected, centrifuged, and assessed immediately. Based on the results, the concentration of solutes in the blood can be determined, and in turn, so can hydration status. The change in freezing or vaporization point can be due to pH changes, carbon dioxide concentration in the blood, lactic acid concentration, or binding of electrolytes to protein (L. E. Armstrong, 2007).This measurement is seen as the current standard to which all other measurements should be compared. When attempting to engineer a simpler, more accessible measurement strategy, the new data should be compared to the data collected via plasma osmolality to ensure validity (Garrett et al., 2018).

b. Total Body Water

Total body water measurements include the isotope dilution and neutron activation techniques, considered to be standards by which to evaluate total body water and body fluid spaces. Unfortunately, these measurements are best performed in a laboratory under controlled conditions. During daily activities, such as exercise, body fluids are rarely at equilibrium, and isotope dilution measurements of total body water require three to five hours for internal isotope equilibrium and analysis (L. E. Armstrong, 2007) Similarly, neuron activation requires time and resources that sports medicine practitioners are not privy to. Neuron activation analysis involves the analyses of total body electrolytes, such as chloride, potassium, and sodium. This information is used to determine extracellular and intracellular volume along with exchangeable sodium in the body. The sum of extracellular and intracellular fluid would then represent the total water within the body (L. E. Armstrong, 2005). Therefore, using total body water measurements in conjunction with plasma osmolality are not realistic for measuring hydration in everyday scenarios (L. E. Armstrong, 2007). The fast-paced nature of collegiate athletes does not allow the time, nor the resources to use plasma osmolality to determine hydration status daily. Instead, practitioners turn to urine specific gravity (USG) to test urine concentration and, in turn, hydration status (Chadha et al., 2001).

c. Urine Specific Gravity (USG)

There are different ways to determine USG, including a refractometer and test strips. When compared to osmolality, the refractometer is a more accurate indicator of the density, or specific gravity of urine. A refractometer works by emitting light that passes through the fluid located within the device. Depending on the refractive factor of the fluid, a measurement is determined (Dorizzi et al., 1987). Since the refractometer has been determined as a more accurate indicator of urine composition, multiple types of devices have been created. The most popular are the manual and digital refractometer (Heileson, SP, USA & Jayne, SP, USA, 2019). A manual refractometer requires pipetting a urine sample into the prism and the device pointed to a light source. Comparatively, there are two types of digital refractometers: one that requires the "pen-like" body to be dipped into the sample, where the prism tip can take a measurement, and the other requires a sample to be pipetted into the prism, like the manual refractometer. However, both digital devices are able to produce readings in around three seconds (Minton MS, ATC et al., 2015). A study conducted by Heilsen and Jayne in 2019 evaluated the validity of the digital refractometer when compared to the manual device. The results showed that while the digital device tended to overestimate hypohydration it was valid when compared to the manual device. In a setting as fast-paced as athletics, it is a feasible option to determine hydration status of athletes (Minton MS, ATC et al., 2015).

Hydration Recommendations

The amount of fluids needed to reach euhydration are different for every individual. The size of the individual, along with energy expenditure and sweat rate are highly specific to each athlete. However, current advise states to hydrate with water and beverages containing sodium, several hours before exercise begins (M. Sawka et al., 2007). The ingestion of sodium allows the body to retain water (Von Duvillard et al., 2004). More specifically, an individual should ingest 500-600 mL of water or sports drink 2-3 hours before practice or competition begins. Similarly, snacks with salt accompanied by water may also be used(M. Sawka et al., 2007). To aid in pre-practice hydration, a well-balanced diet should also be consumed at least 24 hours prior to the practice or game (Casa et al., 2000).

While a balanced diet is essential for overall health of athletes, the meal that immediately precedes practice or competition should be high in carbohydrates. When completing high to moderate endurance or strength-based activities, the main fuel source in the body is glycogen. Since the body has very low storage allowance for glycogen, it is essential that all of the stores are full before exercise begins (Kerksick et al., 2017). In American football, which is a mix of moderate-to-high endurance and strength activities, it is imperative that athletes are given the resources to follow these guidelines to maximize success. Significant differences in hydration status day-to-day can be minimized with consistent meal and hydration timing (L. Armstrong et al., 1994). By consuming the same or similar amounts of fluids or fuel before practice each day, the athlete will start each practice at a consistent hydration level. An athlete that starts each practice at the same hydration level may have fewer variables affecting their performance. Starting every practice in euhydration will allow the athlete a better chance of success than starting some practices in euhydration and some in a state of dehydration. This can also translate to competition.

During exercise, the goal of hydration should be to avoid excessive dehydration, or a loss of 2% or more of total body weight. However, there is no one hydration protocol that fits for all athletes. Drinking during exercise is highly variable, and it can depend on individual sweat rate, duration of exercise, and the availability of fluid (M. Sawka et al., 2007). Fluid replacement needs also vary based on the setting of exercise. A heavier person exercising in a warmer climate will need more fluids compared to that of a smaller person exercising in a more-mild climate (Godek et al., 2005). Along with water, it is suggested that sports beverages containing electrolytes and carbohydrates are consumed in prolonged exercise in warm conditions. Snacks with carbohydrates that are consumed during exercise have been shown to maintain blood glucose levels and allow for greater performance (M. Sawka et al., 2007). Fluid replacement practices during exercise are also highly variable based on the climate and availability of fluids. A study conducted by Clarke ND., et al. investigated the blood glucose response in collegiate male soccer players. Twelve soccer players were asked to preform sport-specific training on three occasions. On two of the occasions, a carbohydrate-electrolyte solution or a placebo were administered at zero and forty-five minutes. On the third occasion, the same total volume of carbohydrate-electrolyte solution was administered by at fifteen-minute intervals for seventy-five minutes. Blood glucose was determined by blood samples taken before the start of practice, at the mid-point of practice, and after practice. Blood glucose was found to remain constant for the placebo trial but increased from the start point to the mid-point of practice for both carbohydrate groups, with the group receiving carbohydrates every fifteen minutes having a highest blood glucose. All groups experienced a decrease in blood glucose from the mid-point of practice to the end of practice. The placebo group experienced a lower blood glucose at the end of practice than at the start of practice. The results demonstrated that the timing of carbohydrate ingestion is not as important as the volume of carbohydrate-electrolyte fluid being consumed (Clarke et al., 2008). Therefore, the volume of ingested carbohydrates should be prioritized over the timing of ingestion during athletic activities.

Following exercise, fluid replacement recommendations vary depending on the speed at which euhydration needs to be achieved. If time allows, eating normal meals and drinking plain water will restore fluids lost. However, if euhydration needs to be achieved much faster, it is recommended that the athlete consume 1.5 liters of fluid per kilogram of body weight lost during exercise (M. Sawka et al., 2007). Regardless of the speed at which euhydration needs to be achieved, it is important that the food consumed contains sodium. Sodium stimulates thirst which allows for the athlete to continue to drink fluids while also replenishing the electrolytes that were lost via sweat during exercise. The ingestion of salt allows for the body to retain the water. The increased body water and thirst sensation, which leads to increased drinking, allows for plasma osmolality to return to normal following exercise (Stachenfeld, 2008). For an individual that has lost greater than seven percent body weight during an exercise bout, intravenous fluid replacement is recommended (M. Sawka et al., 2007). The volume of fluid lost during exercise is only one concern for dehydration, and the composition of the fluid should also be considered when attempting to rehydrate. An athlete should consume a volume of fluid that is greater than the amount lost during exercise, and this fluid should contain a moderately high amount of sodium, potentially accompanied by potassium. While it is not essential, the addition of carbohydrates may assist intestinal absorption of the electrolytes (Maughan & Shirreffs, 1997). A study conducted in 2010 looked at the rate of carbohydrate, sodium, and total solute absorption in the small intestine. The results found that the greater the concentration of carbohydrates in the carbohydrate-electrolyte solution, the greater the rate of absorption in the intestines. However, there is an upper limit to this. Carbohydrate absorption is saturated when the concentration of carbohydrates in solution is greater than the small intestine's transport capacity. Along with this, sodium absorption is related to the types of carbohydrates available in the solution. Glucose produces the fastest rate of sodium absorption in the intestine. A solution containing fructose will lead to the secretion of sodium which slows the absorption ability of the electrolyte. With differing rates of absorption, it is essential for the types of carbohydrates included in a solution to vary. By including multiple types of carbohydrates, more transport mechanisms in the intestine will be stimulated; therefore, increasing the amount of sodium and other electrolytes that may be absorbed (Shi & Passe, 2010).

Heat Acclimatization

In sports that take place during the warmer months, proper heat acclimatization is imperative. Heat acclimatization results in human adaptations to prevent the negative effects of heat stress on the human body and occurs in three stages. The first stage of heat acclimatization occurs at the lower extremes of heat. During this stage, the individual is reliant solely on the natural physiological adaptations of the body, such as sweating. Over time of heat exposure, individuals will reach the second stage of heat acclimatization. This stage is characterized by increased exposure to heat impulse. Individuals respond positively to heat in this stage but are considered low responders. They have not reached the final stage of heat acclimatization. In the final stage, there is habitual responses of the thermoregulation organs in the body. This stage is most often seen in indigenous populations that are highly active. The final stage may take years to accomplish (Taylor, 2014).

Heat acclimatization accounts for reduced core body temperature, improved sweat response, reduced skin temperature, improved skin blood flow, improved fluid balance, improved cardiovascular stability, improved skeletal muscle metabolism, lowered whole-body metabolic rate, and increase acquired thermal tolerance (Periard et al., 2015). Athletes who were able to maintain euhydration tended to have an increased level of sweating compared to athletes that were dehydrated when performing self-paced exercise in the heat (Travers et al., 2020). More efficient sweating leads to more efficient body thermoregulation. An athlete that has accomplished heat acclimatization will have an appropriate sweat response to the heat. Sweating allows the core body temperature to remain at a normal level thereby decreasing the chances of heat-related illness (Periard et al., 2015). Along with increasing thermoregulatory efficiency, practicing in warm conditions when an athlete will be competing in warm conditions benefits the athlete. Since the athlete is accustomed to performing in the heat, there will be no need to alter pre-game or performance hydration habits (Periard et al., 2015).

The National Collegiate Athletic Association (NCAA) recognized that heat acclimatization is important for both the performance and health of the athletes; therefore, a heat acclimatization protocol was established. As of May 2021, the acclimatization period for NCAA football fall camp (see Table 2), which takes place in late-July into early-August, must be seven days long. During these seven days, the first two days may only consist of helmets and spider shoulder pads, or smaller, lighter protective equipment. The next three days may consist of a combination of helmets, spider pads, and shoulder pads. Following these three days, athletes may practice in full pads. The seventh day of this period must be an off day, but it can occur at any point within the first seven days of camp. For the remainder of fall camp, there must be a minimum of seven days in which athletes must wear spider pads and helmets and a maximum of nine days in full pads (*Ncaa.Org*, 2021b)

Table 2. Example Heat Acclimatization Period

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Helmets and Spider Pads	Helmets and Spider Pads	Shoulder Pads	Helmets and Spider Pads	Off	Full Body Pads	Full Body Pads

b. Sickle Cell Trait

A unique aspect of American football is the presence of sickle cell trait (SCT) or disease in a portion of the athletes. Sickle Cell Disease (SCD) is defined by the presence of sickle hemoglobin (HbS) in the blood. A person with homozygous HbS has sickle cell anemia while individuals with heterozygous HbS are described as SCT. The shape of HbS differs from normal hemoglobin in that it is twisted into a spiral, which affects the contact sites needed for polymerization. As HbS is deoxygenated, its affinity for oxygen decreases which makes it an inefficient mover of oxygen throughout the body (Lukens M.D., 1981). In the United States, individuals who present with homozygous sickle cell genes have the highest rate of morbidity and mortality (Mitchell, 2018).

There are variations in the sickling hemoglobin that cause SCD. HbSC and HbS-thalassemia. HbSC is a milder form of the disease characterized by the inheritance of HbS with hemoglobin C. HbSS represents the most common and severe form of SCD. HbSS is characterized by the homozygous inheritance of HbS. In HbSC, patients inherit HbS along with hemoglobin C. While the symptoms are similar between those with sickle cell anemia, complications and severe health events are less likely in those with HbSC (da Guarda et al., 2020). In some cases of SCD, the disease can be present in addition to beta-thalassemia, one of the most common autosomal recessive disorders in the world. Beta-thalassemia is characterized by the reduction or absence of synthesis of the beta globin chains of the hemoglobin tetramer. Beta-thalassemia can be present in three states: carrier, major, and intermedia. A beta-thalassemia carrier is heterozygous and will have no symptoms of the disease. Beta-thalassemia major is severely transfusion dependent anemia, and beta-thalassemia intermedia will have symptoms varying anywhere between asymptomatic and severely transfusion dependent (Cao & Galanello, 2010).

There are two types of SCD characterized by HbS-thalassemia. The first, Hb S/β^0-Thal (absence), halts the production of Hb A, the most common form of hemoglobin in healthy adults (Kato et al., 2018). This molecule is most responsible for transporting oxygen throughout the body. This type of SCD is almost indistinguishable from sickle cell anemia. While the red blood cells have an increased capacity for oxygen transport, they are not any less likely to accumulate in blood vessels than in other sickle cell diseases. The second form, Hb S/β^+-Thal (reduction), is characterized by varying amounts of HbA diluting HbS which prohibits polymerization-induced cellular damage (Figueiredo, 2015). In HbSC, patients inherit HbS along with hemoglobin C.

There are many negative outcomes during sport that occur due to SCT or SCD carrying individuals (Mitchell, 2018). Sickle cell carriers are anywhere between 10-30 times more likely to fall victim to exercise-related sudden death than non-carriers. This can be exasperated by several conditions that are likely to occur in college athletics. These conditions include exposure to heat, dehydration, and intense physical activity (Bonham et al., 2010). These conditions can lead to a sickling event. A sickling event occurs due to misshapen red blood cells that are a characteristic of SCT. As red blood cells begin to move through the body during exercise, the misshapen cells begin to block blood vessels. The blockages can lead to rhabdomyolysis: “a syndrome that involves damage and breakdown of skeletal muscle, causing myoglobin and other intracellular proteins and electrolytes to leak into circulation,” (Bagley et al., 2007). Adverse effects can begin in athletes as quickly as two to three minutes of an all-out effort (Lukens M.D., 1981). An athlete who experiences a sickling event during training can have an emergency medical situation, and without proper medical treatment, the situation can turn deadly. One incidence occurred when an athlete carrying SCT collapsed during a standard conditioning test. This athlete then experienced rhabdomyolysis followed by acute renal failure (Shelmadine et al., 2013).

This is not a unique problem to American football, but it is prevalent due to the population that sickle cell affects. In the United States, one out of every 365 African Americans have SCT, and one out of every 601 have sickle cell anemia (Hassell, 2010). This is important information for the NCAA regarding Division-I football. In 2020, 14,160 or 48% of athletes participating in football were African American (*Ncaa.Org*, 2021)(*Ncaa.Org*, 2021a). Exertional sickling is the leading cause of death in NCAA Division I football. Of the sixteen deaths that have occurred in NCAA Division I football since 2000, none of them occurred during game or practice; all occurred during conditioning. Ten of the sixteen deaths were due to complications of exertional sickling. Even though SCT affects only 3-4% of NCAA Division I football players, it played a role in 63% of the deaths that have occurred in the sport since 2000 (Eichner, 2010). Given this information, the NCAA began mandatory screening for SCT during the 2010-2011 school year. This mandatory testing came directly following the unexpected death of Rice University freshman, Dale Lloyd II (Bonham et al., 2010).

To date, one study has illustrated the differences between SCT players and controlled matches. Results demonstrated that players with SCT have a lower USG before and following practice. Moreover, SCT players had lower concentrations of sodium, potassium, and chloride present in their urine. During the study, the amount and concentration of the sweat was also measured. Overall, the athletes sweat rate did not differ, but athletes with SCT had a higher concentration of sodium, potassium, and chloride present in their sweat (Wang et al., 2021). The likelihood of a sickling event increases as an athlete becomes dehydrated. When athletes were asked to perform a brisk walk, in a warm environment, with no fluid intake, progressive sickling occurred after only 45 minutes (Bergeron et al., 2004). Therefore, the purpose of this study is to evaluate the relationship between SCT and pre-practice hydration status in collegiate football players.

CHAPTER III

Methods

a. Participants

One hundred and thirty-five NCAA Division I American football athletes (≥ 18 years of age) were recruited to participate in a larger study. A subset of sixteen participants were used to assess the main purpose of this present study. Based on obtained medical information provided by the Sports Medicine staff, eight participants had SCT. The other eight participants, the control group (CON), were randomly selected from the larger set of data. This research was approved by the University of Mississippi Institutional Review Board (Protocol #22x004).

b. Procedure

Approximately two hours before practice (0600-0730 am), participants were asked to provide a midstream urine sample for three consecutive days, to assess hydration status. Before measurements, approximately 2-3 teaspoons of deionized water calibrated the refractometer. Using a handheld refractometer (ATAGO, 4410 PAL-10S, Tokyo, Japan), a small sample of urine was used to assess USG. Hydration levels were characterized as follows: well-hydrated (<1.010); minimal dehydration (1.011-1.020); significant dehydration (1.021-1.029); severe dehydration (>1.030) (Casa, D.J, et al., 2000). Participants were not asked to change their normal hydration or meal routine.

c. Statistical Analysis

Descriptive statistics were analyzed for all characteristics (mean ± SD). Pearson correlation coefficient was used to determine the association between hydration status and SCT. Correlation coefficients are as follows: negligible correlation (0.00-0.10), weak correlation (0.10 0.39), moderate correlation (0.40-0.69), strong correlation (0.70-0.89), and very strong correlation (0.90-1.00) (Shober et al., 2018). Chi-Square test was used to determine an association between SCT and CON groups with hydration level. Hydration level was determined as follows: dehydrated $\geq$ 1.011 and hydrated $\leq$ 1.010. Statistical significance set at ($p \leq 0.05$).

CHAPTER IV

Results

Table 3 shows participant characteristics for SCT and CON groups.

Table 3. Demographic Data

	SCT	CON
Number of Participants n	8	8
Age (y)	20±0	20±1
18, n	2	0
19, n	0	3
20, n	2	3
21, n	3	2
22, n	0	0
23, n	0	0
24, n	1	0
BMI (kg/m^2)	35.51±7.76	29.66±3.86
BMI categories		
Underweight, n	0	0
Normal Weight, n	0	1
Overweight, n	2	3
Obese, n	6	4
Year of Eligibility		
1 = Freshman, n	2	2
2 = Sophomore, n	2	3
3 = Junior, n	2	3
4 = Senior, n	2	0
5 = Graduate Student, n	0	0
Position		
1 = Running Back, n	1	2
2 = Wide Receiver, n	1	2
3 = Quarterback, n	0	0
4 = Offensive Line, n	2	1
5 = Defensive Line, n	3	0
6 = Defensive Back, n	1	1
7 = Specialist, n	0	0
8 = Tight End, n	0	1
9 = Linebacker, n	1	1
Race		
Black	8	8

a. Hydration Status

Hydration status of individuals was assessed by measuring the USG of a urine sample and then placing the athlete into a category, well-hydrated, minimally dehydrated, significantly dehydrated, or severely hypohydrated, based on the measurement. More athletes with SCT were found to be well-hydrated while more CON athletes were found to be severely hypohydrated prior to the start of practice. (See Table 4).

Table 4. Number of Participants per Hydration Category each Day

Table 4. Number of Participants per Hydration Category each Day

USG Category	Day 1		Day 2		Day 3	
	SCT	CON	SCT	CON	SCT	CON
Well-Hydrated (n)	3	0	3	1	2	1
Minimally Dehydrated (n)	4	1	4	1	4	4
Significantly Dehydrated (n)	1	5	0	3	1	3
Severely Hypohydrated (n)	0	1	0	1	0	0

b. SCT and Hydration Status

Average USG measurements for both groups can be found in Table 5. Individuals in the SCT group exhibited, on average, a lower USG than those in the CON group. On day one, there was a strong significant correlation between participant groups and USG values (r=0.78, p=0.001). Day two exhibited a moderately significant correlation (r=0.56, p=0.045). Lastly, day three showed a moderately insignificant correlation (r=0.41, p=0.128).

Day one was the shy of presenting with a significant association between groups and hydration level (X^2 = 3.281, p=0.07). However, here was no statistically significant association between groups and hydration level for days two and three (Day 2: X^2 =1.040, p=0.31; Day 3: X^2 =0.603, p=0.44), respectively

Table 5. Average USG Per Day

	Day 1 (Mean ± SD)	Day 2 (Mean ± SD)	Day 3 (Mean ± SD)
SCT	1.013±0.004	1.011±0.004	1.013±0.008
CON	1.024±0.005	1.020±0.009	1.020±0.007

SCT = Sickle Cell Trait; CON = control group

CHAPTER V

Discussion

The purpose of the study was to explore the correlation between SCT and USG measurements along with the association between the groups and hydration level. The key findings of this study show that on average, the USG of the SCT group was lower than the USG of the CON group over the course of the three days. However, the correlation was only significant on days one and two. By day three of camp, both groups should have been focusing more on hydrating and fueling their bodies properly, as research has shown that proper hydration will lead to a lower USG, regardless of SCT (L. E. Armstrong, 2007). Based on our findings, the CON group became more aware of proper hydration, while the effects of SCT on hydration status became muddled. The first two days of camp better display the correlation between SCT and USG without hydration intervention. By the third day of camp, the athletes were more focused on hydrating for performance rather than ad libitum. While the association between SCT and hydration level never met statistical significance, day one was closest to a significant association. On most days, two or three athletes were in the hydrated category for both participant groups. The fact that the sample size was small and not all athletes participated on all three days did not allow for the most accurate representation of the association between SCT and hydration level. The current research, demonstrated by Wang et al. in 2021 shows, that USG is lower in collegiate American football athletes with SCT study also found a higher concentration of creatine kinase (CK) in the blood along with a higher concentration of electrolytes lost via sweat when compared to the control group (Wang et al., 2021). The increased levels of CK in the blood of the SCT group becomes concerning when considering rhabdomyolysis. Rhabdomyolysis involves the breakdown of muscle tissue which releases CK, electrolytes, and other muscle components into the blood stream (Torres et al., 2015). With an already increased level of CK, SCT athletes may have an increased level of muscle damage compared to their counterparts. Similarly, the increased potassium lost via sweat could show increased muscle damage in the SCT group, as potassium is released from muscle cells that have been damaged (Cabral et al., 2020). Our study also found that USG measurements were lower in the SCT group on average; therefore, future research is needed to understand the mechanism behind these findings

There were many strengths of the present study. To date only one other study has identified the trend of lower USG in collegiate athletes with SCT which is concurrent with our findings. Therefore, our protocol is generalizable to other collegiate athletes with sickle cell trait. The refractometer provides quick and valid results to sport practitioners and is more cost effective than other urine assessment methods. However, we are aware that there are limitations to this study. The sample size was small due to the low number of SCT athletes. Along with this, athlete participation was not consistent over the days which caused our sample to be even smaller. In addition, our time frame was short in comparison with the study conducted by Wang et al.

Future research includes exploring the hydration habits of SCT athletes both during exercise and at rest. With a lower USG, SCT athletes could potentially be ingesting more fluids. In addition, exploring the practice outcomes of hydrated SCT versus CON athletes could provide insight to whether USG is a proper hydration assessment for SCT athletes. The use of more accurate assessment methods, such as plasma osmolality or urine osmolality, may provide more insight into SCT athletes' hydration status. While there are obvious increased risks for SCT athletes, such as rhabdomyolysis, exploring the relationship SCT and the likelihood of cramping or the need for intravenous fluids following exercise. Not only is it important for SCT athletes to be aware of their risks, but it is imperative that sports medicine practitioners educate themselves on the dangers of negative implications brough about by SCT. The symptoms of a sickling event are common for any athlete during extended intense bouts of exercise in hot and humid conditions. However, practitioners should be conscientious of those athletes that have SCT and give extra attention should these symptoms arise. Similarly, the fact that SCT athletes present with a lower USG allows for practitioners to cater to their hydration needs. Research has shown that there is potentially increased muscle damage in SCT athletes; therefore, it is imperative that they are intaking adequate water regardless of USG measurements.

Conclusion

This study aimed to determine a correlation between SCT status USG measurements along with an association between SCT and hydration status. The data supports the notion that there is a correlation between SCT and USG, with SCT athletes presenting with lower USG measurements compared to other athletes. However, there was not a significant association between SCT and hydration status. Based on this data, practitioners should consider further hydration education for themselves and for athletes

References

Armstrong, L. E. (2005). Hydration assessment techniques. *Nutrition Reviews*, *63*(6), S40-54.

Armstrong, L. E. (2007). Assessing hydration status: The elusive gold standard. *Journal of the American College of Nutrition*, *26*(5), 575S-584S.

Armstrong, L. E., Giersch, G. E. W., Dunn, L., Fiol, A., Munoz, C. X., & Lee, E. C. (2020). Inputs to Thirst and Drinking during Water Restriction and Rehydration. *Nutrients*, *12*(9). https://doi.org/10.3390/nu12092554

Armstrong, L., Maresh, C., Castellani, J., Bergeron, M., Kenefick, R., LaGasse, R., & Riebe, D. (1994). Urinary Indices of Hydration Status. *International Journal of Sports Medicine*, *4*(3), 265–279. https://doi.org/10.1123/ijsn.4.3.265

Bagley, W., Yang, H., & Shah, K. (2007). Rhabdomyolysis. *Internal and External Medicine*, *2*(3), 210–218. https://doi.org/10.1007/s11739-007-0060-8

Bergeron, M. F., Cannon, J. G., Hall, E. L., & Kutlar, A. (2004). Erythrocyte Sickling During Exercise and Thermal Stress. *Clinical Journal of Sports Medicine*, *14*(6), 354–356

Bonham, V.L., Dover, G.J., & Brody, L.C. (2010). Screening Student Athletes for Sickle Cell Trait-A Social and Clinical Experiment. *The New England Journal of Medicine*, *363*(11), 997–999. https://doi.org/10.1056/NEJMp1007639

Borg, G. (1970). Perceived Exertion as an Indicator of Somatic Stress. *Scandinavian Journal of Rehabilitation Medicine*, *2*(2), 92–98.

Cabral, M. I., Edding, S. N., Portocarrero, J. P., & Lerma, E. V. (2020). Rhabdomyolysis. *Disease-a-Month*, *66*(8). https://doi.org/10.1016/j.disamonth.2020.101015

Cao, A., & Galanello, R. (2010). Beta-Thalassemia. *Genetics in Medicine*, *12*, 61–76. https://doi.org/10.1097/GIM.0b013e3181cd68ed

Casa, D. J., Armstrong, L. E., Hillman, S. K., Montain, S. J., Reiff, R. V., Rich, B. S. E., Roberts, W. O., & Stone, J. A. (2000). National Athletic Trainers' Association Position Statement: Fluid Replacement for Athletes. *Journal of Athletic Training*, *35*(2), 212–224.

Chadha, V., Garg, U., & Alon, U. S. (2001). Measurement of Urinary Concentration: A Critical Appraisal of Methodologies. *Pediatr Nephrol*, *16*, 374 382. https://doi.org/10.1007/s004670000551

Cheung, S. S., McGarr, G. W., Mallette, M. M., Wallace, P. J., Watson, C. L., Kim, I. M., & Greenway, M. J. (2015). Separate and combined effects of dehydration and thirst sensation on exercise performance in the heat. *Scandinavian Journal of Medicine & Science in Sports*, *25*(S1), 104–111. https://doi org.umiss.idm.oclc.org/10.1111/sms.12343

Clarke, N., Drust, B., Maclaren, D., & Reilly, T. (2008). Fluid provision and metabolic responses to soccer-specific exercise. *Europe's Journal of Applied Physiology*, *104*(6), 1069. https://doi.org/10.1007/s00421-008-0864-x

da Guarda, C. C., Yahouédéhou, S., Santiago, R. P., Neres, J., Fernandes, C., Aleluia, M. M., Figueiredo, C., Fiuza, L. M., Carvalho, S. P., Oliveira, R. M., Fonesca, C. A., Ndidi, U. S., Nascimento, V., Rocha, L. C., & Goncalves, M. S. (2020). Sickle Cell Disease: A distinction of two most frequency genotypes (HbSS and HbSC). *PLoS One*, *15*(1). https://doi.org/10.1371/journal.pone.0228399

Dorizzi, R., Pradella, M., Bertoldo, S., & Rigolin, F. (1987). Refractometry, Test Strip, and Osmometry Compared as Measurements of Relative Density of Urine. *Clinical Chemistry*, *33*(1), 190. https://doi.org/10.1093/clinchem/33.1.190

Eichner, R. E. (2010). Sickle cell trait in sports. *Current Sports Medicine Reports*, *9*(6), 347–351. https://doi.org/10.1249/JSR.0b013e3181fc73d7

Figueiredo, M. S. (2015). The Compound State: Hb S/beta-thalassemia. *Revista Brasileira de Hematologia e Hemoterapia*, *37*(3), 150–152. https://doi.org/10.1016/j.bjhh.2015.02.008

Garrett, D. C., Rae, N., Fletcher, J. R., Zarnke, S., Thorson, S., Hogan, D. B., & Fear, E. C. (2018). Engineering Approaches to Assessing Hydration Status. *IEEE Reviews in Biomedical Engineering*, *11*, 233–248.

Godek, S., Bartolozzi, A., Godek, J., & Roberts, W. (2005). Sweat rate and fluid turnover in American football players compared with runners in a hot and humid environment. *British Journal of Sports Medicine*, *39*(4), 205–211. https://doi.org/10.1136/bjsm.2004.011767

Greenleaf, J. E. (1992). Problem: Thirst, drinking behavior, and involuntary dehydration. *American College of Sports Medicine*, *24*(6), 645–656.

Hassell, K. L. (2010). Population Estimates of Sickle Cell Disease in the U.S. *American Journal of Preventive Medicine*, *38*(4), S512–S521. https://doi.org/10.1016/j.amepre.2009.12.022

Heileson, SP, USA, M. J. L., & Jayne, SP, USA, M. J. M. (2019). Validity of Digital and Manual Refractometers for Measuring Urine Specific Gravity During Field Operations: A Brief Report. *Military Medicine*, *184*, e632–e636.

Jones, L. C., Clearly, M. A., Lopez, R. M., Zuri, R. E., & Lopez, R. (2008). Active Dehydration Impairs Upper and Lower Body Anaerobic Muscular Power. *The Journal of Strength and Conditioning Research*, *22*(2), 455–463. https://doi.org/10.1519/JSC.0b013e3181635ba5

Kato, G. J., Piel, F. B., Reid, C. D., Gaston, M. H., Ohene-Frempong, K., Krishnamurti, L., Smith, W. R., Panepinto, J. A., Weatherall, D. J., Costa, F. F., & Vichinsky, E. P. (2018). Sickle Cell Disease. *Natural Reviews Disease Primer*, *4*. https://doi.org/10.1038/nrdp.2018.10

Kerksick, C. M., Arent, S., Shoenfeld, B. J., Stout, J. R., Campbell, B., Wilborn, C. D., Taylor, L., Kalman, D., Smith-Ryan, A. E., Kreider, R. B., Wiloughby, D., Arciero, P. J., VanDusseldorp, T. A., Ormsbee, M. J., Wildman, R., Greenwood, M., Ziegenfuss, T. N., Aragon, A. A., & Antonio, J. (2017). International Society of Sports Nutrition Position Stand: Nutrient Timing. *Journal of the International Society of Sports Nutrition*, *14*. https://doi.org/10.1186/s12970-017-0189-4

Kostelnik, S. B., Rockwell, M. S., Davy, K. P., Hedrick, V. E., Thomas, D. T., & Davy, B. M. (2021). Evaluation of Pragmatic Methods to Rapidly Assess Habitual Beverage Intake and Hydration Status in U.S. Collegiate Athletes. *International journal of sport nutrition and exercise metabolism*, 31(2), 115–124. https://doi.org/10.1123/ijsnem.2020-0125

Lopez, R. M., Ashley, C. D., Zinder, S. M., & Tritsch, A. J. (2021). Thermoregulation and Hydration in Female American Football Players During Practices. *Journal of Strength and Condition*, *35*(9), 2552–2557. https://doi.org/10.1519/JSC.0000000000003180

Lukens M.D., J. N. (1981). Sickle Cell Disease. *Disease-a-Month*, *27*(5), 1–56. https://doi.org/10.1016/s0011-5029(81)80007-7

MacLeod, H., & Sunderland, C. (2010). Previous-day Hypohydration Impairs Skill Performance in Elite Female Field Hockey Players. *Scandinavian Journal of Medicine & Science in Sports*, *22*(3), 430–438. https://doi.org/10.1111/j.1600 0838.2010.01230.x

Magal, M., Cain, R., Long, J., & Thomas, K. (2015). Pre-Practice Hydration Status and the Effects of Hydration Regimen on Collegiate Division III Male Athletes. *Journal of Sports Science and Medicine*, *14*, 23–28.

Maughan, R., & Shirreffs, S. (1997). Recovery from prolonged exercise: Restoration of water and electrolyte balance. *Journal of Sports Sciences*, *15*(3), 297–303. https://doi.org/10.1080/026404197367308

Minton MS, ATC, D. M., O'Neal PhD, E. K., & Torres-McGehee PhD, ATC, T. M. (2015). Agreement of Urine Specific Gravity Measurements Between Manual and

Digital Refractometers. *Journal and Athletic Training*, *50*(1), 59–64. https://doi.org/10.4085/1062-6050-49.3.47

Mitchell, B. L. (2018). Sickle Cell Trait and Sudden Death. *Sports Medicine-Open, 4*(1), 19. https://doi.org/10.1186/s40798-018-0131-6

Nadel, E. (1988). Temperature Regulation and Prolonged Exercise. *Perspectives in Exercise Science and Sports Medicine*, *1*, 125–152.

Ncaa.org. (2021, March). NCAA Demographics Database. https://www.ncaa.org/about/resources/research/ncaa-demographics-database

Ncaa.org. (2021b, May). Report of The NCAA Division I Council. https://ncaaorg.s3.amazonaws.com/committees/d1/council/May2021D1Council Report.pdf

Periard, J. D., Racinais, S., & Sawka, M. N. (2015). Adaptations and Mechanisms of Human Heat Acclimation: Applications for Competitive Athletes and Sports. *Scandinavian Journal of Medicine & Science in Sports*, *25*(Suppl 1), 20–38. https://doi.org/10.1111/sms.12408

Rasouli, M. (2016). Basic concepts and practical equations on osmolality: Biochemical approach. *Clinical Biochemistry*, *49*(12), 936–941.\

Sawka, M., Burke, L., Eichner, E., Maughan, R., Montain, S., & Stachenfeld, N. (2007). Exercise and Fluid Replacement. *Medicine & Science in Sport & Exercise, 39*(2), 377–390. https://doi.org/10.1249/mss.0b013e31802ca597

Sawka, M. N., Francesconi, R. P., Young, A. J., & Pandolf, K. B. (1984). Influence of Hydration Level and Body Fluids on Exercise Performance in the Heat. *JAMA*, *252*(9), 1165–1169. https://doi.org/10.1001/jama.1984.03350090041020

Shelmadine, B. D. P., Baltensperger, A. M., Wilson, R. L. M., & Bowden, R. G. P. (2013). *Rhabdomyolysis and Acute Renal Failure in a Sickle Cell Trait Athlete: A Case Study* (pp. 235–237). Department of Health, Human Performance and Recreation, School of Education, Baylor University, Waco, Texas; Central Texas Nephrology Associates, Waco, Texas. https://oce-ovid com.umiss.idm.oclc.org/article/00042752-201305000

Sherman, W. M., & Lamb, D. R. (1988). Nutrition and Prolonged Exercise. *Perspectives in Exercise Science and Sports Medicine*, *1*, 213–280.

Shi, X., & Passe, D. H. (2010). Water and Solute Absorption from Carbohydrate Electrolyte Solutions in the Human Proximal Small Intestine: A Review and Statistical Analysis. *International Journal of Sport Nutrition and Exercise Metabolism*, *20*(5), 427–442. https://doi org.umiss.idm.oclc.org/10.1123/ijsnem.20.5.427

Shober, P., Boer, C., & Schwarte, L. A. (2018). Correlation Coefficients: Appropriate Use and Interpretation. *Anesthesia and Analgesia*, *126*(5), 1763–1768. https://doi.org/doi:10.1213/ANE.0000000000002864

Stachenfeld, N. S. (2008). Acute Effects of Sodium Ingestion on Thirst and Cardiovascular Function. *Current Sports Medicine Reports*, *7*(4 Suppl), S7–S13. https://doi.org/10.1249/JSR.0b013e31817f23fc

Taylor, N. A. S. (2014). Human Heat Adaptations Comprehensive Physiology, 4(1), 325 365. https://doi.org/10.1002/cphy.c130022

Travers, G., Nichols, David, Riding, N., Gonzalez-Alonso, J., & Periard, J. D. (2020). Heat Acclimation with Controlled Heart Rate: Influence of Hydration Status. *Medicine & Science in Sports & Exercise*, *52*(8), 1815–1824. https://doi.org/10.1249/MSS.0000000000002320

Torres, P. A. M., Helmstetter, J. A. M., Kaye, A. M. P., & Kaye, A. D. M., PhD. (2015). Rhabdomyolysis: Pathogenesis, Diagnosis, and Treatment. *The Oschner Journal*, *15*(1), 58–69.

Volpe, S. L., Poule, K. A., & Bland, E. G. (2009). Estimation of prepractice hydration status of National Collegiate Athletic Association Division I athletes. *Journal of*

Athletic Training, 44(6), 624–629. https://doi.org/10.4085/1062-6050-44.6.624.

Von Duvillard, S., Braun, S., Markofski, M., Beneke, R., & Leithäuser, R. (2004). Fluids and hydration in prolonged endurance performance. *Nutrition*, *20*(7–8), 651–656. https://doi.org/10.1016/j.nut.2004.04.011

Wang, H., Martone, M., Owens, M. E., Lemoine, N. P., Jr, Marucci, J., Calvert, D., Mulleniz, S., Church, T. S., Rood, J., Harrell, B., Irving, B. A., Speilmann, G., & Johannsen, N. M. (2021). NCAA Division I American Football Players with Sickle Cell Trait have AlteredHematological Responses and Hydration Status. *Scientific Reports (Nature Publisher Group); London*, *11*(1). http://dx.doi.org.umiss.idm.oclc.org/10.1038/s41598-021-81473 4

Wittbrodt, M. T., & Millard-Stafford, M. (2018). Dehydration Impairs Cognitive Performance: A Meta-Analysis. *Medicine & Science in Sports & Exercise*, *50*(11),