Hydration Exploration of Right Stuff via the MX3 Sweat Sodium Test in D1 Football Players

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Abstract

Division 1 football players put a lot of strain on their bodies daily and it is imperative that they focus on maximizing their performance. Hydration is a key factor that can help them perform at their best. This study explores football athlete's hydration status, the sodium content of their sweat, and their feelings of thirst, fatigue, and mood after a weightlifting session. Participants were first administered a sweat patch to collect sweat produced during a morning lift. In subsequent lifts, participants were randomized to receive one of three conditions: water at the beginning and water halfway through, water at the beginning and Right Stuff (electrolyte packet) halfway through, or Right Stuff at the beginning and halfway through. Athletes completed the Abbreviated Profile of Mood States (POMS) questionnaire, the Rate of Fatigue scale, and Thirst Distress Scale and Visual Analogue Scale questions. While there were not any significant findings in hydration status between the three intervention groups in regard to fatigue, thirst distress, or total mood disturbance, the results did show that when the athletes were more hydrated after the lift, their esteem-related affect (ERA) score improved. When grouping the athletes based on their sweat rates, there were significant differences in several mood components of the POMS. No correlations between urine specific gravity and the MX3 hydration testing were seen. Continued research needs to be completed on this population of Division 1 football players regarding hydration to help maximize their performance.

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INTRODUCTION

All athletes, no matter what sport, sweat during prolonged physical activity, practice, or competition. However, athletes sweat at different rates and lose different quantities of electrolytes in their sweat. Sweat rates can vary due to body weight, genetic predisposition, heat acclimatization, and metabolic efficiency (American College of Sports Medicine et al., 2007). Sodium is known to be a key factor in maintaining plasma osmolarity and it also helps to regulate fluid moving between intracellular and extracellular spaces (Brown et al., 2024). Replacing the sodium content in an athlete's body is recommended to decrease the risk of cramping and allow the athletes to hold on to more water in their body.

The average athlete will lose 1-3L of sweat per hour (ASPDA., n.d.). Sweat is composed of water and the electrolytes: sodium, chloride, potassium, magnesium, and calcium. Sodium and chloride are the most abundant electrolytes in sweat (MySportsScience., n.d.). American football has been shown to have the highest sweat rates among other sports such as baseball, basketball, and soccer (Barnes et al., 2019). Because American football players wear large protective clothing and have large body mass, they have great sweat losses daily (~8.8 L/day) compared to cross country runners (~3.5 L/day) while training for the same amount of time in a hot environment (American College of Sports Medicine et al., 2007). Since athletes can lose different amounts of sweat each day it is recommended for them to track their body weight changes before and after training or games to estimate how much sweat they lost during that session or competition (American College of Sports Medicine et al., 2007). With high sweat losses it is important to find out what each athlete's hydration needs through hydration testing and personalized hydration plans.

The whole body wash down technique has been shown to provide the greatest accuracy when measuring sodium content in sweat (Brown et al., 2024). This technique is most appropriate in a laboratory setting where participants are sweating during stationary cycling or sweating passively (Brown et al., 2024). Although the whole body wash down technique has been shown to be accurate, it is not suitable to assess sweat sodium content within the rapid pace of collegiate athletics. More recently, the MX3 Diagnostics Company (MX3 Diagnostics, Austin, Texas) has developed a testing method to collect sweat production in sweat pads that can be analyzed with their MX3 Lab Pro device to determine sweat sodium loss during exercise. Determining sweat losses during exertion can inform athletes and their support staff (Dietitians, Athletics Trainers, and Sports Scientists) with knowledge for hydration management.

In addition to sweat sodium content, hydration is crucial to athletic performance. When an athlete is dehydrated it can affect their athletic performance, endurance, breathing, and their mood. Water in the body will cushion the joints which can affect an athlete's flexibility and speed. Fluids are needed in the body for breathing, to moisten the air, making it easier to absorb the oxygen in the lungs. When someone is dehydrated it could affect their mood if they have symptoms such as headaches or a loss of concentration (Nairn, n.d.).

There are multiple different ways athletes can have their hydration status tested such as using a urine specific gravity (USG) test. However, real-time hydration testing through a USG test is not possible on the sideline of a sporting event. Therefore, the MX3 team of medical professionals, engineers, researchers, and athletes came together to create the MX3 hydration testing system (MX3 Diagnostics, Austin, Texas). The MX3 system has been used by a variety of people such as the military, athletes, and companies with employees working in outdoor conditions (MX3 Diagnostics, n.d.). The MX3 hydration system works by getting results for salivary osmolality (SOSM) and qualifies someone as severely dehydrated, moderately dehydrated, mildly dehydrated, or hydrated. Hydration status can also inform the athlete and their support staff about real-time hydration needs to help the athlete perform at their peak.

Athletes can feel fatigued during their training due to general wear and tear on the body, being under fueled, or being dehydrated. Dehydration will make the athlete feel very tired and fatigued and their heart rate will rise. They may even start to have a headache, dry mouth, and feel nauseous. Muscle cramps can also start due to the loss of electrolytes in their body and them not being replenished. Hydration needs to be a priority for an athlete throughout the day because it can help improve their energy level and avoid fatigue (Spain., 2024).

Athletes can feel a range of emotions in their body before a game or even before a very important practice. They can feel a range of emotions such as being happy, sad, fearful, angry, or anxious. If an athlete has an important event coming up and wants to perform well it can cause their heart to beat faster, their breaths can quicken, their muscles might tighten, and their stomach might feel upset. While one athlete may be feeling this way, other athletes on the team may be feeling different emotions and these emotions can spread throughout the team. Learning how to cope with the different feelings in a sport is important to help with consistent performance (Tamminen et al., 2022).

Hydration status, the sodium content of the athlete's sweat, how quickly they fatigue, and their mood can all affect their athletic performance. It is critical to support athletes in every way possible through sports psychology, athletic training, strength and conditioning, sports science, and nutrition. All these groups are needed, working together to aid athletes in reaching their full potential. The main goal during exercise regarding hydration is to prevent excessive (>2% body weight loss from water deficit) dehydration and changes in electrolyte balance so that it will not compromise the athlete's ability to perform their best and put them at risk for injury (American College of Sports Medicine et al., 2007). Keeping a close eye on their hydration status, mood, and fatigue will lead to a healthier overall athlete.

LITERATURE REVIEW

Hydration during Exercise

Dehydration has been shown to impair performance, increase fatigue, and reduce cardiovascular thermoregulatory response (Ashley et al., 2024). Dehydration is usually defined as a 2-3% body mass loss (American College of Sports Medicine et al., 2007). Dehydration can also persist days later. A study by Ashley et al. was completed on American football players ranging in age from 14 to 18 years old on a high school varsity team (Ashley et al., 2024). The participants provided a urine sample before and after practices, while environmental conditions were recorded during practice every 15-20 minutes, for 11 practices. In addition, each athlete was assigned an individual water bottle to drink out of during practice which was refilled when needed. The researchers found that there was a significant difference in their USG from the post practice day 2 score to the pre practice day 3 score ($p=0.011$), indicating that water and electrolyte loss from a previous exercise bout can affect hydration status the next day. A review by Maughan and Shirreffs also found that many athletes begin workouts already dehydrated and that the best way to control their hydration status is to have an individualized hydration plan in place (Maughan & Shirreffs, 2010).

American football athletes are particularly at risk of dehydration. A study of 1303 athletes investigated whole-body sweating rate (WBSR), sweat sodium, and the rate of sweat sodium loss in exercise sessions lasting 1-3 hours (Barnes et al., 2019). WBSR was calculated by taking the difference between their pre and post exercise body mass which was then corrected for fluid and food intake, as well as urine or stool loss during exercise. American football saw the highest losses in WBSR (1.51 ± 0.70 L/h) followed by endurance sports, basketball, soccer, and baseball. In another study, forty-four professional football players were recruited to compare sweat rates, sweat sodium concentrations, and sodium losses in three different position groups (Godek et al., 2010). Before practice each player received a sweat patch on their forearm to determine how much sodium they lost during practice. The patches were collected 40-60 minutes after the start of practice. Sweat sodium losses ranged from 22.5 mmol/h to 291 mmol/h. The findings for group comparisons (backs and receivers [BK] = 73 $\pm$ 4 mmol/h, linebackers and quarterbacks [LB/QB] = 96 $\pm$ 5 mmol/h, lineman [LM] = 121 $\pm$ 8 mmol/h) were close to significance ($p=0.06$). In another study, Horswill et al. recruited 16 members of a professional American football team to participate in a study to look at the effects of physical training and loss of sweat sodium on whole blood sodium concentration (BNa) (Horswill et al., 2009). There were two separate groups: the reference group (R) and the cramp-prone group (C). They all were tested during a training session of 2.2 hours during the first three days of season. The sweat rate and acute weight loss that were recorded at the end of practice were not statistically significant, but group C did tend to sweat more than group R ($p>0.05$). The mean sweat sodium concentration was higher in the C group, but the difference was not significant ($p=0.28$).

Hypohydration can lead to impaired circulatory and thermoregulatory function during endurance exercise. In a recent review by Davis et al., it was shown that $\geq$3% in hypohydration can delay onset and decrease the sensitivity of sweating and skin blood flow response to hyperthermia (Davis et al., 2016). However, drinking breaks in most of the studies reviewed, provided athletes with enough fluids to offset their sweat losses. Drinking breaks can happen on the sidelines or during timeouts. One study in the review tracked body mass during summer training camp and found that there were mild body mass deficits after the first day but the hypohydration levels did not worsen as camp went on.

Hydration during exercise is a core component of keeping an athlete safe while they are on the field practicing or competing. When athletes are not hydrated it can cause a decrease in their performance. The studies above all show that hydration during exercise is an important factor when exercising.

Hydration and the Environment

Sweat rate in athletes can vary day to day with different environmental conditions. Smith et al. looked at thirteen trained endurance athletes that participated in running or biking one time per week for 30 minutes for 24 weeks in varying environmental conditions (Smith et al., 2021). The groups were low temperature (< 10 degrees C), moderate temperature (10-19.9 degrees C), or high temperature (> 20 degrees C). There were significant differences in sweat rate variability between low and high temperature groups and between the moderate and high temperature groups ($p<0.03$) groups with average differences of low = 0.15 L/h, medium = 0.14 L/h, and high = 0.16 L/h sweat rates.

Knowing sweat rates and sodium losses can be used to provide athletes with electrolyte recommendations for consumption and can aid in creating personal hydration plans for them. However, there are variations in sweat rate and sweat composition depending on whether the sweat testing is completed in a laboratory or on the field. Efforts should be made to standardize sweat testing among athletes. Sweat tests need to be interpreted based on where the test took place and what the environmental condition was (Baker, 2017).

A review by Armstrong et al. found that endurance athletes can experience moderate to severe levels of dehydration depending on the type of environment that they are exercising in (Armstrong et al., 2021). This review found that endurance athletes can lose ~11-12% of their body weight in the form of water during an Ironman triathlon in a cool environment but a day long walking or hiking in a desert environment can result in losing ~14-18% of body mass when fluids are restricted or unavailable (Armstrong., 2021).

Athletes all have different sweat rates, and they can all vary day to day based on the environment they are placed in. Collegiate football starts a month-long camp every August but there is not a way to replicate exactly how it is going to be on that first game day. These studies show that when the environment is warmer, the performance team for athletics should focus on acclimatization for the expected environment.

Hydration and Cognition and Mood

It is common that athletes struggle with euhydration during exercise. A systematic review by Dube et al. looked at the effects of hypohydration and fluid balance on the athlete's cognitive function (Dube et al., 2022). The review included five articles where hypohydration was correlated with heat stress and limited fluid intake, which then correlated with impaired cognitive function. The review found that when athletes had 3-5% body mass loss they had impaired cognitive function as well as mood disturbances. D'Anci et al. also found mood disturbances in a dehydrated condition (D'Anci et al., 2009). Thirty-one Tufts University athletes from the men's and women's crew and lacrosse teams volunteered to engage in high levels of aerobic physical activity. Athletes were asked to consume a minimum of 1 L of water on testing day and they weighed in before practice, during practice, and after practice. The participants were split into two groups that were either a dehydration condition or a euhydration condition. All the subjects participated in both conditions and their training lasted for 60 to 75 minutes. Once training was complete, they filled out a thirst and mood questionnaire. Anger ($p<0.001$), fatigue ($p<0.005$), depression ($p<0.05$), tension ($p<.005$), and confusion ($p<.005$) scores were all higher in the dehydration condition, and vigor ($p<.05$) was significantly higher in the euhydrated condition.

Studies have found that mood states and cognitive abilities can be influenced by water consumption. Dehydration and mental performance have been looked at in the military population (Masento et al., 2014). Soldiers in environments of extreme heat led to increased incidences of dehydration. Short-term memory, numerical ability, psychomotor function, and sustained attention were cognitive functions that were assessed. When the soldiers were in a severe state of dehydration all of the cognitive tasks were impaired. In another study, 52 participants were randomized into a low hydration group (<1.2 L/day) or a high hydration group (>1.2 L/day) for two days (Pross et al., 2014). Then the groups switched on days 3, 4, and 5, where the low hydration group increased their fluid consumption, and the high hydration group decreased their consumption. Mood and sensation assessments were repeated multiple times during the day. When the high hydration group had restricted water intake it significantly increased their thirst ($p<0.01$), decreased their contentedness ($p<0.05$), decreased their calmness ($p<0.01$), decreased their positive emotions ($p<0.05$), and decreased their vigor/activity ($p<0.001$).

Emotions throughout the day can change on a whim and they can be caused by lots of different factors. However, in sport, emotions could be affected by the athlete's hydration status. Athletes often have a hard time staying in euhydration status throughout games and practices. The studies above support that when athletes are in a dehydrated state it can cause them to feel more negative emotions than positive.

Hydration and Performance

Hydration status has been correlated with perceived performance. Cesanelli et al. took eight males that are competitive junior category cyclists, and performed similar team monitored training sessions for 2 to 5 days a week (Cesanelli et al., 2021). This study categorized the training load levels into training impulse (TRIMP) values of low (<100), medium (100-200), or high load (>200). Fluid loss was tracked by athletes weighing in before and after their training sessions as a non-invasive fluid loss marker. Rate of Perceived Exertion (RPE) was negatively correlated with pre to post exercise fluid loss, independent from training load. A recent review article focused on how sweat losses and fluid balance changes are reported in team sport athletes (Nuccio et al., 2017). The authors reviewed how the potential mechanisms of dehydration can impact team sport performance. Reductions in aerobic capacity and muscle endurance that occur when athletes are dehydrated can contribute to their impaired physical performance. American football, rugby, and basketball are a few sports that reported the highest sweating rates and fluid balance disturbances. Their mean body mass loss however was <2% which is considered mild. Dehydration is typically a bigger problem when their mean body mass loss is between 3-4% and includes heat stress.

In another study, eight males volunteered to be in a study that investigated the effects of pre-warming (cycling to warm up the body) and then they would consume a specific amount of fluid (Marino et al., 2003). In this study, pre-warming was initially achieved by cycling for 20 minutes and heating up the body temperature. The first fluid regimen was fluid replacement equal to sweat rate (FF), the second fluid replacement regimen was equal to half the sweat rate (HF), and the third regimen was no fluid replacement (NF). The study found that when the two 20-minute active pre-warming periods were completed, the exercise time to fatigue was significantly reduced for NF ($p<0.01$) compared to the FF and HF group. However, there were no differences in time to fatigue between the FF and HF groups.

A lot of components are needed for an athlete to perform at their best. These components can be weightlifting to become stronger, or sprint training to become faster, but hydration is also an important factor to consider. Several hydration studies on athletes' performance found that when they are dehydrated this caused them to fatigue quicker and have reductions in aerobic capacity and muscle endurance. For athletes to compete at a high level they must make sure they are hydrated.

Sodium Supplementation on Hydration and Performance

Sodium supplementation to improve hydration is one strategy employed with athletes. In a study by Stofan et al., ten football players in NCAA Division 1 with a history of cramping issues were recruited to receive in their own 32-oz water bottle during practice with either water, Gatorade, or Gatorade with additional electrolytes (Stofan et al., 2005). The players had the choice to drink as little or as much as they wanted to during practice. Before each practice a USG test was administered, and their body weights were taken pre and post practice. Gross sweat loss for the 2.5-hour practice was very similar in the crampers versus non crampers group (p=0.250). Fluid intake was also very similar in the cramper and non cramper group (p=0.734). The season prior to this study each subject in the crampers group experienced at least one but no more than five episodes of heat cramps (mean 3.0 ± 1.9, n=5) that needed to be treated. The season following this study the cramping group saw in total only ten episodes that required treatment. The non cramper group did not experience any cramping episodes the season prior or the season following this study.

In another study, twelve ranked tennis players from the University of Hull Men's tennis team took part in a study looking at how consuming a 250 ml beverage with or without sodium twenty minutes before a training session would affect their body mass, urine osmolality, groundstroke and serve performance, thirst, rating of perceived exertion, and gastrointestinal discomfort (Munson et al., 2020). The beverages they were provided were either a sodium containing beverage (10, 20, 50 mmol/L) or a placebo. They continued to drink 1000 mL of the same beverage during certain intervals of training. The study found that there was a significant main effect of the condition for the pre and post change in urine osmolality. The reduction in urine osmolality was significantly greater in the 50 mmol/L group compared to the placebo ($p=0.037$) and improved groundstroke performance ($p<0.001$) compared to placebo. There was a reduction in RPE ($p=0.029$), perception of thirst ($p=0.012$), and GI discomfort ($p=0.019$) when consuming 50 mmol/L compared to the placebo group.

Looking at urine output with varying sodium containing beverages, eight moderately trained healthy male runners participated in a study to determine if pre-exercise ingestion of high concentrations of sodium beverages (659-870 ml split into 7 equal portions) would increase plasma volume and reduce the physiological strain in the heat (Sims et al., 2007). Treadmills were set to 70% of the participant's temperate-environment VO2max. There was an increase in urine output in the low sodium trial compared to the high sodium trial which could put an athlete at greater risk for dehydration.

Greenleaf et al. investigated different rehydration drinks on endurance exercise (Greenleaf et al., 1994). Five men participated in three sitting cycle ergometer endurance tests until exhausted, at weekly intervals. After each test the participants either drank a commercial rehydration drink (P1), a specifically formulated hyperhydration drink (HA), or nothing (N). The HA drink was a sodium-chloride-citrate hyperhydration drink where the P1 drink was a multi-ionic-glucose rehydration drink. It was found that the mean endurance was significantly longer with the HA drink compared to drinking N ($p<0.05$), but not with P1. All of the subjects except for one had longer endurance cycling with the HA beverage compared to P1 or N highlighting the possible beneficial effect of sodium supplementation for endurance events.

In a randomized cross-over study with 15 collegiate athletes, personal or standard hydration plans were investigated (Ayotte Jr & Corcoran., 2018). Athletes were given a prescription hydration plan (PHP) where the athlete's sweat rate and sodium loss were considered, or they followed their normal habits (NHP). Athletes exercised 45 minutes or more of moderate to hard training and their attention, awareness, and lower body anaerobic power were assessed. Athletes who followed the PHP jumped 4.53 $\pm$ 3.80 inches farther and had a faster heart rate recovery following a moderate to hard training session indicating that consuming adequate hydration and electrolytes tailored to each athlete led to an increased performance in these areas.

In a study with ultra marathon runners, Hoffman et al. recruited 233 people competing in a 161.3 km ultramarathon race (Hoffman & Stuempfle., 2016). Weight measurements were taken on the race participants the day before the race, within 1.5 hours of the race starting, and again when the runners reached the 47.8, 89.6, and 125.5 km distances during the race, and then immediately once the participants finished the race. The runners completed a questionnaire at the end of the race which included questions on how they train for the race, when do they drink for hydration, and what type of fluid they were consuming. The tests revealed that there was significant weight loss for both groups but there was a greater loss of weight at the 90 km point ($p=0.016$) and at the finish ($p=0.014$) for the participants consuming zero sodium supplements compared to those using sodium supplements. Also, the group using sodium supplementation during the race had significantly less weight loss ($p=0.0003$) at the finish line than they did at 48 km. The main finding in this study was that there was a significant relationship between the percent of body weight change and the amount of sodium the runners consumed and those that consumed more sodium maintained higher proportions of their initial body weights.

In an investigation on post-exercise hydration, Ly et al. recruited twenty-six athletes to complete three 90-minute interval training sessions (treadmill, stationary bike, or elliptical machine) where they did not consume any fluids (Ly et al., 2023). After exercise all participants replaced their body mass lost and were observed for 3.5 hours to see change in % fluid retained. The groups either drank water only, an oral rehydration solution (ORS), or a sports drink (SD). The % fluid retained for the ORS and SD groups were similar and more than the water-only placebo group ($p<0.05$). Once the 3.5 hours of observation concluded the ORS and SD beverages rehydrated the participants more than the water only group with a trend towards the ORS rehydrating the participants earlier.

Athletes constantly hear from professionals to hydrate throughout the day but if they are just drinking water this still may not solve all their hydration problems. Sodium supplementation may play a bigger role in staying hydrated. The studies above support that when supplementing with sodium during practice or competition, the athletes can hold on to more of their initial body weights and decrease their urine osmolality.

Hydration Test Products

Due to the importance of hydration and knowledge of sweat sodium production, companies are actively designing products that will further this area of research. The reliability of these hydration testing and sweat sodium testing tools is actively being researched. In a recent study, thirty-one recreationally active and non-heat acclimated adults volunteered for a trial to run for 45 minutes in a warm environment (Brown et al., 2024). MX3 patches were placed on the participants arm, back, and thigh to read their sweat sodium that they lost. Between the measurement sites, the mean difference between the first measurement and the second measurement was 0.1 mmol/L. The MX3 device had a coefficient of variation (CV) of 5.6%

compared to the Horiba LAQUAtwin-NA-11 that had a CV of 9.8%. The MX3 device was shown to have a good single trial reliability and good CV compared to the Horiba LAQUAtwin-NA-11.

In another study testing the MX3 saliva hydration instrument, seventy-five adults gave two saliva samples 3 to 5 minutes apart from one another to test reliability and minimal detectable change (MDC) of the MX3 hydration testing system (Winter et al., 2024). The participants filled out a questionnaire providing information for their age, sex, race/ethnicity, exercise, physical activity, and recent fluid intake. The amount of fluid the participant had consumed 4 hours prior was also recorded. The first and second saliva osmolality (SOSM) mean measurements averaged 64.3 ± 19.0 mOsm and 65.5 ± 17.9 mOsm ($p = 0.423$). Overall, this study found that the MX3 instrument system demonstrated moderate to good reliability for the assessment of SOSM.

Another method for sweat loss monitoring is through ion-sensitive field effect transistor (ISFET) sensor patches and ion-selective electrode (ISE) printed patches, which both transmit data on ion (Na^+ and K^+) fluxes in real-time. During endurance exercise these non-invasive patches were used to evaluate the physiological conditions of athletes (Wang et al., 2023). Three athletes participated in this study. The ISE patch was worn on two of the subjects while the ISFET sensor patch was worn on one subject while they cycled for exercise in the lab. Patches with ISFET sensors were able to give more reliable measurements while the ISE printed sensor patches had the benefit of integrating a simpler fabrication process and they were more flexible. In conclusion, it was found that the wearable patch technologies were promising for real time portable monitoring.

Athletes focus on their performance every single day but practicing and nutrition are not the only areas that can aid in helping improve their performance. Hydration is a key factor that can affect an athlete's performance, cognition, and mood. If the environment is cooler or hotter an athlete will need to change their hydration plan based on the weather and environment, they will be competing in. Sodium supplementation has been shown to help keep an athlete hydrated because when they are sweating, they need to replenish what they are losing. Overall, hydration test products such as MX3 are new to the sports field and will need more research conducted to test their validity.

Purpose and Specific Aims

Virginia Tech Athletics supports eleven men's sports programs including NCAA Division 1 football. Athletes focus not only on what they eat but also how they hydrate. Athletes need to consume adequate hydration and electrolytes so that they are not dehydrated which could put them at risk for an injury or a decrease performance. Sports dietitians work closely with their athletes to determine sweat rates and sodium loss. Athletes can decrease their risk of cramping during practices and games when they know how much sodium to consume beforehand. New tests and methods are coming out each year to find out how much sodium athletes are losing in their sweat. The purpose of this study was to evaluate sweat rates and hydration status of Virginia Tech football players. Specifically, the specific aims were to:

1. Classify the players as a low, moderate, high, or very high sweat producers
2. Assess how the MX3 hydration system compared with Urine Specific Gravity
3. Determine whether consuming the Right Stuff packet compared to water would affect hydration status, perception of hydration, fatigue, and mood of the athletes

METHODS AND MATERIALS

Study Design

14 Division 1 Football players (n=14) participated in a 4-week randomized cross-over design study to assess sweat rates and hydration status at 4 morning lift sessions. In session 1, all participants were administered a sweat patch to collect sweat produced during their lift. In sessions 2-4, participants were randomized to receive one of 3 conditions during their lift: 1) water at the beginning and water halfway through, 2) water at the beginning and Right Stuff halfway through, or 3) Right Stuff at the beginning and halfway through (see Figure 1). To control for differences in food intake, participants were served the same breakfast which consisted of one Musselman's applesauce pouch and one Gusher's pack to provide the athletes with quick carbohydrates for energy for their lift session. Upon arrival, participants did a urine analysis to determine urine specific gravity (USG). Before and after each lift, participants donated a saliva sample using the MX3 hydration system (MX3 Ventures, Newport Beach, CA). In addition, after each lift day the athletes were required to fill out the abbreviated POMS survey, Thirst Discomfort Score and Visual Analogue Scale, and the Rate of Fatigue scale. There was no monetary incentive given to the participants; however, they could receive their sweat sodium results to share with their team dietitian to receive their own personal hydration plan. This study was approved by the Institutional Review Board (IRB) at Virginia Tech.

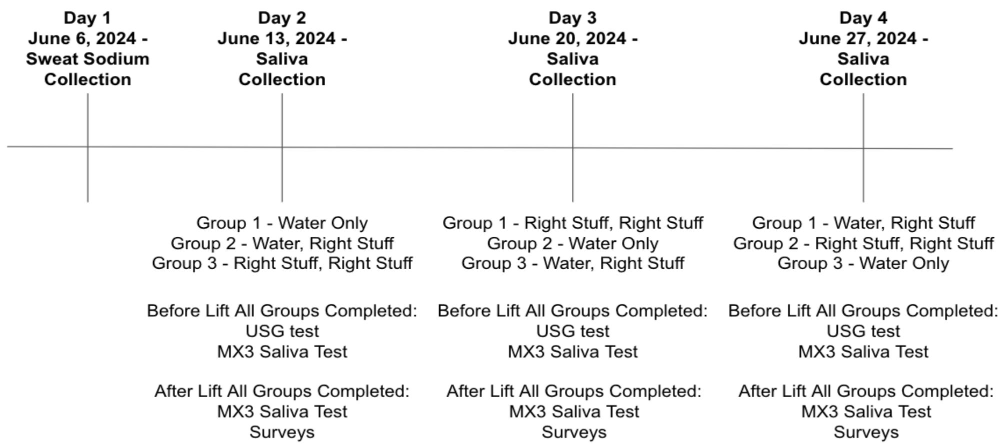


Figure 1. Study design.

The Right Stuff

The Right Stuff (Wellness Brands Inc., Tampa Bay, FL) is an electrolyte packet that can be given to the athletes to aid with hydration during their training. This packet was developed by NASA and is NSF certified for sport which means it has been third party tested (The Right Stuff, n.d.). The packet is 0.8 oz and is 10 calories and contains 3 g of carbohydrates. This packet aids with hydration because it contains 1780 mg of sodium and 1379 mg of chloride. The right stuff packet was added to a 16.9 fl oz Dasani water bottle for training. The athletes consumed the entire water bottle before and/or during their training session. Each group was assigned to drink either water only for the entirety of the lift, one right stuff bottle and one water bottle, or two right stuff bottles (see Figure 1).

Urine Specific Gravity

On day 1 of data collection, all participants completed an USG test prior to starting their lift. The USG test was read using a pocket refractometer ATAGO PAL-10S (Atago Co., Bellevue, WA). Urine samples were recorded. If the score was equal to or greater than 1.025 indicating that they were not fully hydrated, the athlete would then receive 5 ml/kg body weight to drink before the lift started to normalize hydration status between the participants (Brown et al., 2024). Supplementation ranged from 460 mls to 730 mls, depending on the player's weight.

Sweat Collection

MX3 sweat sodium patches (MX3 Ventures, Newport Beach, CA) were used to identify the participants sodium loss in their sweat. Each participant's forearm was wiped down thoroughly with an alcohol wipe and then the patch was applied to their inner forearm. The participants wore the sweat sodium patch during lift. The patches were collected at the conclusion of lift or if it was very saturated with sweat before lift ended it was collected earlier. All the patches were assigned their own syringe, the sweat patch was placed in the syringe and the sweat was pushed out onto a sample tray. Once there was about a dime sized amount of sweat on the tray, it was read by the MX3 analyzer. The score was sent to the MX3 app and which determined participant sweat content in mg/L. The number was then categorized as low, moderate, high, or very high sweat sodium content.

Saliva Collection

Prior to and after lifts 2-4, each participant's hydration status was tested using the MX3 hydration test strips. The MX3 saliva tests are administered by having the athlete swallow the saliva in their mouth and then generating new saliva on their tongue. They then stick their tongue out and the test strip in the device will be tapped on their tongue. The device will then connect with the MX3 app and will record the hydration results. The hydration score is based on mOsm and is categorized into one of four categories: hydrated, mildly dehydrated, moderately dehydrated, or severely dehydrated.

Abbreviated POMS Survey

The Abbreviated Profile of Mood State Questionnaire (POMS) was used to capture the participants current feelings across different emotional states. There were 40 different emotional states on the survey and the participants would rate how they were feeling on a scale of 0-4 ("Not at all" to "extremely") (see Table 1). The categories for the abbreviated POMS survey are tension (TEN), anger (ANG), fatigue (FAT), depression (DEP), esteem-related affect (ERA), vigour (VIG), and confusion (CON). These categories will leave you with the participants total mood disturbance (TMD) score. The formula for total mood disturbance is TMD = [TEN+DEP+ANG+FAT+CON]-[VIG+ERA]. This formula is the total of negative subscales and then subtracting the total of positive subscales (Grove et al., 1992).

Table 1. Abbreviated POMS.

TEN	Tense	On-Edge	Uneasy	Restless	Nervous	Anxious	
ANG	Angry	Grouchy	Annoyed	Resentful	Bitter	Furious	
FAT	Worn Out	Fatigued	Exhausted	Weary	Bushed		
DEP	Unhappy	Sad	Hopeless	Discouraged	Miserable	Helpless	Worthless
ERA	Proud	Ashamed	Competent	Confident	Satisfied	Embarrassed	
VIG	Lively	Active	Energetic	Full of Pep	Vigorous		
CON	Confused	Can't concentrate	Bewildered	Forgetful	Uncertain		

Thirst Discomfort and Score and Visual Analogue Scale

The thirst discomfort score (TDS) and visual analogue scale (VAS) were used to assess participant's perception of how dry they perceived their mouth was after they were finished working out and how much they would like to drink fluids at the moment. The TDS consisted of seven different questions and the VAS had two questions (Carey et al., 2021) (see Table 2).

Table 2. Thirst Distress Scale and Visual Analogue Scale Questions.

TDS Questions	VAS Questions
My mouth is dry	How intense is your thirst at the moment?
My lips are dry	How distressing (or bothersome) is your thirst at the moment?
My tongue is thick	
My saliva is thick	
My throat is dry	
I have a bad taste in my mouth	
I want to drink water	

Rating of Fatigue Scale

The rating of fatigue scale allowed participants to choose how fatigued they felt after the lift had concluded (see Figure 2). The scale consisted of a range of numbers from 0-10, with 0 meaning not fatigued at all and a 10 meaning total fatigue and exhaustion - nothing left. If the participant picked 0 that would be equivalent to them feeling as though they had a very easy lift and a good night's rest before. If the participant picked 10 this would mean it was one of the hardest workouts they have ever completed, and they do not have any more energy to exert. Each participant circled one number on the survey to show how tired they felt (Micklewright et al., 2017).

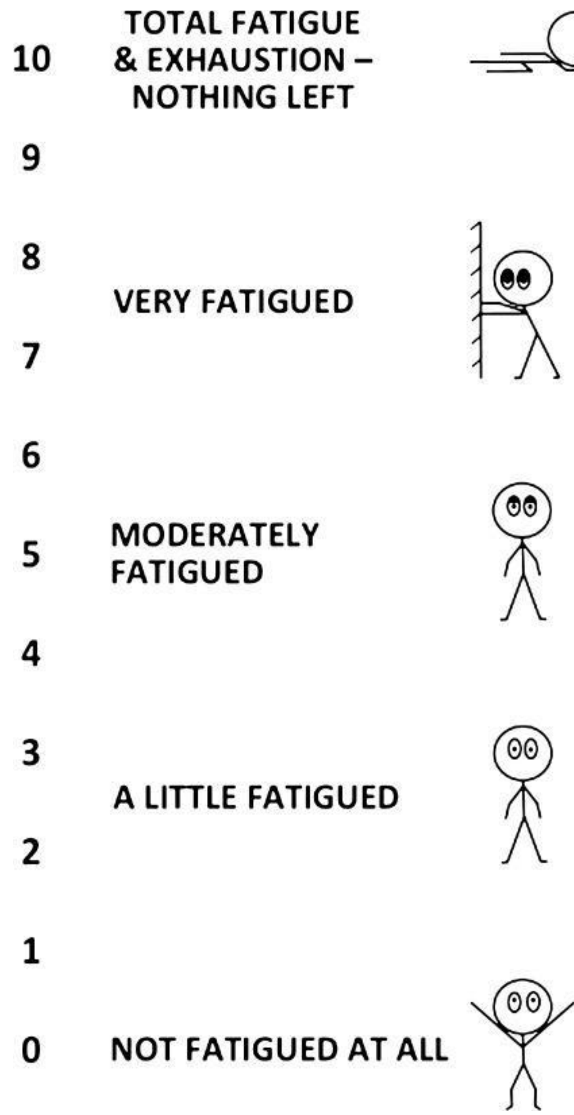


Figure 2. Rating of Fatigue Scale. 0-10 scale assessing perception of fatigue.

Statistics

Correlations with Pearson's R were completed to look at correlative data between POMS scores, rate of fatigue, thirst distress, VAS scores, with MX3 reported hydration, as well as the correlation between USG and MX3 reported hydration, and the correlation between sweat content and current weight. A two-way ANOVA was used to assess group and time interactions between the water only group, Right Stuff/water group, and the Right Stuff/Right Stuff group. One-way ANOVA was completed for mean comparisons between the three intervention groups and the POMS scores, rate of fatigue, thirst distress, VAS scores, as well as the three sweat rate groups and the POMS scores, rate of fatigue, thirst distress, VAS scores. Data analysis was completed with GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). Significance was set at a $p \leq 0.05$.

RESULTS

Participants

The participants for this study were recruited voluntarily to take part in the 4-week study. In order to participate in this study, the participants had to be on the current roster of the Virginia Tech football team. The study recruited and consented 17 participants. Three participants dropped out during week 1 of the study; one participant due to not having enough sweat in the patch to collect a reading, and the other two dropped due to not being able to complete the USG test. 14 total participants completed the study. All the participants were male, and they represented five different position groups in football (see Table 3). Most of the participants were upperclassmen.

Table 3. Participant demographics.

Gender	Male- 14 (100%)				
Race	Black or African American - 8 (57.1%)	White - 6 (42.8%)			
Year in College	Graduate Student - 1 (7.14%)	Senior - 2 (14.29%)	Redshirt Senior - 3 (21.4%)	Redshirt Junior - 4 (28.57%)	Redshirt Freshman - 4 (28.57%)
Position	Defensive Line - 4 (28.57%)	Offensive Line - 5 (35.71%)	Tight End - 2 (14.29%)	Wide Receiver - 2 (14.29%)	Running Back - 1 (7.14%)
Average Weight of Position	Defensive Line – 267.0 $\pm$ 27.1 lbs	Offensive Line - 303.4 $\pm$ 10.3	Tight End - 242.5 $\pm$ 2.1 lbs	Wide Receiver - 201 $\pm$ 9.9 lbs	Running Back - 207 lbs

MX3 Saliva Hydration Testing and Urine Specific Gravity

Participants provided a USG sample upon arrival as well as a saliva hydration test prior to their lifts. There was no significant correlation between their hydration status as measured with a USG test compared to the MX3 saliva hydration test over the three lift intervention days (14 players providing 3 samples each) (see Figure 3a). However, to standardize hydration status prior to starting the lift, players with a USG score equal to or greater than 1.025, received 5 ml/kg body weight of water to drink before the lift (Brown et al., 2024). When removing these samples (n=17) from the correlation to account for recent water consumption interfering with the salivary MX3 hydration sample, there was still no correlation with USG and MX3 saliva samples (see Figure 3b).

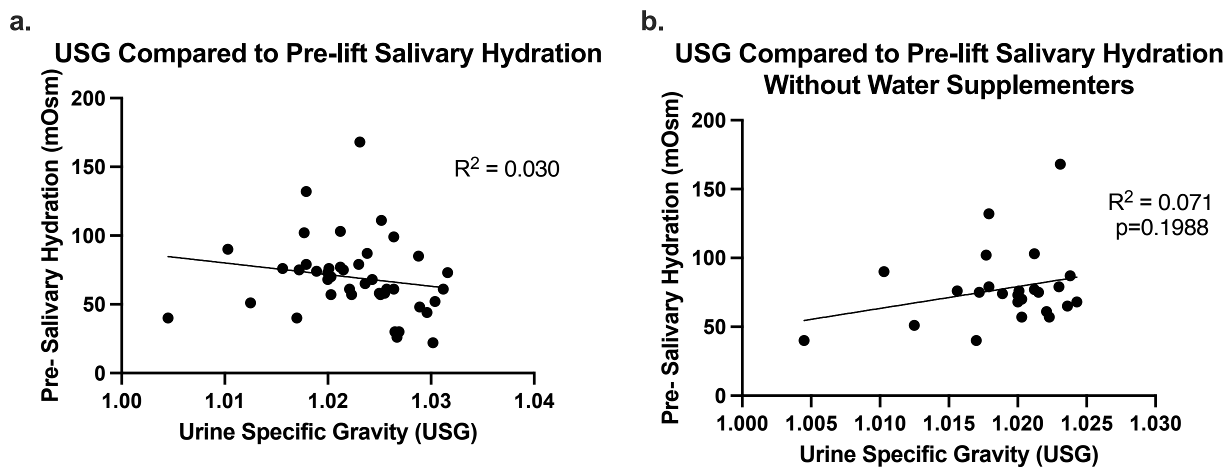


Figure 3a and b. USG Correlations with MX3 Salivary Hydration. a. No correlation was seen with the 42 samples from the 3 lifts between USG samples and the salivary MX3 hydration tests administered before the weightlifting session. **b.** When removing the 17 samples from players who supplemented with water prior to the lift due to a USG score of 1.025 or greater, there still was no correlation between the USG samples and the salivary MX3 hydration tests.

MX3 sweat sodium results

MX3 sweat sodium patches were used to identify the participants sodium loss in their sweat in mg/L. Participants were then categorized as having low, moderate, high, or very high sweat sodium content. Five players were categorized as having low sweat sodium content, six as having moderate sweat sodium content, two as having high sweat sodium content and one as having very high sweat sodium content (see Table 4). No significant differences were seen between the three groups for their scores on the Rate of Fatigue scale or VAS during lift days 2-4. There was a trend (p=0.0840) towards a decrease on the Thirst Distress Scale for the high sodium sweat group (see Figure 4).

Table 4. MX3 Sweat Test Results

Low (<750 mg/L)	5 subjects (35.7%)
Moderate (750-1100 mg/L)	6 subjects (42.8%)
High (1100-1450 mg/L)	2 subjects (14.3%)
Very High (>1450 mg/L)	1 subject (7.14%)

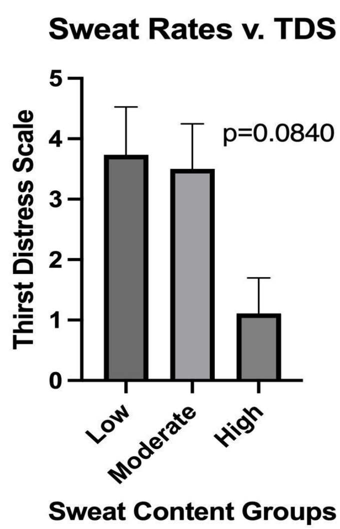
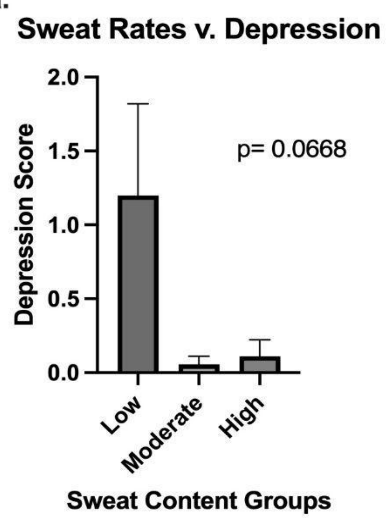


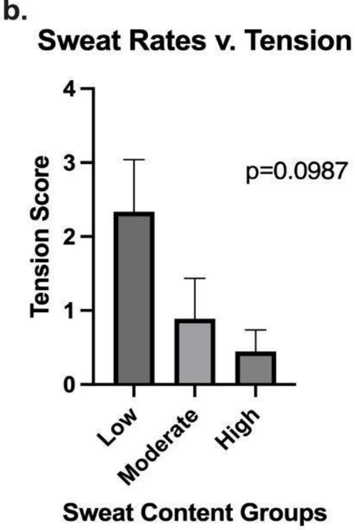
Figure 4. Sweat Sodium Content Group's Thirst Distress Scale Scores. This figure shows how each sweat content group scored on the Thirst Distress Scale. The high sweat content group trended towards lower thirst distress scale scores than the low and moderate groups.

The POMS indicated that there was a trend towards a decrease in depression (p=0.0668) and anger (p=0.0987) in the high sodium sweat group (see Figures 5a and b). The high sodium sweat group in figure 4 was combined with groups high and very high. The high sodium sweat group represented those who lost 1100 mg/L or more of sodium in their sweat. The moderate group represented those who lost 750-1100 mg/L of sodium in their sweat and the low group lost <750 mg/L. There was a significant decrease in anger for both the moderate and high sodium sweat groups compared to the low sodium sweat group (see Figure 5c), a significant decrease in esteem-related effect in the high sodium sweat group compared to the moderate sodium sweat group, but not compared to the low sodium's sweat group (see Figure 5d), and a significant decrease in total mood disturbance in the moderate sodium sweat group compared to the low sodium sweat group with no difference in the high sodium sweat group compared to the others (see Figure 5e).

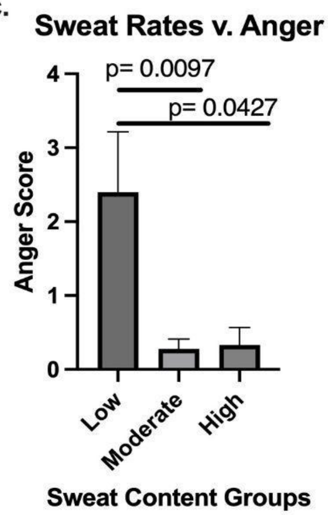
a.


Sweat Rates v. Depression
Depression Score
2.0
1.5
1.0
0.5
0.0
p= 0.0668
Low
Moderate
High
Sweat Content Groups

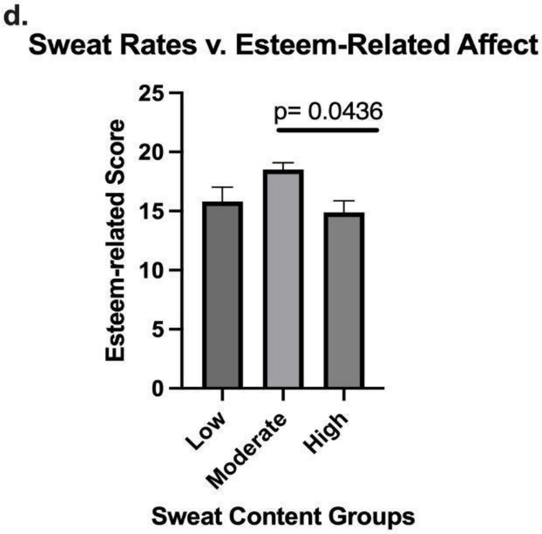
b.


Sweat Rates v. Tension
Tension Score
4
3
2
1
0
p=0.0987
Low
Moderate
High
Sweat Content Groups

c.


Sweat Rates v. Anger
Anger Score
4
3
2
1
0
p= 0.0097
p= 0.0427
Low
Moderate
High
Sweat Content Groups

d.


Sweat Rates v. Esteem-Related Affect
Esteem-related Score
25
20
15
10
5
0
p= 0.0436
Low
Moderate
High
Sweat Content Groups

e.

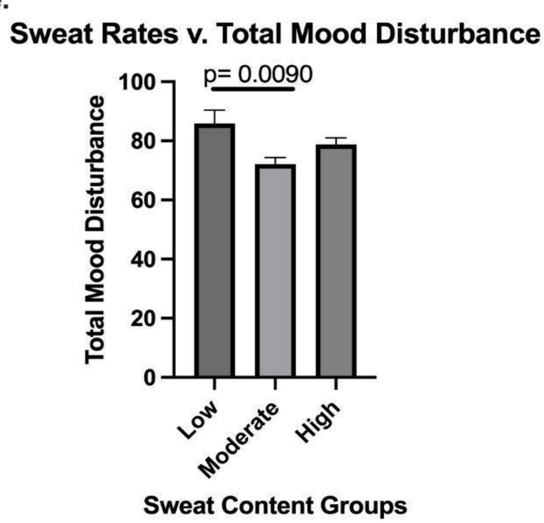

Sweat Rates v. Total Mood Disturbance
Total Mood Disturbance
100
80
60
40
20
0
p= 0.0090
Low
Moderate
High
Sweat Content Groups

Figure 5a-e. Sweat Sodium Content Group's POMS Scores. The low sweat content group trended towards higher scores for depression and tension, and was significantly higher for anger after training concluded. The moderate sweat content group had the highest esteem-related affect score and the low sweat content group had the highest score for total mood disturbance.

MX3 saliva results

Participants donated MX3 saliva samples pre and post lift. A score of less than 65 mOsm is considered hydrated, 65-100 mOsm is midly dehydrated, 100-150 mOsm is moderately dehydrated, and a score >150 mOsm is considered severely dehydrated. On lift day 2, all of the participants in the water only group were hydrated at the end of the lift (see Table 5). Participant 64 was the only athlete in the water only group where their score pre lift was more hydrated than their post lift score. In the water/ right stuff group participants 61 and 62 had scores post lift that were mildly dehydrated while the other three in the group were hydrated post lift. Participant 62's post lift score was more dehydrated than their pre lift score (see Table 5). In the right stuff/ right stuff group, there were two participants that had post lift scores that were mildly dehydrated. All the scores in the right stuff/ right stuff group improved and became more hydrated by the end of the lift (see Table 5). The participants with an asterisk next to their saliva score indicated they had a score of $\geq$ 1.025 on the USG test and needed to consume 5 mL/kg before lift began. No significant differences were seen between the three groups in their change in hydration status (see Figures 6a-c). There was a significant time effect pre-post lift for all the conditions ($p \leq 0.001$), indicating all three conditions increased hydration status in the players.

Table 5. MX3 Pre and Post Saliva Test Scores

ID	Water only pre	Water only post	Change in MX3 Score	Water+Right Stuff pre	Water+Right Stuff post	Change in MX3 Score	Right Stuff only pre	Right Stuff only post	Change in MX3 Score
50	90	26	-64	132	32	-100	103	29	-74
51	22*	33	11	87	54	-33	40	25	-15
52	61*	40	-21	58*	55	-3	73	52	-21
55	30*	64	34	48*	84	36	99*	72	-27
56	51	55	4	102	38	-64	40	8	-32
54	76	46	-30	75	48	-27	111*	46	-65
57	76	51	-25	74	61	-13	79	62	-17
63	168	35	-133	70	63	-7	57	62	5
64	26*	37	11	61*	27	-34	73*	33	-40
53	57	30	-27	52*	23	-29	68	58	-10
58	61*	48	-13	44*	18	-26	58*	24	-34
60	79	38	-41	65	45	-20	30*	35	5
61	68	31	-37	77	61	-16	75	131	56
62	85*	38	-47	61	68	7	57*	48	-9

* Asterisks denote water supplementers

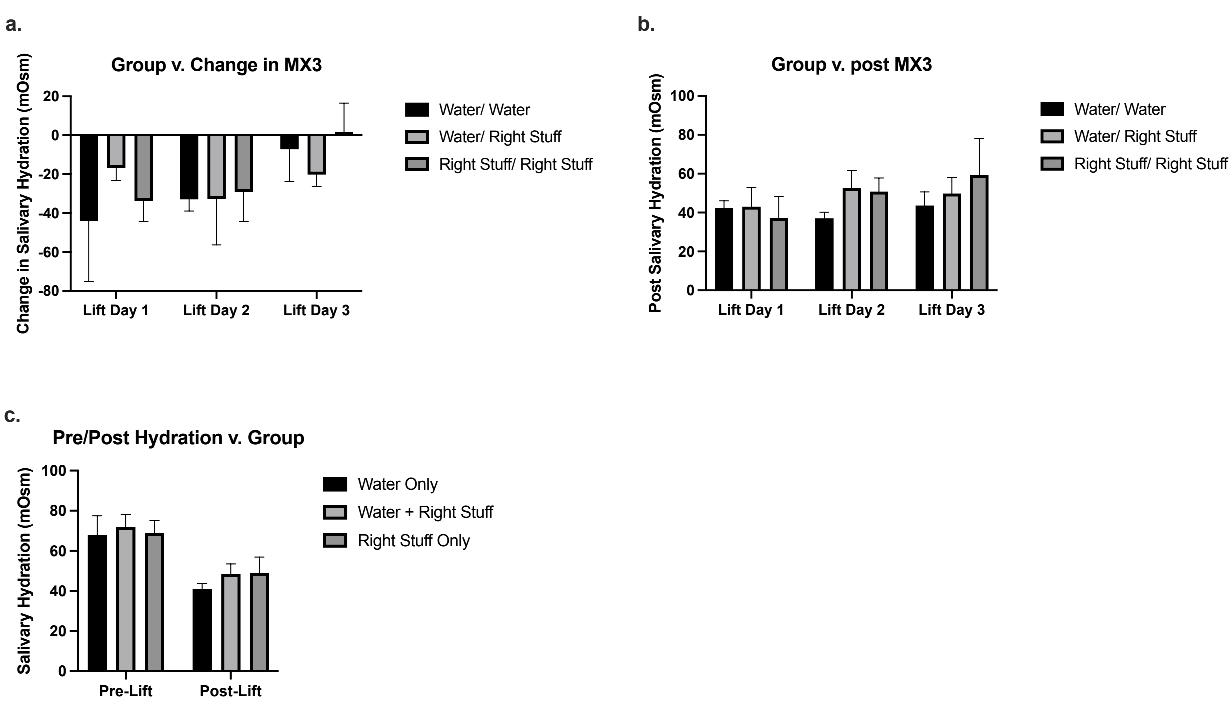


Figure 6a-c. Salivary Hydration Changes Between Groups. a. Group changes in pre-post lift change in salivary hydration status. **b.** Group changes in post-lift salivary hydration status. **c.** Changes in salivary hydration grouped by condition.

All the participants completed the abbreviated POMS survey. One of the categories in the POMS survey was the esteem-related affect (ERA). The emotions that are in the ERA category are proud, ashamed, competent, confident, satisfied, and embarrassed. If the post lift salivary hydration test was lower in score, then there was an increase in the esteem related affect score (see Figure 7). The esteem-related affect was higher when the participants were more hydrated which had a significance of p=0.04. No other significant findings in mood were correlated with hydration status.

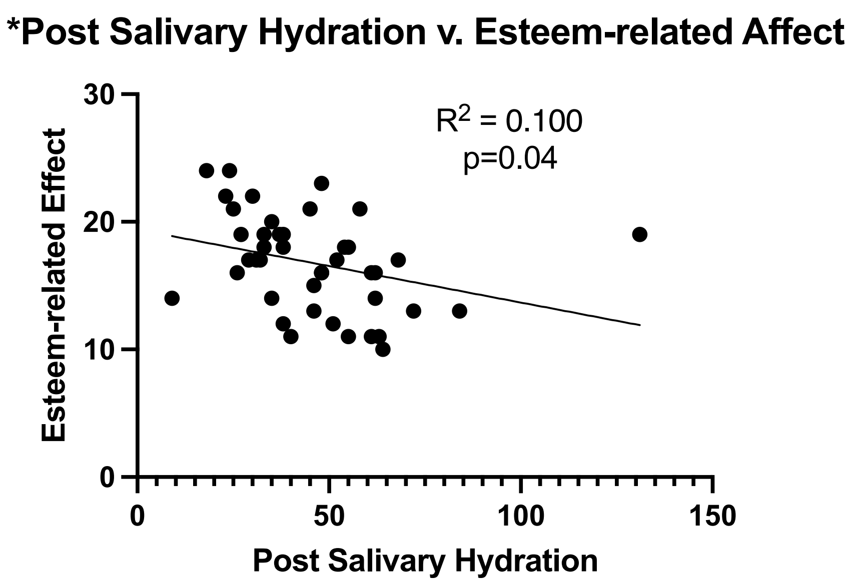


Figure 7. Post Salivary Hydration Compared to Esteem-Related Affect. Decreased post salivary hydration scores (more hydrated) were correlated with a higher esteem-related affect.

After each lift the participants filled out the Rate of Fatigue survey, the Thirst Distress Scale, and the VAS. The right stuff/ right stuff group trended toward lower scores of fatigue compared to the water only group and the water/right stuff group (p=0.1077, see Figure 8). No significant differences were seen with the Thirst Distress Scale or the VAS when comparing the hydration of the three groups

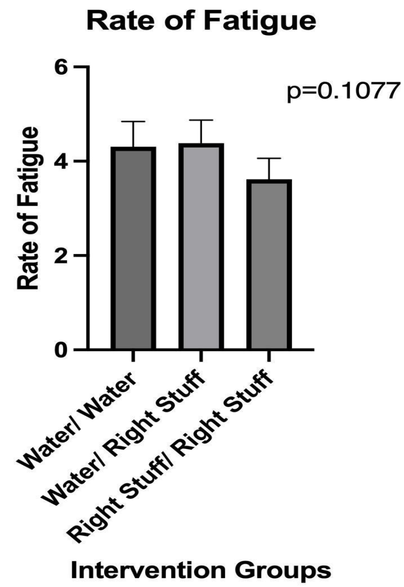


Figure 8. Rate of Fatigue. This figure shows that the right stuff/ right stuff group trended towards lower scores on the rate of fatigue scale after lift compared to the water/ water group and the water/ right stuff group.

DISCUSSION

This study tested the amount of sodium sweat content that Division 1 football players lost during a lift and if the Right Stuff sodium packet would hydrate the players more during a lift compared to water only. The specific aims focused on classifying the players as low, moderate, high, or very high sodium sweaters, assessing how the MX3 hydration correlated with a USG sample, and whether the Right Stuff packet compared to water would affect hydration status, perception of hydration, fatigue, and mood of the athletes. Overall, there was not enough evidence that the Right Stuff packet hydrated the athletes better during a lift than only water.

In this study there was no correlation between the MX3 pre lift hydration tests and the USG tests. In a blog post written by the team at MX3 diagnostics, when looking at studies that compared USG spot checks with plasma osmolality measurements the studies found that the USG measurement overestimated the number of athletes that were dehydrated before exercise (MX3 Diagnostics., 2020). In another study that the MX3 Diagnostics team reported on, 65% of hydrated individuals were dehydrated on a USG test. More research needs to be done to assess whether MX3 salivary hydration testing reports accurate hydration status and what confounders when testing could alter the results.

Regardless of what intervention group the subjects were in, there was no significant change on the thirst distress scale (TDS) scores. In a study from Carey et al., that tested the validity of the TDS and visual analogue score (VAS) found that there was a trend observed of higher TDS scores when fasting time was increased (Carey et al., 2021). Since the players in this study were fed prior to the interventions, this could have affected their reporting on the TDS and VAS scales.

Throughout the three separate MX3 saliva testing days there was no significant data found between the right stuff/ right stuff, water only, or right stuff/ water groups in hydration status post lift or change scores, with all three groups ending more hydrated than beginning. Scores varied each day, and some athletes dropped to lower scores on the saliva testing with only drinking water than when they had two Right Stuff packets during a lift. These results contrast with a study using a men's tennis team that showed a decrease in pre and post change in urine osmolality when the groups were supplemented with sodium during exercise (Munson et al., 2020). The results from this study are not consistent with the results found in our study where using the Right Stuff packet was not significantly different than water alone.

Significant data was found with one of the profiles of mood constructs, that when the athletes were more hydrated, they had a higher-esteem related affect. This aligns with a study by D'Anci et al., who found that when athletes were dehydrated, they had higher scores in anger, fatigue, depression, tension, and confusion, and when they were euhydrated, they had an increase in vigor (D'Anci et al., 2009). These findings also align with what Pross et al. found when assessing mood and sensation assessments throughout the day (Pross et al., 2014). When the hydration group had their fluid consumption reduced it decreased the subjects' positive emotions. When we broke the players into their sodium sweat content groups, the low sweat content group trended towards higher scores for depression and tension and had significantly higher feelings of anger after training concluded, than the higher sweat groups. The moderate sweat content group had the highest esteem-related affect score and the low sweat content group had the highest score for total mood disturbance. These findings could have been due to the players that were in each group and more research with a larger sample size is needed to confirm these results.

Lastly, the right stuff/ right stuff group trended towards a lower rate of fatigue on the questionnaire compared to the water only and right stuff/ water group. The results however were not significant. These results align with Greenleaf et al., who used a cycle ergometer endurance test study to assess hydration (Greenleaf et al., 1994). This study found that when drinking a formulated hyperhydration drink (HA) compared to the commercial hydration drink or nothing, the athletes who drank the HA beverage were able to have longer endurance.

Conclusion

Hydration testing and sweat sodium testing is an up-and-coming tool that dietitians, sports scientists, and athletic trainers will use in the future to learn more about their athletes and be able to help the athlete perform at their best. However, due to the little research on the new products, there are varying results that need to be tested. In summary, saliva hydration testing and sweat sodium testing can be helpful to learn more information about the athlete, but the Right Stuff packet may not have a huge impact on the athlete's hydration status when they are lifting inside a temperature-controlled room. More research needs to be conducted before recommending the MX3 device for saliva hydration testing.

Limitations

Despite not having many significant outcomes, some limitations were identified. There were only 14 participants in the study that represented 5 different position groups in football. When using the MX3 device to measure hydration status you must wait 5 minutes after the athlete has consumed fluid or food. Due to time restrictions with the athletes, there was not always 5 minutes to wait before testing the athlete. This could have skewed the results during their post lift hydration tests, as athletes were sometimes finishing their second beverage when it was time for testing. This could also have had an effect in pre-lift hydration testing in those athletes that had to drink extra water before lift due to a high USG. After speaking with a MX3 scientist post study, it was recommended that the athletes should rinse their mouth with water after consuming high amounts of sodium before completing a MX3 saliva test. This was not completed during this study. The athletes also had different weightlifting routines during each lift which could have affected the saliva scores due to varying intensities of the routines. Lastly, some of the emotions that the athletes answered on the abbreviated POMS may have been unfamiliar to them.

Future Directions

More research on hydration status with athletes using the MX3 device and sweat sodium patches needs to be completed. Moving forward it would be interesting to see how different the sodium amounts the athletes lose in lift compared to out on the field either during a conditioning session in a hot environment or with pads on during a practice. It would also be beneficial to research whether the athlete wearing pads and helmets during a conditioning session would affect their sweat sodium concentration compared to a conditioning session without pads and helmets. A larger population for these studies would be helpful to look at and compare different position groups hydration status and their sweat sodium loss.

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